Taxonomy, Ecology and Processing of Red Seaweeds

Based on a Regional Study and Workshop on the Taxonomy, Ecology and Processing of Economically Important Red Seaweeds

Food and Agriculture Organization (of the United Nations)
Network of Aquaculture Centres in Asia-Pacific
IFREMER

Bangkok, Thailand
June 1996
Report on a Regional Study and Workshop on the Taxonomy, Ecology and Processing of Economically Important Red Seaweeds

(GCP/INT/553/FRA)

Food and Agriculture Organization (of the United Nations) Network of Aquaculture Centres in Asia-Pacific

IFREMER

Bangkok, Thailand
June 1996
This volume contains the study report and proceedings of the Regional Workshop on the Taxonomy, Ecology and Processing of Economically Important Red Seaweeds, which was held in Bangkok, Thailand from 24 to 27 January 1995. The Workshop was supported by the Government of France Trust Fund with the Food and Agriculture Organization of the United Nations (FAO) and implemented under GCP/INT/553/FRA. The Network of Aquaculture Centres in Asia-Pacific (NACA) provided financial assistance and implemented the project. The government representatives who submitted country reports spoke as official representatives of their respective governments. The views contained in this publication do not necessarily represent those of FAO or NACA.

FOREWORD

NACA takes pleasure and pride in bringing the results of the regional study on the Taxonomy, Ecology and Processing of Red Seaweeds to farmers, governments, research and development organisations, the private industry support sector, investment agencies and regional and international assistance organisations. This report comprises:

i. the results of studies conducted in the nine participating countries and the status of red seaweed development in two other countries that attended the final workshop;

ii. technical reviews by resource persons from collaborating organisations and institutions in the Asia-Pacific region and France; and

iii. recommendations for follow-up action by countries and for regional collaboration in support of national efforts.

The study focused on *Gracilaria* species, which was of common interest among the countries because of:

- the many products that can be derived or extracted from it, such as human food, protein, and various bio-medical and industrial materials;
- its many uses, including as a phytosanitation agent of aquaculture waters and as a polyculture species with mollusc, shrimp and fish which are important contributions to environmental and natural resources management;
- its production and processing lends itself very well to community or family based small-scale activities which adds to rural employment and income; and
- the potential value of *Gracilaria* to national economies; the export or local consumption of *Gracilaria* in its dried raw, semi-processed, and processed forms could earn (or save) a sizeable amount of foreign exchange.

The country studies and workshop results identified activity areas and methods for upgrading *Gracilaria* stocks to:

i. improve agar yield and quality;

ii. improve systems of growing the species to increase production levels and make better use of natural resources; and

iii. identify other economic uses of the seaweed to enhance its utility or value.

The recommendations highlight regional co-operative action, in view of the relatively uneven state of advancement in *Gracilaria* research and development among the countries and the presence of a number of national and regional institutions that could be strengthened to serve as focal points and sources of expertise and technology for regional sharing. Specific areas and ways to co-operate in research, training, and information and expert exchange are recommended. The workshop however has recognised that upgrading the regional capacity from its present strength to that of self-reliance -- a long-term objective -- would require intensified assistance from organisations external to the region. In particular, the French Government which has for
a long time provided assistance in seaweed development to various countries through collaboration with scientists and technologists in several institutions in the region, was identified as a potential long-term collaborator in seaweed culture and processing research and development.

NACA, on behalf of the participating countries in the Asia-Pacific region, would like to express its gratitude to 1FREMER and the Food and Agriculture Organization of the United Nations (FAO) for endorsing the project, and the Government of France for providing funding and technical support. It is hoped that the conclusions and recommendations of this study and workshop will provide a baseline from which continued collaboration, with FAO, the Government of France and further co-operation with other concerned agencies and institutions, can be successfully developed.

I take this opportunity to commend the former NACA Co-ordinator, Dr Banchong Tiensongrusmee, on his role in bringing this study and workshop to a successful conclusion.

Hassanai Kongkeo
NACA Co-ordinator
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FOREWORD</strong></td>
<td>lii</td>
</tr>
<tr>
<td><strong>TABLE OF CONTENTS</strong></td>
<td>V</td>
</tr>
<tr>
<td><strong>EXECUTIVE SUMMARY</strong></td>
<td>vii</td>
</tr>
<tr>
<td>Part I Workshop background and organisation</td>
<td>1</td>
</tr>
<tr>
<td>Part II Country presentations</td>
<td>5</td>
</tr>
<tr>
<td>Part III Technical sessions</td>
<td>9</td>
</tr>
<tr>
<td>Part IV Discussion</td>
<td>13</td>
</tr>
<tr>
<td>Part V Recommendations</td>
<td>17</td>
</tr>
<tr>
<td>Part VI Closing remarks</td>
<td>23</td>
</tr>
<tr>
<td><strong>Annexes</strong></td>
<td>25</td>
</tr>
<tr>
<td>Annex I</td>
<td></td>
</tr>
<tr>
<td>Annex I-1 Summary of Training Workshop 21-28 April 1992</td>
<td>27</td>
</tr>
<tr>
<td>Annex I-2 List of participants</td>
<td>35</td>
</tr>
<tr>
<td>Annex I-3a Speech - Dr Kitjar Jaiyen</td>
<td>41</td>
</tr>
<tr>
<td>Annex I-3b Speech - Dr Veravat Hongskul</td>
<td>42</td>
</tr>
<tr>
<td>Annex I-3c Speech - Mr Alex Brayle</td>
<td>43</td>
</tr>
<tr>
<td>Annex I-3d Speech - Dr Banchong Tiensongrusmee</td>
<td>44</td>
</tr>
<tr>
<td>Annex I-4 Workshop programme</td>
<td>47</td>
</tr>
<tr>
<td>Annex II Country Reports</td>
<td></td>
</tr>
<tr>
<td>Annex II-1 Bangladesh</td>
<td>49</td>
</tr>
<tr>
<td>Annex II-2 Peoples Republic of China</td>
<td>53</td>
</tr>
<tr>
<td>Annex II-3 India</td>
<td>73</td>
</tr>
<tr>
<td>Annex II-4 Indonesia</td>
<td>87</td>
</tr>
<tr>
<td>Annex II-5 Malaysia</td>
<td>99</td>
</tr>
<tr>
<td>Annex II-6 Myanmar</td>
<td>107</td>
</tr>
<tr>
<td>Annex II-7a Philippines (Part I)</td>
<td>125</td>
</tr>
<tr>
<td>Annex II-7b Philippines (Part II)</td>
<td>143</td>
</tr>
<tr>
<td>Annex II-8 Thailand</td>
<td>151</td>
</tr>
<tr>
<td>Annex II-9 Vietnam</td>
<td>163</td>
</tr>
<tr>
<td>Annex II-10 Iran</td>
<td>183</td>
</tr>
<tr>
<td>Annex II-12 Sri Lanka</td>
<td>185</td>
</tr>
<tr>
<td>Annex III Summary of Country Reports</td>
<td>191</td>
</tr>
<tr>
<td>Annex IV Technical Sessions</td>
<td></td>
</tr>
<tr>
<td>Annex IV-1 An overview of the regional study on Taxonomy and ecology of Gracilaria. Khanjanapaj Lewmanomont</td>
<td>215</td>
</tr>
<tr>
<td>Annex IV-2 Gracilaria culture and utilisation. R. Perez and O. Barbaroux</td>
<td>225</td>
</tr>
<tr>
<td>Annex IV-3 A review of the culture of Gracilaria in Asia-Pacific and directions for future development. GavinoC. Trono Jr</td>
<td>245</td>
</tr>
</tbody>
</table>
| Annex IV-4 | Taxonomy and culture of *Gracilaria* in the Asia-Pacific region.  
*Chen Jiaxin and Xia Bang Mei* | 253 |
| Annex IV-5 | Delimitation of species and population genetic structuring of *Gracilaria verrucosa*: consequences for cultivation.  
*C. Destombe, R. Wattier, D. Bulke and M. Valero* | 301 |
| Annex IV-6 | *Gracilaria* studies at SEAFDEC/AQD.  
*A. Q. Hurtado-Ponce* | 311 |
| Annex IV-7 | An overview of seaweed processing technology for *Gracilaria* with reference to agar yield and quality.  
*S. Chandrkrachang* | 319 |
| Annex IV-8 | Future directions in *Gracilaria* research and valorisation.  
*A. Alfsen* | 327 |
| Annex IV-9 | Phytosanitation - Utilisation of *Gracilaria* in reclamation of shrimp pond effluents.  
*K. Chaiyakam* | 331 |
| Annex IV-10 | International and regional trade in seaweeds and seaweed products, with special reference to *Gracilaria* and agar quality standards  
*S. Wongwai* | 333 |
| Annex IV-11 | Socio-economics of a coastal community in Philippines with *Gracilaria* seaweed production as an alternative livelihood.  
*N. Taw* | 339 |
| Annex IV-12 | *Gracilaria* production and trade  
*INFOFISH* | 347 |
EXECUTIVE SUMMARY

I. INTRODUCTION

This Regional Study and Workshop on the Taxonomy, Ecology and Processing of Economically Important Red Seaweeds was supported by the Government of France Trust Fund with the Food and Agriculture Organization of the United Nations (FAO) and is one of three regional projects endorsed by IFREMER and implemented under GCP/INT/553/FRA. Nine countries from the region participated in the study, namely: Bangladesh; China; India; Indonesia; Malaysia; Myanmar; Philippines; Thailand and Vietnam. Iran and Sri Lanka prepared summary reports for the workshop, but did not participate fully in the study. Red seaweeds of the genera *Porphyra*, *Gracilaria* and *Eucheuma* are important cultured seaweeds in the region, but in view of the common interest and regional importance of the *Gracilaria* seaweed, it was recommended that the study focus on *Gracilaria* species.

This report presents the activities of the Final Workshop on the Taxonomy, Ecology and Processing of Commercially Important Red Seaweeds held at the Conference Room of the National Inland Fisheries Institute, Department of Fisheries, Kasetsart University Campus, Bangkok, Thailand from 24-27 January, 1995.

The objectives of the workshop were to:

- present country reports on the ecology, taxonomy and processing aspects of the *Gracilaria* species studied by the country participants;
- present regional overviews and resource papers on taxonomy, ecology, processing, environmental, socio-economic and marketing aspects of *Gracilaria* culture; and
- discuss the results and regional summary documents presented and recommend suitable follow up actions at national and regional level.

II. RECOMMENDATIONS

**National level follow up actions and priorities**

**Bangladesh:** Follow-up action included a more extensive and thorough inventory and preparation of a prospectus of seaweed resources in the country. Bangladesh also proposed: (i) introducing appropriate *Gracilaria* species from Myanmar or India for culture in shrimp ponds; (ii) to study the extraction of phycocolloids from *Hypnea* and other important species found in Bangladesh; and (iii) to culture *Hypnea* in shrimp ponds as a means of improving water quality.

**China:** Follow-up studies aim to develop new genetic varieties of *Gracilaria* with fast growth and high agar quality. Tissue culture studies to improve spore collection techniques are also planned.

**India:** Improving the yield and agar quality of *G. edulis* will be a priority activity. Experimental culture in different areas will be conducted using different techniques to develop commercial-scale culture. Other seaweed species, such as *Hypnea*, will be studied for their potential for culture.

**Indonesia:** The present study will be expanded to formulate a sound basis for planning and establishing a national agar industry.

**Iran:** In line with the overall programme to develop Iranian aquaculture, seaweed
resources will be surveyed to determine species for culture and identify suitable culture sites.

**Malaysia:** A complete seaweed laboratory to screen species with commercial potential and research on culture, post-harvest and quality improvement of products from phycocolloids will be established.

Research will include control of epiphytes in *Gracilaria* culture ponds as well as control of predators in open water sites. Inventory studies of species, site selection and habitat suitability will be conducted. Information on population genetics, biotypes and ecotypes of tropical seaweeds are lacking and Malaysia plans to adopt molecular taxonomy for further confirmation of the identified species.

**Myanmar:** The follow-up programme will include confirmation of the identified *Gracilaria* species and research on processing techniques and purification of products. Better culture techniques to attain higher yields will be included in the fanning studies. Further training of the research and field personnel will also be needed.

**Philippines:** Immediate needs include training in processing, specifically on the chemistry of agarophytes for the purpose of developing new value-added products. Training and expert assistance is required. The follow-up programme to this regional project includes: (i) continuing the research on processing and agar extraction techniques suitable for each of the *Gracilaria* species identified as suitable for culture and maintaining standard qualities; (ii) introduction of village-level processing techniques; (iii) follow-up studies on taxonomy and ecology; (iv) further development of culture techniques; and (v) continuing studies on stock assessment of the seaweed resources of the country. The Philippines plans to establish a research and development centre for seaweeds to serve national needs and which could also become a regional resource for collaborative activities.

**Sri Lanka:** A national study on the taxonomy and ecology of commercially important seaweed species in Sri Lanka is planned. The programme will include biofiltration and phytosanitation and polyculture studies with other aquatic species. Training and advice on taxonomy and ecology are requested.

**Thailand:** The regional project has identified three species for further development, namely, *G. fisheri, G. tenuistipitata* and *G. firma*. The scope of the follow-up project will include more intensive ecological assessments of these species, stock assessment and management, development of culture techniques including polyculture, and development of other applications of the algae The proposed programme will require training as well as advice on ecological studies and stock assessment techniques; taxonomy, particularly molecular taxonomy; culture methods and farming systems. Co-operative research on biotechnology is planned.

**Vietnam:** The follow-up plan includes: a more intensive taxonomic and resource inventory of all seaweed species; development of culture techniques for the species that have been identified as having a good growth rate and high quality agar; and application of better processing techniques to produce higher quality standards of agar for the domestic and international markets. Vietnam requests assistance in establishing a centre for *Gracilaria* research and development to co-ordinate and conduct the national programme and to serve as the national focal point for regional collaborative research.

**Regional co-operation**

In the regional context, the analysis of needs showed that there were several common
areas of interest, particularly in training (in taxonomy, culture and processing), but also in research and requests for certain technical assistance. The national studies have also shown that countries were at different stages in the development of research capacity and their *Gracilaria* industries. The analysis of capacities and needs also showed that there was good potential to use the existing centres within the region, such as exist in China, the Philippines and Thailand, to assist countries less well developed in their research capacity or seaweed industries, using the principle of Technical Cooperation among Developing Countries (TCDC). It was also recognised that expertise and appropriate technical assistance from countries and institutions outside of the region was still needed in some technologies and skills. The objective of such activities would be for countries to become self-reliant in the taxonomic, ecological, culture, and processing studies of *Gracilaria* and other economically important seaweeds. The workshop recognised the valuable regional co-ordinating role played by NACA and requested that NACA co-ordinate the regional seaweed programme. The further upgrading of expertise and manpower through appropriate training was considered a high priority. It was further requested that NACA co-ordinate the organisation of assistance to national training courses, through provision of suitable expertise and information.

The workshop recognised a number of important research priorities, which could be undertaken through a regional co-operative programme.

The workshop considered the need for improving post-harvest methods. The social and economic impact of *Gracilaria* culture to coastal communities could be improved considerably by: (i) development and promotion of methods for adding value to products through small-scale processing, which could be undertaken by farmers; and (ii) diversification of the products which could be processed from *Gracilaria*. The workshop concluded that small-scale demonstration plants could be developed, for the dissemination of technically feasible processing methods.

The workshop considered that information exchange should form an important part of the regional programme. There was a need for regular collation and dissemination of information on environmentally sound seaweed culture and processing methodologies, perhaps through a regular newsletter. Marketing and promotional efforts were also needed, whilst keeping in mind the potential market constraints. It was recommended that the UP Marine Science Institute database on seaweed, which has a very extensive collection of seaweed information, should be tapped for a regional seaweed information exchange under NACA’s regional information programme.

The workshop discussed the preparation of a monograph on *Gracilaria*, based on the results of the regional study and other related information generated by the workshop. The monograph will describe the present status of knowledge on taxonomy, species descriptions, ecology, distribution and processing technology (especially agar yield and quality).

**Recommendations**

Following the discussions of the workshop participants, the following recommendations for follow up actions were adopted:

1. In recognition of the potential contributions of *Gracilaria* culture, as well as that of other economic seaweeds, for social and economic uplifting of coastal communities and environmental improvement, the workshop recommended that national governments give priority to further strengthening programmes in each country to upgrade facilities for seaweed research and development and to
promote the culture and processing of *Gracilaria* and other seaweeds. If not already established, each country should establish a research laboratory and culture and processing facilities to continue research and development on the processing and quality of their identified *Gracilaria* resources.

2. In support of the national programmes, and in view of the benefits of a regional co-operative approach, the workshop **recommended** that a regional programme be developed by NACA. The workshop further **recommended** that NACA should ensure that the regional co-operative programme make efficient use of regional institutions and expertise.

3. The workshop **recommended** that the regional programme on seaweed culture should give emphasis to training priorities, research, information exchange and technical assistance to upgrade national capacity:

4. In implementing the programme, the participants **recommended** continued full utilisation of the two regional referral centres on taxonomy and processing. The programme should further expand to include other centres with expertise, such as SEAFDEC Aquaculture Department, UP Marine Science Institute, the Yellow Sea Fisheries Research Institute, and other regional centres.

5. In support of the regional and national programmes, the workshop **recommended** that NACA seek the collaborative assistance of the French Government and other concerned agencies to meet the needs of the governments and private sector, as identified during the workshop. The participants further requested that the participants from France provide their support to the recommendations and to bring them to the attention of their respective agencies and government authorities. The workshop further **recommended** that NACA seek the collaborative assistance of FAO.

6. The workshop further **recommended** NACA to give high priority to the use of TCDC mechanisms for support of the national programmes. It was strongly **recommended** that Cambodia, whose development efforts could be further assisted by the development of its seaweed resources, be included in the regional seaweed development programme.
REPORT OF THE WORKSHOP
PART I: WORKSHOP BACKGROUND AND ORGANISATION

A. Introduction

1. This Regional Study and Workshop on the Taxonomy, Ecology and Processing of Economically Important Red Seaweeds (GCP/INT/553/FRA) was one of the activities formulated during the Regional Workshop on Seaweed Development held in Cebu City, the Philippines in August 1990, under the auspices of the UNDP/FAO Regional Seafarming Demonstration and Development Project. The regional seaweed programme was absorbed by the Network of Aquaculture Centres in Asia-Pacific (NACA) after the termination of the Seafarming Project and it is now a component of NACA’s holistic programme on environment and aquaculture development.

2. This regional study and workshop was supported by the Government of France Trust Fund with the Food and Agriculture Organization of the United Nations (FAO) and is one of three regional projects endorsed by IFREMER and implemented under GCP/INT/553/FRA. Of the other two projects, one is a study of ice-ice syndrome in seaweeds and the other is a project on small-scale colloid extraction and processing.

3. Nine countries from the region participated in the study, namely: Bangladesh; China; India; Indonesia; Malaysia; Myanmar; Philippines; Thailand and Vietnam. Iran and Sri Lanka prepared summary reports for the workshop, but did not participate fully in the study. Project activities commenced with a training-workshop, held from 21-28 April 1992 in Bangkok, on standard methods of seaweed taxonomy and analysis of phycocolloids. Eighteen participants (two from each country) were trained at the workshop and an action plan for the two-year study was formulated.

4. Red seaweeds of the genera *Porphyra*, *Gracilaria* and *Eucheuma* are important cultured seaweeds in the region, but in view of the common interest and regional importance of the *Gracilaria* seaweed, the training workshop recommended that the study focus on *Gracilaria* species. The conclusions of the Training Workshop and the Plan of Action appear as Annex 1-1.

5. A project status review meeting in March 1994, consisting of NACA personnel and resource persons, subsequently recommended that the period of the study be extended. Accordingly, a request was made to FAO to extend the period of the study until January 1995 and for additional funds to support the research and conduct the final workshop. This request was granted.

6. This report presents the activities of the Final Workshop on the Taxonomy, Ecology and Processing of Commercially Important Red Seaweeds held at the Conference Room of the National Inland Fisheries Institute, Department of Fisheries, Kasetsart University Campus, Bangkok, Thailand from 24-27 January, 1995.

B. Attendance

7. Workshop participants included government personnel from the countries participating in the regional study, as well as representatives from the National Aquatic Research Agency (NARA) of Sri Lanka and the Iranian Fisheries Research and Training Organisation (IFRTO). Resource persons came from the Yellow Sea Fisheries Research Institute of China, IFREMER and CNRS in France, University of the Philippines’ Marine Science Institute, UNDP/FAO Seaweed Production Development Project in the Philippines (PHI/89/004) and SEAFDEC Aquaculture Department in Iloilo, Philippines.
Resource persons from Thailand represented the Biopolymer Research Unit of Srinakarinvirot University, the Faculty of Fisheries of Kasetsart University, the National Institute of Coastal Aquaculture, and the Chemical Goods Section of Bangkok Port Authority. Also represented were FAO RAPA, the Mekong Secretariat, the Science and Technology Centre of the French Embassy in Bangkok, Chulalongkorn University, the Department of Fisheries of Thailand, the private sector, and NACA. INFOFISH sent a resource paper on marketing and trade of agar.

The list of participants appears as Annex 1-2.

C. Opening ceremony

8. The workshop was opened by Dr. Kitjar Jaiyen, Deputy Director General of the Department of Fisheries, Thailand. He considered it significant that many of the activities being reported under this project have taken place through co-operation between the participating member countries of NACA. He disclosed that the efforts began with the FAO/UNDP Seafarming Project, under the NACA umbrella. He hoped that the results of the present study would be further utilised to expand *Gracilaria* and other seaweed culture and bring benefits leading to the improved socio-economic status of coastal communities in countries in the region, as envisaged under the holistic aquaculture development programme of NACA. Dr. Kitjar represents the Thai Government on the NACA Governing Council and was formerly its Chairman.

9. Dr. Kitjar noted the enthusiasm and interest in this regional seaweed study among the country study participants and the unselfish expert assistance of the resource persons and their institutions. He expected that this spirit of co-operation would enable the successful conclusion of the study, the application of its results and the implementation of follow-up activities. He looked forward to the results contributing to the further development of the seaweed industry in Thailand and the other countries.

*The welcoming and opening speech of Dr. Kitjar appears as Annex I-3a*

10. Dr. Veravat Hongskul, Regional Fisheries Officer of FAO RAPA, noted that the study was a joint effort between FAO, NACA and the Government of France. He then cited recent production and trade figures to emphasise the importance of seaweed and focused on the rapid growth in red seaweed production in the Asia-Pacific Region, which he said has been stimulated by various scientific and development efforts of governments with the collaboration of FAO and UNDP. He mentioned the UNDP/FAO Seaweed Production Development Project in the Philippines as one example and noted that the Chief Technical Adviser of the project was participating as a resource person in the workshop.

11. Dr. Veravat said FAO is convinced that the future of the seaweed industry is bright and mentioned the numerous applications, both traditional and newly emerging, which would be further enhanced by research in biotechnology. He urged that research should also address problems associated with marketing products from seaweed. He looked forward to the conclusions and recommendations of the workshop for guiding future activities in promoting seaweed culture in the region.

*Dr. Veravat’s speech appears as Annex I-3b.*

12. The Director of the Scientific and Technological Centre of the French Embassy in Bangkok, Mr. Alex Brayle, said he found it gratifying to see that the funds provided for the project through the French Government Trust Fund with FAO has enabled the Network of Aquaculture Centres in Asia-Pacific and its participating countries in the
region to complete an important aspect of the study on red seaweeds.

13. He envisaged that this study would stimulate the development of the agar industry in the region and thus further develop the economies of the countries with the production of valuable products. He noted the fruitful collaboration among the countries, which was enhanced by the guidance and expert inputs of scientists from various institutions that co-operated with NACA in the study.

14. He said that advanced technology and high applications of phycocolloids are available in developed countries, like France, and he was pleased that French experts from IFREMER, Universite Rene Descartes and University of Lille, are among the resource persons guiding the activities of the workshop, providing advice on the outputs and helping to evolve suitable follow-up activities.

*M. Brayle's speech (delivered in French) appears as Annex I-3c.*

15. The NACA Co-ordinator, Dr. Banchong Tiensongrusmee, welcomed the participants on behalf of the NACA Organisation. He said that the project is a clear example of the close and strong collaboration among various national sectors, including the academe and regional and international agencies, in a regional activity. He expressed NACA's gratitude to the French Government and FAO for their support of the project, the collaborating institutions, and the governments for their co-operation in the implementation of the project. He looked forward to closer and continuing relationships with all concerned. Dr Banchong expected that the workshop, apart from the technical results which will be useful for developing the agar industries of countries, will also provide guidelines for future regional collaborative activities in seaweed development. NACA would be most keen to collaborate further with the participating countries and various regional and international organisations, he stated.

*Dr. Banchong's speech appears as Annex I-3d.*

**D. Procedural matters**

16. Dr. Gavino Trono, Jr. of the Marine Science Institute of the University of the Philippines served as the workshop Chairman. The workshop agenda was adopted without revision.

*The workshop agenda appears as Annex 1-4.*

17. Dr. M. N. Kutty, NACA Senior Aquaculturist (Research and Training) explained the objectives and outputs of the workshop as well as the arrangements and procedures planned to achieve the objectives and produce the outputs. The objectives of the workshop were described as follows:

- present the country reports on the ecology, taxonomy and processing aspects of *Gracilaria* species studied by the country participants;
- present regional overviews and resource papers on taxonomy, ecology, processing, environmental, socio-economic and marketing aspects of *Gracilaria* culture; and
- discuss the results and regional summary documents presented and recommend suitable follow-up actions at national and regional level.
PART II: REPORTS OF COUNTRY STUDIES

18. The participants presented the results of the country studies, highlighting the species identified, their ecology and the yield and quality of agar. Abstracts of the country reports are presented below:

The full country reports appear as Annex II-I to II-9.

19. **Bangladesh:** Bimonthly sampling of seaweed was carried out in the coastal area of Bangladesh during: July, September and November 1992; January, March and May, 1993; and July and September 1994. *Gracilaria* spp. was not found during the period of study. Data regarding ecological conditions were presented. Among 19 different groups of seaweeds collected, most of them were found only from November to March and *Asparagopsis taxiformis, Hypnea* spp. And *Sargassum* spp. were most abundant. Agar extraction from *A. taxiformis* showed that the lowest yield (4.23% ± 0.70) was obtained during January and the highest (12.70% ± 0.53) was obtained during March. The report recommends the introduction of *Gracilaria* spp. from neighbouring countries for the specific purpose of improving water quality in shrimp culture farms and managing and exploiting existing resources of *Hypnea* spp. and *Sargassum* spp.

20. **China:** In 1993, China produced 450 tonnes of agar, 260 tonnes of which came from *Gracilaria* spp. More than half the agar produced is consumed locally. *Gracilaria* is cultured in Hainan, Guangdong and Fujian, with Hainan as the major producer. Twenty-eight species of *Gracilaria* have previously been reported. The present study concentrated on *G. lemaneiformis, G. asiatica* and *G. tenuistipitata.* Within the limited time available, ecological conditions (mainly salinity and temperature) of the various species (including varieties in some cases, as in *G. tenuistipitata*) were correlated with extracted agar yield and quality. The measured agar content was high and gel strength was good (<500-608 g/cm²). Different methods of extracting agar were compared. No species is specifically prioritised for culture, but the three species described are being cultured. As the study was limited in time (June-September 1994), further study is recommended.

21. **India:** Studies were made on the yield and physical properties of agar from *Gracilaria corticata* var. *corticata, G. corticata* var. *cylindrica, G. crassa* and *G. edulis,* growing in four localities near Mandapam. The quality of agar (gel strength) obtained from *G. edulis* is higher than the other three species, but the yield of agar in this species is slightly less than that in *G. corticata* var. *corticata* and *G. corticata* var. *cylindrica.* This may be due to the repeated commercial harvesting of *G. edulis* in the study area during recent years. There were no marked variations in the data collected on environmental and hydrological parameters from the four study areas as all of them are located in the vicinity of Mandapam.

22. Thirty two species of *Gracilaria* have already been reported from Indian waters and their occurrence in different parts of the Indian coast were described. Descriptions and ecology of four species of *Gracilaria* (*G. edulis, G. corticata* var. *corticata, G. corticata* var. *cylindrica* and *G. crassa*) were also given. Studies carried out on seasonal variations in growth, spore output, agar processing, yield and physical properties of agar of *Gracilaria* spp., *Gelidiella acerosa* and *Gracilariopsis sjoestedtii* by different workers, were reviewed. The results of work carried out on experimental cultivation of *Gracilaria* spp. in different field environments using various culture techniques were given. It was recommended that *G. edulis* be selected for commercial scale cultivation in India because of its high yield and good quality agar.
23. **Indonesia:** Five species of *Gracilaria*, namely: *G. edulis*, *G. lemaneiformis*, *G. salicornia*, *G. eucheumoides* and *Gracilaria* sp., were studied. The first two occur more commonly and also yield good quality agar (565-880 g/cm², gel strength). Most of the species have low gelling temperatures, which is an important characteristic of agar quality preferred for microbiological medium. The study recommends *G. edulis* and *G. lemaneiformis*, which have robust thallus and higher biomass production, for culture in both pond and field areas.

24. **Malaysia:** Studies were undertaken to determine suitable *Gracilaria* species for culture. Specimens were collected from five locations throughout the west coast of Peninsular Malaysia. Physical parameters for each location were noted. The samples were brought back to the laboratory for preparation of the herbarium specimens and agar yield analysis. Agar extraction was carried out using the hot water technique. The results showed that *Gracilaria changii* has the following desirable characteristics for culture purposes, namely: high yield and quality of agar; abundant in many locations; euryhaline (22-32 ppt); rapid growth rate; and easily cultured, either using vegetative cuttings or spores. It was recommended that *Gracilaria changii* be given the highest priority for culture in Malaysia.

25. **Myanmar:** Taxonomic studies identified seven species, namely: *G. verrucosa* (Hudson); *G. edulis* (Gmelin) Silva; *G. crassa* (Harvey) J. Agardh; *G. foliifera* (Forsskal) Boergesen; *G. millardetii* (Montagne) J. Agardh; *G. textorii* Suringer; and *G. eucheumoides* Harvey. The first six were studied for yield and agar quality. Four species, namely: *G. crassa*; *G. foliifera*; *G. verrucosa*; and *G. edulis* were cultured in a small-scale experimental system. The results of the culture experiment showed *G. edulis* to be most promising. The study recommends its culture in inland ponds and coastal waters. Identification of the species reported as "verrucosa" is not confirmed.

26. **Philippines:** Results of taxonomic and ecological studies were reported for eleven species, namely: *G. arcuata*; *G. changii*; *G. edulis*; *G. eucheumoides*; *G. firma*; *G. gigas*; *G. heteroclada*; *G. lemaneiformis*; *G. manilaensis*; *G. salicornia*; and *G. tenuistipitata*, identified from the materials collected in Philippine waters between June 1992 and December 1994. Collection sites were located in several areas in eight provinces of the Philippines, namely: Cagayan, La Union, Cavite and Sorsogon in Luzon Island; Aklan Samar and Bohol in the Visayas region; and Sulu (Tawi-Tawi and Sitankai) in Southern Mindanao. Of the species reported, five (*G. changii*, *G. edulis*, *G. firma*, *G. heteroclada* and *G. tenuistipitata*) have been identified as potential species for farming, based on the quality of their phycocolloid as well as a number of ecological considerations. Of these five, *G. firma*, *G. heteroclada* and *G. tenuistipitata* were recommended as priority culture species considering their wide distribution in the country.

27. Laboratory analysis of the colloid extracts showed that the mean yield was between 16.2% and 24.7%, with *G. tenuistipitata* obtaining the lowest yield and *G. changii* the highest yield. Gel strength was highest in *G. heteroclada* (892 g/cm²) and lowest in *G. edulis* (250 g/cm²). Gel strength values obtained for the other species were 726 g/cm² for *G. tenuistipitata*, 606 g/cm² for *G. firma*, 583 g/cm² for *G. changii* and 287 g/cm² for *G. salicornia*. The melting temperature of agar ranged from 79°C in *G. edulis* to as high as 92°C in *G. firma*. Most of the species showed high-quality agar that can be used for bacto-agar and agarose production. The report included a discussion of the present status of the country's seaweed industry and a brief review of previous related studies on Philippine *Gracilaria* spp.
28. **Thailand:** Seven species were studied for their taxonomy and ecology, namely: *G. changii*, *G. edulis*, *G. firma*, *G. fisheri*, *G. irregularis*, *G. salicornia*, and *G. tenuistipitata*. In terms of agar quality, *G. fisheri* and *G. tenuistipitata* had the highest gel strength of 768 and 758 g/cm\(^2\), respectively. This is in accord with the results reported by Dr. Suwalee Chandrkrachang of BRU, that *G. fisheri* from Songkhla and *G. tenuistipitata* from Pattani have the highest potential as sources of agar in Thailand. It was recommended that further studies be conducted on these two species with a view to developing them as the main sources of agar for the processing industry.

29. **Vietnam:** During this study, eight *Gradlaria* species of the thirteen reported by Nguyen Hun Dinh and Nguyen Ban Tien (1992) were identified from specimens collected in Northern Vietnam, namely: *G. asiatica* Chang et Xia; *G. tenuistipitata* Chang et Xia; *G. blodgettii* Harvey; *G. arcuata* Zand.; *G. hainanensis* Chang et Xia; *G. chorda* Holm.; *G. gigas* Harv.; and *G. bursa-pastoris* (Gmelin) Silva.

30. Taxonomic identification of *Gracilaria* species usually meets with the following difficulties: the variability among species is not clearly seen; morphological features of the species are influenced by environmental conditions and tend to change at different locations; and some of the specimens collected lacked reproductive organs. For this reason, synonyms are often used. Among the above species, four were determined to have high economic value. Of these four species, three were studied and cultivated, namely: *Gracilaria asiatica*; *G. blodgettii*; and *G. tenuistipitata*. The fourth, *G. chorda*, will also be studied in trial cultivation.

31. Analysis of results from six *Gracilaria* species collected from Hai Phong and Quang Ninh showed that the dried/fresh ratio, agar yield and gel strength are highest in *Gracilaria asiatica*. It had also the lowest ash content and is a widely distributed species in Vietnam. It could be cultivated in the brackish water swamps of the Northern region. The other highly promising species for cultivation and agar processing in Vietnam are *G. blodgettii* and *G. tenuistipitata*.

**Status reports of other governments**

*The presentations from other governments appear as Annex 11-10 and 11-11.*

32. **Iran:** The representative from Iran reported the studies done on inter-tidal algae in the Southern Coasts of Iran along the Oman Sea and Persian Gulf. A number of surveys and studies have been carried out to identify and map the distribution of Iranian coastal algae. The first attempt to identify algae in the Persian Gulf was made by Diesing and Eadlicher in 1845. The results of this study were confirmed by Borgesean and Koie (1939) and Newton (1955).

33. During four study trips in 1993, 23 species of brown algae, 16 species of green algae and 29 species of red algae were identified in coasts of Persian Gulf and Oman Sea. *Sargassum* sp., *Ulva lactuca*, *Chondrus crispus* species in Oman Sea and *Padina* spp., *Enteromorpha intestinalis*, *Chondrus crispus* species were quite well distributed in the Persian Gulf. The Iranian Fisheries Company has a programme to develop the culture of economic and commercial algal species in Iranian waters and establish a seaweed processing industry based on agar, alginate and carrageenan. Any co-operation with relevant companies and organisations would be accepted and appreciated.

34. **Sri Lanka:** There are nearly 260 species of seaweeds growing along the Sri Lankan coast, which is some 1700 km long. *Gracilaria* spp. is the most commonly used seaweed for food. Two species, *G. verrucosa* and *G. edulis* occur in commercially
valuable quantities in Sri Lanka. *Gracilaria edulis* was reported in 1950's in the Kalpitiya area. The presence of larger quantities of *G. verrucosa* (*G. confervoides*) has been recorded in Kaddiayar Bay near Trincomalee. Recent studies have shown that *G. edulis* gives the highest constitution (48.7%) of best quality agar when extracted at pH 5. Comparison of cultured and wild seaweeds showed that the latter give satisfactory gel content, strength, moisture content, ash content and insoluble ash contents. Comparative studies on three of the most common species found in the northern part of Sri Lanka, namely *Hypnea* sp., *Gracilaria lichenoides* and *Gelidium* sp. have shown a seasonal variation in agar content in these species, the plant showing the highest agar content in January.

35. Sri Lanka used to export an average of 100 tonnes/year of dried raw *Gracilaria*, but exports have dwindled through the years for various reasons. At present, exporting companies are reporting a very high renewed demand for agar, which they find difficult to satisfy. The National Aquatic Resources Agency launched a project in 1988 at Kalpitiya (north west coast) to find out optimum growth conditions and best culture sites for *G. edulis* in Puttalam lagoon. Two methods were followed for the cultivation, namely vegetative and spore setting techniques. The culture of *Gracilaria* spp. on a commercial scale and improvement of processing methods were considered to be of immense importance to national development from seaweed products and have cut down the import expenditure on refined agar.

36. **Summary of the results of the country studies.** At a pre-workshop meeting of the resource persons, held in Bangkok from 21-23 January, 1995, the findings of the country papers were discussed and summarised. Comments were made on the taxonomy, ecology and distribution of *Gracilaria* spp. in the region and the results of the agar analysis from each country are presented. The agar extraction methods used in each country were also presented.

*The Summary of Results of the Country Papers appear as Annex III*
PART III: TECHNICAL SESSIONS

37. To provide comprehensive guidelines for discussion and the formulation of recommendations, resource persons were invited to present and discuss specialist papers in the fields of taxonomy, ecology and culture, processing, phytosanitation and environment, marketing and trade, and socio-economics. Summaries of the papers are presented below:

38. A review paper on the taxonomy of *Gracilaria* in Asian countries. Presented by Prof. Khanjanapaj Lewmanomont, Faculty of Fisheries, Kasetsart University, Thailand. In reports from countries in the region, only Bangladesh reported the absence of *Gracilaria*. Reports from the eight other countries, namely: China; India; Indonesia; Malaysia; Myanmar; Philippines; Thailand; and Vietnam, included 23 identified species with 3 varieties and 6 unidentified species. Among these, 10 species seem to be correctly identified, while the rest have to be confirmed. The most common species was *G. edulis*, followed by *G. tenuistipitata*, *G. salicornia* and *G. changii*. The species recommended for culture were *G. edulis*, *G. changii* and *G. tenuistipitata*.

39. The problem in identifying the species of *Gracilaria* is the lack of sexual reproductive organs. Only a few species can be identified by their morphological characteristics, such as *G. eucheumoides* and *G. salicornia*. The paper by Professor Khanjanapaj appears as Annex IV-1.

40. Cultivation and uses of *Gracilaria* Presented by Dr. Olivier Barbaroux, IFREMER, Centre de Nantes, Nantes Cedex 01, France. The phycoculture of *Gracilaria* has four objectives: (i) fodder for fish and mollusc aquaculture; (ii) human consumption; (iii) fertiliser; and (iv) high quality agar. Chinese and Philippine fish farmers were probably the first to cultivate it by throwing the algae (cut in little pieces) into ponds where herbivorous fishes (such as milkfish) are grown. *Gracilaria verrucosa* was not considered an agarophyte before 1949 when Japanese agar ran short and agar extraction using alkali treatment was developed. Since that time, production has steadily increased. Despite the simplicity of culture, two major problems restrict the production of a high commercial quality agar biomass. Firstly, taxonomic problems due to the difficulties encountered in reliably determining species and secondly increasing the culture yields usually lowers the agar quality and *vice versa*. The presentation was supplemented by a slide presentation showing various culture methods and processing techniques in various countries including China, the Philippines, Vietnam, and Chile. The paper by Dr Perez and Dr Barbaroux appears as Annex IV-2.

41. A review of *Gracilaria* culture in Asia-Pacific and directions for future development. Presented by Dr. Gavino Trono, Jr., Marine Science Institute, College of Science, University of the Philippines. This paper described the current status of the culture of *Gracilaria* in the region, the different methods presently used in its culture and production of planting materials. It outlined various courses of action for future development. The paper by Dr Trono appears as Annex IV-3.

42. Taxonomy and culture of *Gracilaria* in the Asia-Pacific region. Presented by Prof. Chen Jiaxin, Yellow Sea Fisheries Research Institute, Qingdao, China. This review combines the country reports presented by China, India, Myanmar and Vietnam and literature on the genus *Gracilaria*. Detailed biological characteristics of *Gracilaria* species, including basic features for taxonomy, life cycle and ecological conditions for
cultivation are described. Sixty five species of *Gracilaria* and their distribution in the region are listed and 25 species are described in detail. Culture methods, such as pond culture and floating-raft culture, are briefly described.

*The paper by Prof. Chen Jiaxin and Xia Bang Mei appears as Annex IV-4.*

43. Delimitation of species and population genetic structure in *Gracilaria verrucosa*: Consequences for cultivation. Presented by Dr. Christophe Destombe. Laboratoire de Genetique et Evolution des Populations Vegetales. URA CNRS. Universite de Lille I, Ascq Cedex, France. Species of *Gracilaria* are some of the most useful algae in the world, combining the production of valuable agar with a fast growth rate, ease of vegetative production and other attributes favouring their cultivation. When planning to farm these red algae in new areas, it is essential to identify the species and to be aware of the biology of the species considered. In agarophytes, delimitation of species is very difficult using available morphological characteristics and the biology of the species generally depends on local adaptation.

44. The paper describes (i) the new molecular tools to delineate species of *Gracilaria* using sequences of cytoplasmic and nuclear DNA, (ii) the level and pattern of population genetic structuring and the possibility for local adaptation in *Gracilaria verrucosa* using two methods. First, crossing experiments to study the crossing compatibility depending on the distance between male and female gametophytes and, secondly, molecular analysis to study the distribution of the genetic markers within and between populations. These studies reveal that Rubisco spacer analysis is a good method to delimit the species and that genetic divergence could occur between populations of *Gracilaria verrucosa*, even at short distances (about 100m). Consequently, it is very important before planning cultivation to be aware of the origin of the *Gracilaria verrucosa* inoculum to use in breeding experiments and farming.

*The paper by Dr Destombe appears as Annex IV-5.*

45. *Gracilaria* studies at SEAFDEC Aquaculture Department. Presented by Dr. A. O. Hurtado-Ponce. Scientist SEAFDEC/AQD. Tigbauan, Iloilo, Philippines. Research on seaweed during 1988-1994 focused on *Gracilaria* species. Studies were made on the following areas: inventory of seaweed resources; production-ecology; farming systems; and agar characterisation. Six species of *Gracilaria* and one *Gracilariopsis* abound in the Western Visayas region. Monthly variations in biomass and agar quality were recorded in *G. changii*, *G. manilaensis*, and *Gracilariopsis heteroclada* collected at different places. However, only *G. heteroclada* was found throughout the year.

46. Most of the studies were carried out on *G. heteroclada* because of its wide distribution, fast growth characteristics and good quality agar. Its reproductive state was seasonal with tetrasporophyte abundant in May and carposporophyte in January. Harvesting 75% of the available biomass was sufficient to maintain "seedstock" for the next cropping season. Harvesting using "arana" was not appropriate in places where the biomass is exposed to air during lowest tide. The addition of nutrients to the stock increased the growth rate of the plant and gel strength of its agar. *G. heteroclada* grown at lower stocking density in hapa nets, both in floating cages and in ponds, gave higher growth rates and production than at higher stocking densities. Polyculture with *Penaeus monodon* at lower stocking density combinations gave the highest growth rate and income. Encouraging results were observed when cultured vertically in ropes inside a floating cage. Culture at 24-25 ppt under tank conditions produced the highest gel strength.
47. An overview of seaweed processing technology for *Gracilaria* with reference to agar yield and quality. Presented by Dr. Suwalee Chandrkrachang, Asst. Professor, Biopolymer Research Unit of the Department of Chemistry, Faculty of Science, Srinakarinwirot University, Thailand. Seaweeds are valuable to both the economy and environment of many countries. The processing of seaweeds into phycocolloids became the foundation of a major world-wide industry involving the manufacture of food, cosmetics, drugs and products of biotechnology. The most valuable product of phycocolloids is agar, which is produced from red seaweeds of the genus *Gracilaria*, involving different species. Agar consists of heterogeneous biopolymers containing galactose units and their derivatives. Varying market prices of the agar result from different grades, which depend on the gel strength, clarity and the quantities of the charged particles in the agar.

48. Different agar extraction techniques were reviewed. Samples of seaweed and agar from the countries participating in the regional study were analysed at BRU. The results will be used to develop guidelines for improving both the quality and quantity of the seaweed raw materials and their agar products which are essential information for research and industry development. The presentation offered advice on the essential considerations in setting up a processing plant for agar.

49. Future directions in *Gracilaria* research and valorisation. Presented by Prof. Annette Alfsen, Directeur de Recherche, CNRS, Université Rene Descartes, Paris. The research currently carried out in co-operation with Dr. Suwalee Chandrkrachang from Bangkok (Srinakarinwirot University) deals with the protein content of different samples of *Gracilaria* spp. growing or cultivated in Thailand. Amino acids were analysed to select the best species for preparation of proteins on an industrial scale from these algae. Such proteins could be used in human and animal food with the same advantages as vegetal proteins, at low cost to countries lacking in animal proteins in Asia. The extraction of proteins has to be carried out after the extraction of agar, adding value to the production of cultivated *Gracilaria* spp. Study and extraction of lipid with polyunsaturated fatty acids essential in children's nutrition have to be planned also from *Gracilaria* sp. Further, the preparation of enriched endocytic vesicles, cell organelles previously isolated and studied in other algae, has to be set up from these algae to develop their use in pharmacology. Studies have shown that there are possibilities for using such materials as drug carriers.

50. Phytosanitation - Utilisation of *Gracilaria* in reclamation of shrimp pond effluents-Presented by Mr. Kanit Chaiyakam, National Institute of Coastal Aquaculture, Songkhla, Thailand. Experiments using *Gracilaria fisheri* for biological wastewater treatment of shrimp pond effluent was conducted by static bioassay with indoor tanks of 200 litres in size. The results of the first experiment showed that values of B.O.D., C.O.D., total ammonia, nitrate, total settleable solids and chlorophyll a decreased over 24 and 48 hrs. The second experiment showed similar trends and confirmed the results of the first experiment. Experiments have also been done using *Gracilaria* sp. in combination with green mussel (*Mytilus* sp.) for biological water treatment and also some field trials in Lake Songkhla for reducing nutrient load in proximity to the finfish cages. The results are encouraging and confirm the phytosanitation value of *Gracilaria* spp.
International and regional trade in seaweeds and seaweed products, with special reference to *Gracilaria* and agar quality standards. Presented by Mr. Suchart Wongwai, Chemical Goods Section. Port Authority of Thailand, Bangkok. *Gracilaria* is the major raw material for the agar industry in the world. Chile is the largest *Gracilaria* producer and Japan the largest agar producer and consumer. World agar production is estimated at 10,000 tonnes a year and about half is from *Gracilaria*. Thailand, Malaysia and Indonesia are major importers of agar and are also attempting to produce agar locally, but lack of raw materials and know-how are major drawbacks. Vietnam can produce a great deal of *Gracilaria* and has an agar industry, but agar quality needs to be improved. The price of *Gracilaria* is increasing year by year and agar, both of commercial and bacterial grade, is expensive. Japan, South Korea and Taiwan are top agar producers, labour and land costs in these countries are among the reasons for the increase in the price of agar. To save on these costs Japan is now setting up agar factories in other Asian countries.

The socio-economics of a coastal community in the Philippines with *Gracilaria* seaweed production as an alternative livelihood. Presented by Nyan Taw, Chief Technical Adviser, Seaweed Production Development Project Philippines. The estimated income level of coastal households in Sorsogon ranges from 2000 to 3560 Pesos\(^1\) per month. About 39% of the population are engaged in fishing, which is their main source of income. Studies in the coastal areas of Sorsogon have revealed that the area is rich in *Gracilaria* resources both in quality and quantity. Three types of areas with their specific environmental conditions were found for *Gracilaria* farming purposes. They are: (i) open sea coraline flats with high salinity (ii) bays along coast line with sandy muddy substrate and with high salinity and (iii) brackishwater ponds with muddy substrates and low salinity. Three methods of farming have been developed, namely - fixed bottom line, floating raft line and pond culture. Simple (family sized) processing technology to produce one kg per day of food agar powder was also developed. Utilising the technologies that developed coastal communities have initiated, *Gracilaria* farming and processing activities have been promoted as an alternative livelihood, with local government support. The unit cost to produce one kg of dried *Gracilaria* is estimated at 3.20 Pesos per kg with the selling price of between 6.00 to 10.00 Pesos, while the unit cost to produce one kg of agar powder is estimated at 406.20 Pesos with the selling price of between 750.00 and 1000 Pesos. The potential for farming and processing *Gracilaria* has not yet been fully utilised in the Philippines.

Total world production of agar is estimated at 7,000-10,000 tonnes, half of it originating in Japan and Republic of Korea. The major producing countries are Japan, Spain, Chile and Republic of Korea. Several leading agar producers export most of their production. Emerging producers in the Asia-Pacific region are Indonesia, the Philippines and Thailand.

\(^1\) 25 Pesos = 1 US$ Approximately
PART IV: DISCUSSION

54. The country studies and the presentation of the regional overviews and resource papers were followed by discussions, which are summarised below.

A. Country papers

55. The Bangladesh study, which was restricted to shallow waters around St. Martin’s Island, did not reveal the presence of any species of Gracilaria. The Bangladesh participant requested the introduction of Gracilaria species from Myanmar or India. In discussions, caution was recommended because of concerns over the possible negative effects on biodiversity which might arise.

56. Bangladesh wanted studies to be initiated on Hypnea spp. and Sargassum spp. Prof. Alfsen, resource person from CNRS, Paris, advised that the countries should utilise the seaweed resources that are available locally. Dr. Suwalee, regional resource person in processing, indicated that Hypnea spp. is a source of carrageenan used in the food, cosmetics and pharmaceutical industries. Research on this topic was not being carried out in Bangladesh owing to a lack of trained personnel, equipment and material supplies. Remediying this situation required international co-operation and support.

57. The China study presentation was made by a researcher from Shanghai Fisheries University, who replaced the original study participant. It was pointed out that studies on seaweed taxonomy, processing technology and production are well established in Chinese institutions and that there is a need to share expertise with other member countries of NACA. This point was re-iterated in the resource paper presented by Prof. Chen Jiaxin, resource person from Yellow Sea Fisheries Research Institute, China.

58. In India, Gracilaria edulis was the most common species. Agar production is based on wild stocks. The culture of seaweeds is still largely experimental. There is considerable information in India on the taxonomy of Gracilaria, but the present study was restricted to the southern part of India (around Mandapam). Commercial culture of Gracilaria and other seaweeds is yet to be established and the support of suitable extension programmes will be needed.

59. In the Indonesian study, Prof. Khanjanapaj, regional resource person on taxonomy, pointed out that the scientific names applied to G. eucheumoides and G. salicornia are correct, but the other species (G. edulis and G. lemaneiformis) need verification. There was a need to collect specimens with reproductive structures for examination. Dr. Destombe, resource person from University of Lille, France, advised of the need for collecting information on growth rates and its relationship with agar content and quality. The need for estimates based on larger samples was also suggested. Indonesian Gracilaria showed desirable characteristics of high gel strength, and low gelling temperatures of agar extracted. The comparative methods of extraction are described in Annex III.

60. It was suggested that taxonomic descriptions in the Malaysian study were incomplete, that there should be complete descriptions of each species and that the G. changii identification had to be verified. There is a need for assistance in taxonomic identification by the referral centre (Kasetsart University, Faculty of Fisheries), through NACA, and more training in taxonomy and processing in Gracilaria. It was pointed out by Prof. Alfsen that countries with capabilities, such as Malaysia, should have some of these training programmes organised within their national centres, with possibly some
external assistance through organisations such as NACA. Dr. Destombe also pointed out the need for collecting samples all through the year to obtain a better picture of seasonal effects on agar yield and quality of *Gracilaria*

61. The Myanmar study gave complete taxonomic descriptions. The regional resource person in taxonomy pointed out that the name *G. crassa* should be changed to *G. salicornia*, and that the specimen identified as *G. verrucosa* could be *G. lemaneiformis*. There was no male plant in the collection to verify the reproductive structure and so it could not be confirmed. The resource person in processing technology indicated that Myanmar should have more development in processing techniques and product development. The agar quality observed was not good and needed improvement. The regional resource person from the Philippines, Prof. Trono, wanted to know details of the post-harvest methods adopted. He noted that high moisture content in dried *Gracilaria* can result in poor quality of agar. Former Chief Technical Adviser, FAO-UNDP Seaweed Project in Philippines, and resource person from Myanmar, Dr. Nyan Taw, indicated that the post-harvest methods could be below standard and that there was need for improvement.

62. For the Philippines’ study, the regional resource person for taxonomy advised that the species identified as *G. fastigiata* should be changed to *G. edulis*. *G. heterooclada*, *G. tenuistipitata* and *G. firma* give good quality of agar and high yields (16-20%) and are recommended for fanning. A separate study on *Gracilaria* from the Philippines was reported in a resource paper from SEAFDEC Aquaculture Department. This was a study over the period 1988-1994 and gave a good description of the production ecology, agar yield and quality and culture of selected *Gracilaria* species, including socio-economics aspects of culture. Polyculture of *Gracilaria* with finfish (*Lates calcarifer*) and also with *Penaeus monodon* was explained. An interesting aspect was that variations in agar quality measured over the year showed a seasonal difference of 10-fold, suggesting the need for seasonal studies for all candidates for culture and agar production. Prof. Chen Jiaxin commended the study of the economics of culture and pointed out that more attention must be paid to studies of economics in the countries.

63. Prof. Alfsen observed that the SEAFDEC study is a model study for all countries and the training facilities and technology available should be made use of by the other countries. Socio-economic benefits of the operations of culture should be made available to the countries and the producers, the small farmers and fishermen. On the issue of species names used, it was pointed out that different taxonomic schools adopt different methodologies, but the workshop agreed on the need for standardisation.

64. In the Thailand study, of the seven species reported *G. fisheri* and *G. tenuistipitata*, occurring in the southern coastal area, were considered best for culture. To a question from the Malaysian participant, the resource person in taxonomy stated that there are 13 species of *Gracilaria* in Thailand. Prof. Trono indicted that there are several ecotypes in *G. firma*. Prof. Alfsen suggested that agar yield and quality of *Gracilaria* species should be studied over different seasons and correlated with growth rates. The resource person for processing pointed out that *G. fisheri* from southern Thailand is known to yield good quality agar (700-900 g/cm², gel strength) during December to June.

65. The Vietnam study had the benefit of a visit and advice by the regional resource person in taxonomy. The samples studied came from wild stocks. *G. verrucosa* (*G. asiatica*) was known to give high yields of agar and high gel strength but agar quality could be improved. Vietnam made a proposal that a Centre of *Gracilaria* Studies be
established at Hai Phong, Vietnam. The Research Institute of Marine Products, Hai Phong, has several different activities in Gracilaria culture and processing, including a Gracilaria physiology laboratory.

66. **Sri Lankan** and **Iranian** government nominees participated as special invitees, but were not study participants. The paper from **Sri Lanka** reported very early studies on Gracilaria (1977-78), and more recent studies in 1994. Sri Lanka would like to conduct further studies as well as culture trials of Gracilaria. As Sri Lanka's Gracilaria areas are contiguous to India's the studies made in India would be relevant (to an extent) to Sri Lanka. Iran plans to develop their aquatic seaweed resources, including Gracilaria, and would welcome assistance in formulating the development plan, as well as organising research and training of personnel.

**B. Presentations by resource persons**

67 The following summarises the discussions which followed the presentations from the resource persons.

68 The major problems in Gracilaria taxonomy were discussed by the resource person in taxonomy. She indicated that lack of reproductive structures in samples sent by the participants to the Referral Centre made the identification of the species difficult. Also the number of specimens, especially of male plants, was often limited. She noted that the variation in Gracilaria was also high. The resource person advised the following action on country reports from the participants: Some species names should be changed according to the synonymies established. The names of uncertain species should remain the same as shown in the reports, with a notation, "uncertain species". These would need re-examining to determine the correct nomenclature. Some countries - Indonesia, Myanmar and Vietnam - need more training in taxonomy. As a general need, it would be advisable to select trainees based on their basic background and subject experience.

69 Dr. Barbaroux's resource paper explained the global aspects of the culture of Gracilaria. While agar is used basically for food in Asia, in developed countries it is used for bacteriological and other high-tech purposes. He advised that the socio-economics and trade of agar should be closely watched, especially in expanding culture and production of Gracilaria, to avoid market saturation and low profit.

70 Prof. Trono's review clearly indicated the future directions for development in Gracilaria culture, which included: selection and development of fast growing seed stocks with high quality agar; polyculture; appropriate culture methods and sites; environmental aspects of polyculture; and improvement of post-harvest methods. The NACA Adviser asked if seaweed culture has been associated with fish stock enhancement in any country. Prof. Trono indicated that fish do congregate in these culture sites, especially in floating rafts, possibly because of the cleaner environment. Dr Phillips wanted to know if there was any quantified information in this. Prof. Trono observed that there have been no studies carried out.

71 Dr. Destombe explained the use of Rubisco spacer analysis in the speciation study of Gracilaria. The Malaysian participant wanted to know how much agreement there was between the morphological and molecular study. Dr. Destombe explained that the range of speciation can be known. Dr. Destombe remarked that the level of studies were very different among the participating countries and in some reports details of analytical and statistical methods were missing. Further improvement in the regional study can be effected through: co-operation between the NACA member countries; an
annual or bi-annual NACA publication providing information on new techniques, listing new publications on genetics, taxonomy, biology and physiology of *Gracilaria*; and the creation of a Centre of Reference, and a library with books, journals and other publications.

72. Prof. Suwalee, resource person in processing, reviewed the processing aspects of the studies, also indicated in the comments in Annex III. Prof. Suwalee wanted each country to set up its own laboratory to continue research and development of agar processing, possibly with funding assistance through co-operative efforts for training personnel, equipment supply and other needs, emphasizing the regional co-operation among the member countries. She also wanted progress to be monitored to evaluate the activities and problems.

73. Prof. Alfsen, explaining her involvement in research in algal chemistry, observed that a difficulty she has faced in trying to help through training in developing countries is in locating those who need it. She emphasised that countries should work towards self-reliance. As a general observation on the country studies on *Gracilaria*, Prof. Alfsen observed that each country should choose for itself the species recognised as giving the best agar yield and gel strength, using whatever species name identified. Standard methods should be used for describing the species, location and season of growth, season for harvest to obtain best yield, growth stages, etc. Standard methods for agar extraction and analysis should be used. The geographical range of each species within the country should be known. Long-term plans for algae production, according to the needs of the country (nutrition, pharmacological, therapeutic), the involvement of manpower and needs for training, should be made for each country.

74. Phytosanitation through utilisation of *Gracilaria* was explained in the paper by Mr. Kanit Chaiyakham, resource person from the National Institute for Coastal Aquaculture (NICA) in Thailand. The environmental aspects of algal culture was of great interest, also with respect to earlier references to this in Prof. Trono's directions for development. Dr. Nyan Taw wanted to know the objective of the utilisation of *Gracilaria*. In Malaysia, cockle and *Gracilaria* were cultured in effluent water to reduce coastal water pollution, he said. In the study described in Thailand, treated water is recirculated for re-use in the shrimp pond. Prof. Trono stated that there could be problems with bio-accumulation of heavy metals and chemicals/pesticides in *Gracilaria*, grown in the shrimp pond, which should be looked into.

75. Mr. Suchart Wongwai, resource person from industry, explained the marketing and trade in *Gracilaria* and agar. Dr. Barbaroux observed that Mr. Suchart indicated world demand for agar (10,000 tonnes) was attained by 1993 and that the growth rate of the agar industry between 1990-93, was 40%. Prof. Trono and Dr. Destombe cautioned the increasing production and need for agar in the world, leading to possible over production.

76. Dr. Nyan Taw explained the activities of the FAO/UNDP Project on *Gracilaria* culture in the Philippines, specifically the socio-economic aspects. Dr. Hurtado-Ponce observed that the socio-economic study done in SEAFDEC was more elaborate, to which Dr. Nyan Taw replied that the elaborate socio-economic study in his project was done by Mr. Tagarino, whose work is cited in his (Taw's) paper.
PART V: RECOMMENDATIONS

77. Following the discussions, the workshop recommended follow-up actions which could be taken at national and regional level.

National level follow-up actions and priorities

78. **Bangladesh:** Follow-up actions should include a more extensive and thorough inventory and preparation of a prospectus of the seaweed resources in the country. Bangladesh also proposed to: introduce appropriate *Gracilaria* species (i.e. *G. edulis*) from Myanmar or India for culture in shrimp ponds; study the extraction of phycocolloids from *Hypnea* spp. and other important species found in Bangladesh; and test the culture of *Hypnea* spp. in shrimp ponds as a means of improving water quality. The assistance of a taxonomist and a culturist will be needed. Samples of *Hypnea* spp. Could be sent to the Biopolymer Research Unit (BRU) in Thailand for determination of yield and quality of the colloid. Results of the programme will be published to attract the interest of prospective entrepreneurs.

79. **China:** Follow-up genetic studies aimed at developing new varieties of *Gracilaria* spp. With fast growth and high agar quality and tissue culture studies to improve spore collection techniques, are planned.

80. **India:** Improving the yield and agar quality of *G. edulis* will be a priority follow-up activity. Experimental culture in different areas will be conducted using different techniques to develop commercial-scale culture of the species. Other seaweed species, such as *Hypnea* spp., will be studied for their potential for culture.

81. **Indonesia:** The present study will be expanded to formulate a sound basis for planning and establishing a national agar industry. The project will focus on Lampung, West Java and Central Java. A more thorough survey of the *Gracilaria* species in the country will be conducted. A field guide for taxonomic identification, as well as the assistance of a taxonomist and training in taxonomy for local scientists, is required.

82. **Iran:** In line with the overall programme to develop Iranian aquaculture, seaweed resources will be surveyed to determine species for culture and suitable sites. The assistance of a taxonomist and culture expert will be required. The planned start of initial activities is August 1995.

83. **Malaysia:** There is a need to set up a complete seaweed laboratory to screen species with commercial potential, conduct research on culture, post-harvest and quality improvement of products from phycocolloids. Research will include control of epiphytes in *Gracilaria* spp. culture ponds as well as control of predators in open water culture sites. Inventory studies of species and site selection and habitat suitability will be conducted.

84. Information on population genetics, biotypes and ecotypes of tropical seaweeds are lacking and Malaysia plans to adopt molecular taxonomy for further confirmation of the identified species. As a follow-up to the regional study, confirmation of the identified *Gracilaria* species will be needed, for which a taxonomist is requested for attachment with the Fisheries Research Institute, Penang. Collaboration will be worked out with the Universiti Sains Malaysia. Technical advice on setting up a small-scale processing plant (capacity of 50-200 tonnes dried seaweed per day) is requested. Further training for two local scientists in taxonomy, culture and processing is also planned.

85. **Myanmar:** The follow-up programme to this regional study will include confirmation of the identified *Gracilaria* species and research on processing techniques and purification of products. Better culture techniques to attain higher yields will be
included in farming systems studies. Further training of research and field personnel will also be needed. A seaweed research team could be organised to develop and conduct a research programme aimed at further developing the local processing industry by obtaining an adequate, reliable and high quality supply of raw materials to produce high quality products for domestic and export markets.

86. **Philippines:** Immediate needs include training in processing, specifically on the chemistry of agarophytes, for the purpose of developing new value-added products. Training and expert assistance is required. The follow-up programme to this regional project includes: (i) continuing the research on processing and agar extraction techniques suitable for each of the *Gracilaria* species identified as suitable for culture and maintaining standard qualities; (ii) introduction of village-level processing techniques; (iii) follow-up studies on taxonomy and ecology; (iv) further development of culture techniques; and (v) continuing studies on stock assessment of the seaweed resources of the country. The Philippines plans to establish a research and development centre for seaweeds to serve national needs and which could also become a regional resource centre for collaborative activities.

87. **Sri Lanka:** A national study on the taxonomy and ecology of commercially important seaweed species in Sri Lanka is planned. The programme will include biofiltration and phytosanitation as effluent (particularly from the growing shrimp farming industry) is becoming a problem, and polyculture studies with other aquatic species. Training and advice on taxonomy and ecology are requested.

88. **Thailand:** The regional project has identified three species for further development, namely, *G. fisheri, G. tenuistipitata* and *G. firma.* The scope of the follow-up project will include: a more intensive ecological assessment of these species; stock assessment and management; development of culture techniques (including polyculture); and development of other applications of algae that include bio-filtration or bio-remediation in shrimp farms, feed for aquaculture species such as abalone, and biotechnologically-enhanced products. The proposed programme will require training as well as advice on ecological studies and stock assessment techniques, taxonomy (particularly molecular taxonomy), culture methods and farming systems. Co-operative research on biotechnology is planned.

89. **Vietnam:** The national follow-up plan to the regional project includes: a more intensive taxonomic and resource inventory of all seaweed species; development of culture techniques for the species that have been identified as having good growth rates and high quality agar; and application of better processing techniques to produce higher quality standards of agar for the growing domestic market and the requirements of the international market. Vietnam requests assistance in establishing a centre for *Gracilaria* research and development to co-ordinate and conduct the national programme and to serve as the national focal point for regional collaborative research. The Research Institute of Marine Products in Hai Phong has been identified as the host institution for such a centre.

**Regional co-operation**

90. Following the presentation of national priorities, the country representatives and resource persons considered future directions for regional co-operation in support of the national efforts for further development of *Gracilaria* culture and processing. The analysis of needs showed that there were several common areas of interest, particularly in training (in taxonomy, culture and processing), but also in research and requests for certain technical assistance.
The national studies and identified national needs have also shown that countries were at different stages in the development of research capacity and their *Gracilaria* industries. The analysis of capacities and needs showed that there was good potential to use the existing centres within the region, such as exist in China, the Philippines and Thailand, to assist countries less well developed in their research capacity or seaweed industries, using the principle of Technical Co-operation among Developing Countries (TCDC). It was also recognised that expertise and appropriate technical assistance from countries and institutions outside of the region was still needed in some technologies and skills. The objective of such activities would be for countries to become self-reliant in the taxonomic, ecological, culture and processing studies of *Gracilaria* and other economically important seaweeds.

In view of the above, the workshop participants considered that it was important to enhance regional co-operation, emphasising TCDC and through working together to exchange experiences and solve common problems. The workshop emphasised that proper and effective co-ordination of regional activities was vitally important for accelerated development of the seaweed industry in the region. The workshop recognised the valuable regional co-ordinating role played by NACA, and requested NACA to co-ordinate the regional seaweed programme.

The further upgrading of expertise and manpower through appropriate training was considered a high priority. The training activities could be undertaken at national and regional level. The following priorities were identified:

- integrated seafarming and marine polyculture (all countries);
- research methodologies for seaweed research;
- advanced approaches to *Gracilaria* taxonomy; and
- improved techniques in processing technology.

It was further requested that NACA co-ordinate the organisation of assistance to the national training courses, through provision of suitable expertise and information.

The workshop recognised a number of important research priorities, which could be undertaken through a regional co-operative programme. There was a need for the selection of fast-growing seedstocks with high quality agar. The importance of further studies of the performance of local strains and species was recognised, particularly under culture conditions. The workshop emphasised that further attention should also be given to the development of research methodologies for such work, particularly in countries where *Gracilaria* culture and research methodologies were less developed.

The workshop recognised that *Gracilaria* culture (and "searanching") could contribute to environmental improvement in coastal waters (e.g. through cleaning up effluents, attraction of fish to culture areas). It was also noted that the culture of seaweeds with other species (polyculture and integrated farming) in coastal waters would contribute to a balanced use of natural resources, whilst increasing economic benefits to farmers from sustainable use of coastal resources. Such techniques could make an important contribution to the integrated management of coastal resources. As the techniques for this new form of coastal farming are not well developed, the workshop recommended co-operative research be undertaken for the development of integrated marine farming systems. The workshop also recognised the benefit of collation and dissemination of existing experiences through regional training and requested the implementation of this recommendation as soon as possible.
96. The workshop considered the need for improving post-harvest methods. The social and economic impact of *Gracilaria* culture to coastal communities could be improved considerably by: (i) development and promotion of methods for adding value to products through small-scale processing, which could be undertaken by farmers; and (ii) diversification of the products which could be processed from *Gracilaria*. The workshop considered that small-scale demonstration plants could be developed for the dissemination of technically feasible processing methods. Similarly, further research is necessary to extract suitable products, such as protein. The research would help enhance the productivity and utilisation of an important marine living resource for food and income.

97. In addition, further studies were necessary to quantify the species and strains of *Gracilaria*. New technology should be applied to further identify species and strains with the view of improving economic outputs from *Gracilaria* culture systems.

98. The workshop considered that information exchange should form an important part of the regional programme. There was a need for regular collation and dissemination of information on environmentally sound seaweed culture, and processing methodologies, perhaps through a regular newsletter. Attention should also be given to research methodologies and dissemination of results. Marketing and promotional efforts were also needed, keeping in mind potential market constraints. It was recommended that the UP Marine Science Institute database on seaweed, which has a very extensive collection of seaweed information, should be tapped for a regional seaweed information exchange under NACA’s regional information programme.

99. The workshop noted that, in some cases, a low priority had been given to *Gracilaria* in national aquaculture development plans. In view of the potential benefits of *Gracilaria* culture, the workshop considered that further efforts should be made to sensitise governments and the private sector and to promote the benefits of seaweed culture. Promotional work was also required to encourage entrepreneurs to go into seaweed culture and phycocolloid extraction and processing.

100. The regional programme should also give emphasis to the provision of technical assistance to promote national self-reliance in *Gracilaria* research and development programmes.

101. The workshop considered that the regional programme should be based on existing centers within the region, which could be further strengthened as necessary. The workshop welcomed the initiative of the Government of Vietnam to establish a centre on *Gracilaria* culture and processing. The workshop suggested that the French government might be an appropriate source of technical assistance and requested that NACA approach the French government to enquire of this possibility. The workshop also noted that there were other centres within the region who might host training and research activities, including those in China, the Philippines and Thailand. In organising the regional programme, NACA was requested to make efficient use of such centres.

102. The workshop further considered the mechanisms for development of the regional programme. The importance of technical co-operation among developing countries (TCDC) was emphasised, which would be one effective mechanism for utilisation of manpower resources within the region.

103. It was also recognised that additional support would be required for follow-up activities. In recognition of the lead role played by the French government in supporting seaweed culture development within the region, the workshop requested the
The French participants were requested to bring the recommendations of the workshop to the notice of the concerned authorities with a view to obtaining French government assistance to strengthen the regional programme. The workshop recommended that other donor governments and collaborating institutions should also be requested to further strengthen the regional effort in seaweed development.

104. The workshop further discussed the preparation of a monograph on *Gracilaria*, based on the results of the regional study and other related information generated by the workshop. The monograph would describe the present status of knowledge on taxonomy, species descriptions, ecology, distribution, processing technology (especially agar yield and quality) and status of culture - mainly based on information available from the present studies, resource papers and other available information. The participants felt the monograph would be extremely valuable to national development efforts. The workshop expressed some concern about the large amount of work involved, the limited availability of time and the need to synthesise the country reports to shorter documents. The workshop requested that NACA consider using any savings from the project to further hire personnel to assist with the task. NACA was requested to make the necessary arrangements to complete the monograph as a matter of urgency.

**Recommendations**

105. In summarising the above discussions, the workshop adopted the following recommendations for follow-up actions:

1. In recognition of the potential contributions of *Gracilaria* culture, as well as that of other economic seaweeds, for social and economic uplifting of coastal communities and environmental improvement, the workshop recommended that national governments give priority to further strengthening programmes in each country to upgrade facilities for seaweed research and development. Priority should also be given to promoting the culture and processing of *Gracilaria* and other seaweeds. If not already established, each country should establish a research laboratory and culture and processing facilities to continue research and development on the processing and quality of their identified *Gracilaria* resources.

2. In support of the national programmes, and in view of the benefits of a regional co-operative approach, the workshop recommended that a regional programme be developed by NACA. The workshop further recommended that NACA should ensure that the regional co-operative programme make efficient use of regional institutions and expertise.

3. The workshop recommended that the regional programme on seaweed culture should give emphasis to the following aspects:

**Training priorities:**

- integrated seafarming and marine polyculture;
- research methodologies for seaweed research;
- advanced approaches to taxonomy of *Gracilaria* and other commercially important seaweeds; and
- improved techniques for processing.
Research priorities:

- polyculture and integrated seafarming;
- comparative performance of *Gracilaria* as well as other species under farming conditions;
- taxonomy of *Gracilaria*; and
- improved processing of *Gracilaria* and other seaweeds, specifically the extraction of protein, and to explore other potentially valuable by-products.

Information exchange:

- regular collation and dissemination of information on environmentally sound seaweed culture and processing methodologies;
- research methodologies and results; and
- promotional activities to encourage farmers/entrepreneurs.

Technical assistance to upgrade national capacity:

- for research and development programmes on *Gracilaria* and other seaweed species.

4. In implementing the programme, the participants recommended continued full utilisation of the two regional referral centres, on taxonomy and processing. The programme should further expand to include other centres with expertise, such as SEAFDEC Aquaculture Department, UP Marine Science Institute, the Yellow Sea Fisheries Research Institute, and other regional centres.

5. In support of the regional and national programmes, the workshop recommended that NACA seek the collaborative assistance of the French Government, and other concerned agencies to meet the needs of the governments and private sector, as identified during the workshop. The participants further requested the participants from France to provide their support to the recommendations and to bring them to the attention of their respective agencies and government authorities. The workshop further recommended that NACA seek the collaborative assistance of FAO.

6. The workshop further recommended NACA to give high priority to the use of TCDC mechanisms for support to the national programmes; it was strongly recommended that Cambodia, whose development efforts could be further assisted by the development of its seaweed resources, be included in the regional seaweed development programme.

7. The workshop requested NACA to organise the early publication and dissemination of the monograph on *Gracilaria* taxonomy, ecology, processing and culture. It recommended that any savings from the project be used to fund the preparation and publication of this important output from the project, and also to fund the other urgent follow-up activities as noted above.
PART VI. CLOSING REMARKS

106. The participant from Malaysia, Dr. Mashhor Bin Mansor of the Universiti Sains Malaysia, Penang, gave the vote of thanks on behalf of the country participants. He thanked the French Government for its support to the study and FAO for its assistance in the inception and organization of the regional study. He conveyed the deep appreciation of participants and the governments to NACA for its successful implementation. He thanked the resource persons and their respective organisations for their guidance and expert assistance to the study and for the deliberations of the workshop.

107. The NACA Co-ordinator closed the workshop. In his closing speech, he reiterated his thanks to the Government of France, FAO, the resource persons and the study participants for their contributions to the project. He assured everyone of NACA's follow-up actions to the recommendations. He conveyed his gratitude to the governments for their co-operation in this regional effort.
ANNEXES
1. TRAINING WORKSHOP RECOMMENDATIONS

In a plenary session on 28 April 1992, the Training-Workshop discussed the concept and methodology of the regional study. After a thorough discussion, the participants developed and endorsed a Plan of Action for the Regional Study on Taxonomy, Ecology and Analysis of Commercially Important Red Seaweeds.

Several genera of red seaweeds were reported as occurring in the participating countries, including: *Euchewna; Hypnea; Gelidiales; Gracilaria;* and *Porphyra*. In view of the common interest and the regional importance of *Gracilaria* species, the workshop recommended that the regional study would concentrate on *Gracilaria* species. The species to be studied will depend on the countries, but the number would be narrowed down to the ones that are shown to have high commercial potential after the initial analyses.

To expand the development of the seaweed industry in the region, the participants recommended that urgent problems for national studies be tackled. They identified priority problems that NACA could assist in developing proposals to address and in seeking assistance for their implementation. The problem areas were identified as follows:

**Bangladesh**  Taxonomy, ecology and analysis of *Hypnea* species.

**China**  Taxonomy, ecology and analysis of *Gelidiales* spp.
Genetic studies on agarophytes and carrageenophytes.
Protection of natural seaweed resources from destruction caused by industrial expansion and other conflicting activities on the coastal environment.

**India**  Taxonomy, ecology and analysis of *Hypnea* spp.
Conservation and management of natural seaweed stands.
Alternative use of seaweeds *i.e.* as fertiliser.

**Indonesia**  Concentrating on *Gracilaria* for both regional and national activities.
National activities will include technology transfer of appropriate processing techniques at village level.

**Malaysia**  Conversion of unproductive shrimp ponds to seaweed farms.

**Myanmar**  Concentrating on *Gracilaria edulis* for both regional and national activities.

**Philippines**  Conservation and management of natural seaweed resources.

**Thailand**  Taxonomy, ecology and analysis of *Porphyra* and *Gelidiales*.
Conversion of unproductive shrimp ponds to seaweed farms.
Use of seaweeds for wastewater treatment in shrimp farms.
Use of seaweeds as feed for abalone.
Protection of seaweed resources from industrial/shrimp farming expansion.
Conservation of natural seaweed resources.

Vietnam  Taxonomic studies on and culture techniques of *Eucheuma.* Conservation of natural seaweed resources.

In addition to the above national-level activities, it was recommended that socio-economic studies of the seaweed industry be included. The participants requested NACA to officially advise their respective Government authorities of the results and recommendations of the Training Workshop and to reiterate the support needed from the Government to successfully implement the regional study.

A final regional technical workshop will be held to analyse at the regional level the results of the study, develop the required outputs and formulate recommendations for further action.

2. **PLAN OF ACTION**

1. NACA would advise Governments of the results of the Training-Workshop, participants would consult with the concerned authorities on the support required to implement the study.


3. Samples would be collected once every two months or bimonthly over a period of one year for a total of six samplings. Identification of samples and agar yield and quality analysis would be carried out

   - Researchers should report to NACA the results of the bi-monthly collection.

   - Analysis should be done immediately.

   - Analytical results should be accompanied by a report on the status of the study, problems encountered and assistance required from NACA, including fielding of experts to monitor the status of study and provide on-site advice.

4. The Kasetsart University Faculty of Fisheries, and the Biopolymer Research Unit of the Srinakarinvirot University will be the referral centres for taxonomic identification and agar quality determination, respectively.

5. Herbarium specimens and agar samples extracted from the collected seaweeds, properly labelled and packed, shall be sent to NACA for onward referral to these institutions.

6. The data collection forms as shown in this annex, include: (i) data sheet/field notes for the taxonomic and ecological aspects of the study; (ii) data sheet for the analytical work done on the samples; (iii) recommended labelling for the herbarium specimens; and (iv) the standard analytical procedure to agar quality. These forms are to be used for reporting results.

7. The participants will draft a final report based on the results of the field research.

8. NACA, with the assistance of resource persons, will review the country reports and develop a format for the required study outputs.

9. The Final Workshop will draft the study outputs and develop
recommendations for further action.

10. NACA, with assistance from the referral centres and Resource Persons, will finalise the outputs from the study, and publish and disseminate them to governments and interested parties.
Regional Study On The Taxonomy, Ecology
And Analysis Of Red Seaweeds

DATA SHEET I

FIELD NOTES

(To be completed for each sample)

Note: Collect and prepare a minimum of 5 complete samples for each species for the herbarium and 1 kg (wet weight) of seaweed for analysis.

Sample code number: ______

<table>
<thead>
<tr>
<th>A. Map of sampling site</th>
<th>B. Date</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time</td>
</tr>
<tr>
<td></td>
<td>Locality</td>
</tr>
<tr>
<td></td>
<td>Collector(s)</td>
</tr>
<tr>
<td></td>
<td>State of tide</td>
</tr>
<tr>
<td></td>
<td>Season</td>
</tr>
<tr>
<td></td>
<td>Weather</td>
</tr>
</tbody>
</table>

C. Species

Substratum (rocky, sandy, muddy)

Depth (m)

Current speed

Turbidity

Salinity (%)

Temperature (°C)

Reproductive stage

Abundance

Epiphytes

Grazing

Exposure (exposed or sheltered)

Tidal exposure (exposed to air during low tide?)

Others?
(e.g., sources of pollution, evidence of over harvesting)
REGIONAL STUDY ON THE TAXONOMY, ECOLOGY
AND ANALYSIS OF RED SEAWEEDS

DATA SHEET II
ANALYSIS
(To be completed for each sample)

<table>
<thead>
<tr>
<th>Sample code:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species:</td>
</tr>
<tr>
<td>Collected by:</td>
</tr>
<tr>
<td>Date of collection:</td>
</tr>
<tr>
<td>Place of collection:</td>
</tr>
<tr>
<td>Date of arrival:</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B. Results of analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date of analysis:</td>
</tr>
<tr>
<td>Wet weight:</td>
</tr>
<tr>
<td>Dry weight:</td>
</tr>
<tr>
<td>Description of sample</td>
</tr>
<tr>
<td>(e.g., colour, presence of epiphytes)</td>
</tr>
<tr>
<td>Clean anhydrous weight (CAW)</td>
</tr>
<tr>
<td>Moisture content (%)</td>
</tr>
<tr>
<td>Ash (%)</td>
</tr>
<tr>
<td>Yield (%)</td>
</tr>
<tr>
<td>Gel strength</td>
</tr>
<tr>
<td>Total sulphate content</td>
</tr>
<tr>
<td>(hydrolysis method)</td>
</tr>
<tr>
<td>Melting temperature (°C)</td>
</tr>
<tr>
<td>Species:</td>
</tr>
<tr>
<td>------------------</td>
</tr>
<tr>
<td>Locality:</td>
</tr>
<tr>
<td>Date of collection:</td>
</tr>
<tr>
<td>Collector:</td>
</tr>
<tr>
<td>Identified by:</td>
</tr>
<tr>
<td>Code no:</td>
</tr>
</tbody>
</table>

Recommended Labelling for Herbarium
EXTRACTION OF AGAR

20 gram for BRU
20 gram for lab

20 grams dried *Gracilaria* 5% NaOH (w/v)
ratio 1:5

Heat to 90°C for 1 hour

Wash alkali, acidify by 1M HCl
pH x 7

Boil further at 90° - 100°C, 1 hour

Filter through the bag by pressing tool

Gel to set at room temperature

Freeze for 2 days

Thaw and wash

Oven dry at 50° - 55°C

Powder agar

Analysis

% moisture of dried seaweed - % yield
- % moisture
- % yield
- % ash
- gel strength
- total sulphate content
  (hydrolysis method)
- melting temperature
LIST OF PARTICIPANTS

I. COUNTRY PARTICIPANTS

Bangladesh
Mr. Manmatha Nath Sarker, Fax No. (880-341)4078
Scientific Officer, Marine Fisheries Survey, Tel No. (880-341) 3620
Management and Dev. Project, Motel Road, Cox’s Bazar 4700.

China
Ms. Kuang Mei, Fax No. (86-21) 543 4358
Assistant Professor, Department of Aquaculture, Tel No. (86-21) 543 1090
Shanghai Fisheries University,
334 Jungong Road, Shanghai 200090.

Ms. Cen Feng, Fax No. (86-1) 500 2448
National Fisheries Technology Extension Centre, Tel No. (86-1) 500 1886
No. 11, Nong Zhan Guan Nan Li, Beijing.

India
Mr. Subramanian Kalimuthu, Tel No. (91-457)-41443
Technical Officer, Regional Centre of CMFRI, (91-457)-41456
Marine Fisheries Post, Mandapam Camp 623 520,
Ramanathapuram District, Tamil Nadu.

Mr. Janaki Ramachary Ramalingam, Tel No. (91-457)-41443
Technical Assistant, Regional Centre of CMFRI, (91-457)-41456
Marine Fisheries Post, Mandapam Camp 623 520,
Ramanathapuram District, Tamil Nadu.

Indonesia
Mrs Hendarni Mulyani, Fax No. (62-021) 667 5593
National Centre for Fish Quality Control and Processing Devt,
Tel No. (62-021) 669 5586
Directorate General of Fisheries,
Jl. Muara Baru, Pluit, Jakarta 14440.

Mr. Suci Antoro, Fax No. (62-721)480898
National Seafarming Development Centre, Tel No. (67-721) 63533
P.O. Box 74, Telukbetung 35401,
Bandar Lampung.

Iran
Mr. Hossein Shougi, Fax No. (98) 05423 3537
Offshore Fisheries Research Center Chabahar, Tel No. (98) 05423 3452
Fisheries Co. of Iran, Ministry of Jehad-e-Sazandegi,
No. 52 Naderi (soheil) St. Keshavarz Blvd., Tehran.

Malaysia
Mr. Ramli bin Saad, Fax No. (60-4) 6572323
Fisheries Officer, Fisheries Research Institute, Tel No. (60-4) 6572777
11700 Gelugor, Penang.
Dr. Mashhor Bin Mansor,  
School of Biological Science, 
Universiti Sains Malaysia (USM), Penang 11000.  
Fax No. (60-4) 6565125  
Tel No. (60-4) 6577888  
(60-4) 6573125

Myanmar  
U Kyi Shwe,  
Principal Scientist, Applied Chemistry Research Department, 
Myanmar Scientific and Technological Research Department, 
No. 6 Kaba Aye Pagoda Road, Yangon.  
Fax No. (95-1) 65292  
Tel No. (95-1) 63262, 63310  
Cable CEREORG

U Aung Khine,  
Scientist, Pharmaceutical Research Department, 
Myanmar Scientific and Technological Research Department, 
No. 6 Kaba Aye Pagoda Road, Yangon.  
Fax No. (95-1) 65292  
Tel No. (95-1)63262, 63310  
Cable CEREORG

Philippines  
Mrs. Ma Ethel Liana,  
Chief, Seaweeds Section, 
Bureau of Fisheries and Aquatic Resources, 860 Quezon Avenue, 
Quezon City, Metro Manila 3000.  
Fax No. (63-2) 987871  
Tel No. (63-2) 965428;  
(63-2) 991249  
(63-2) 987075

Mrs Purita de la Pena,  
Bureau of Fisheries and Aquatic Resources, 
860 Quezon Avenue, Quezon City, Metro Manila 3000.  
Fax No. (63-2) 967790  
Tel No. (63-2) 967790

Sri Lanka  
Ms. Vasantha Pahalawattarachchi,  
Research Officer, National Aquatic Resources Agency, 
Crow Island, Mattakkuliya, Colombo 15.

Thailand  
Dr. Vithya Srimanobhas,  
Faculty of Environment and Resources Studies, 
Mahidol University, Buddhamongkol 4 Road Salaya, 
Nakhon Pathom 73170.  
Fax No. (66-2) 441 9509-10  
Tel No. (66-2) 4410211-6

Dr. Attaya Kungsuwan,  
Food Technologist, 
Fishery Technological Development Division, 
Charoenkrung Road, Yannawa, Bangkok 10120.  
Fax No. (66-2) 2129446  
Tel No. (66-2) 2115272  
(66-2) 211 1261

Vietnam  
Dr. Do Van Khuong,  
Research Institute of Marine Products, 
170 Le-Lai St., Hai Phong.  
Fax No. (84-31) 45153  
Tel No. (84-4) 46664, 46656

Mr. Nguyen Van Thuc,  
Head-Processing Research Department, 
Research Institute of Marine Products, 170 Le Lai St., Hai Phong.  
Fax No. (84-31) 45153  
Tel No. (84-4) 46664, 46656

II. RESOURCE PERSONS

Ecology, Taxonomy and Culture
Prof. Khanjanapaj Lewmanomont,
Faculty of Fisheries,
Kasetsart University, Jatujak, Bangkok 10900, Thailand.
Fax No. (66-2) 579 5579
Tel No. (66-2) 579 5576

Dr. Olivier Barbaroux,
IFREMER, Centre de Nantes,
Rue de l'Ile d'Yue, B.P. 1049,
44037 Nantes Cedex 01, France.
Fax No. (33-40) 37 40 01
Tel No. (33-40) 37 40 00

Dr. Gavino C. Trono, Jr.,
Professor, Marine Science Institute,
College of Science, University of the Philippines,
P.O. Box 1, Diliman, Quezon City 1101, Philippines.
Fax No. (63-2) 924 3735
Tel No. (63-2) 97 60 61

Prof Chen Jiaxin,
Director, Yellow Sea Fisheries Research Institute,
19 Laiyang Road, Qingdao, China 266003.
Fax No. (86-532)287 0702
Tel No. (86-532)286 9307

Dr. Christophe Destombe,
Laboratoire de Genetique et Evolution,
des Populations Vegetales, URA CNRS. 1185, SN2,
University of Lille I, 59655 Villeneuve d'Ascq,
CEDEX, France.
Fax No. (33-20)43 49 91
Tel No. (33-20)43 69 79

Dr. Anicia Q. Hurtado-Ponce,
Scientist, Aquaculture Department,
Southeast Asian Fisheries Development Center,
5021 Tigbauan, Iloilo.
Fax No. (63-33)271008
Tel No. (63-33) 27-1009

Processing

Dr. Suwalee Chandrkrachang,
Asst. Professor, Biopolymer Research Unit (BRU),
Faculty of Science, Srinakarinvirot University,
Prasanmitre, Sukhumvit, Bangkok 10110, Thailand.
Fax No. (66-2)2591151
Tel No. (66-2)259 1151

Phytosanitation and Environment

Mr. Kanit Chaiyakam,
Senior Fisheries Biologist,
National Institute of Coastal Aquaculture,
Kao Saen Soi 1, Muang District, Songkhla, Thailand 90000.
Fax No. (66-74)311
Tel No. (66-74)311895

Marketing and Trade

Mr. Suchart Wongwai,
Chemicals Goods Section, Bangkok Port,
Port Authority of Thailand,
444 Tha-rua Road, Prakanong, Bangkok 10110.
Fax No. (66-2) 249-0399
Tel No. ext 2148,2558

Socio-Economics

Mr. Nyan Taw,
Former CTA, Seaweed Production Development Project,
III. REPRESENTATIVES OF COLLABORATING ORGANISATIONS

French Government

Mr. Alex Brayle,  
Director, Scientific and Technological Centre,  
French Embassy, 29 Sathorn Tai Rd., Bangkok 10120.

Tel No. (66-2) 287 1592 to 8
Fax No. (66-2)287-1019

Mr. Duffillot,  
Deputy to the Regional Delegate,  
French Regional Co-operation Services,  
29 Sathorn Tai Rd., Bangkok 10120.

Tel No. (66-2) 287 1592 to 8
Fax No. (66-2)287-1019

Food and Agriculture Organization (of the United Nations)

Dr. Veravat Hongskul,  
Regional Fisheries Officer,  
FAO Regional Office for Asia and the Pacific,  
39 Phra Atit Road, Bangkok 10200 Thailand.

Tel No. (66-2)2817844
Fax No. (66-2)280 0445

Mekong Secretariat

Mr. Jorgen Jensen,  
Chief, Agriculture, Irrigation, Forestry and Fisheries,  
Mekong Secretariat, Kasatsuk Bridge,  
Rama I Road, Bangkok 10330, Thailand.

Tel No. (66-2)225 0029
Fax No. (66-2)252 2796
Telex 21322 MEKONG

Department of Fisheries, Thailand

Mr. Sutheewat Somsueb,  
Coastal Aquaculture Division, Department of Fisheries,  
Kasetsart University Campus, Ladyao, Jatujak, Bangkok 10900.

Tel No. (66-2)579 3681
Fax No. (66-2)579-3683

Ms. Jamaree Phungoen,  
Coastal Aquaculture Division, Department of Fisheries,  
Kasetsart University Campus, Ladyao, Jatujak, Bangkok 10900.

Tel No. (66-2)579 2421
Fax No. (66-2)579 4496

Mr. Wiwat Singthaweesak,  
Chanthaburi Coastal Aquaculture Development Centre,  
Department of Fisheries,  
Thachalab, Chanthaburi Province 22000.

Tel No. (039)391025

Mr. Suchart Tachanarawong,  
Biologist, National Institute of Coastal Aquaculture,  
Muang District, Songkhla 90000.

Tel No. (66-74)311 895

Miss Amara Cheunpan,  
Aquatic Natural Resources Museum,  
Polthep Building, Department of Fisheries,  
Kasetsart University Campus, Ladyao, Jatujak, Bangkok 10900.

Fax No. (66-2)562-0600-15 ext6103

Purified Agar Co. Ltd.

Dr. Phornchai Sakullelarasmi,  
Purified Agar co. Ltd.

Tel No. (66-2)225 8439
Fax No. (66-2)225-828-10
888 Songwad Rd, Bangkok 10100.

IV. **Network of Aquaculture Centres in Asia-Pacific**
Kasetsart University Campus,
Ladyao, Jatujak, Bangkok 10900, Thailand.

FaxNo. (66-2)561-1727
Tel No. (66-2) 561-1728 to 9
Telex 84267 NACATH

Dr. Banchong Tiensongrusmee. Co-ordinator
Dr. M. N. Kutty. Senior Aquaculturist
Mr. Pedro Bueno. Information Specialist
Dr. Michael J. Phillips. Environment Specialist
Mr. Chen Foo Yan. Adviser
I am pleased to note the enthusiasm and interest in this regional seaweed study among the country study participants. I am sure that, with the continuing co-operation and guidance of the regional and inter-regional resource persons, this workshop will achieve its objectives and appropriate recommendations for follow-up activities for the further
development of seaweed production in Thailand and other countries in the region will emerge.
I wish all the participants a very successful workshop and our visitors a pleasant stay in Thailand.
ADDRESS BY THE REPRESENTATIVE OF FAO

Dr. Veravat Hongskul,
Regional Fisheries Officer, FAO/RAPA, Bangkok.

Mr. Deputy Director General, distinguished participants, ladies and gentlemen:

It is a great pleasure to welcome you, on behalf of FAO and the Regional Representative, to the Workshop of the Regional Study on Red Seaweeds which is a joint effort between FAO, NACA and the Government of France.

The importance of seaweeds is now generally recognised world-wide. Various seaweeds have been utilised throughout the world for centuries. Recent development and discoveries on the use of seaweed colloids has further expanded new areas for seaweed utilisation. World production of red seaweeds, which accounted for 20% of the total world seaweed production of 6.2 million tonnes in 1992, for example, has increased by 24% from about 1 million tonnes in 1983 to 1.26 million tonnes in 1992. More important is the fact that about 90% of production of red seaweeds came from Asia and the Pacific.

While China and Japan remain the principal producers in the north-west Pacific, the Philippines continues to be the main producer in Southeast Asia. The production of red seaweeds in this region also increased considerably, by about 143%, in the last decade that is, from 200,000 tonnes in 1983 to 538,000 tonnes in 1992, accounting for 43% of the world's seaweed production in 1992. Undoubtedly, such success has been a result of various efforts at promoting seaweed production in the region, including a FAO/UNDP project on seaweed production development in the Philippines of which Dr Nyan Taw, is the Chief Technical Adviser. I am happy to note that he is also participating and hope to hear more from him. I also note the participation of Prof Kanjanapaj and Dr Suwalee, the noted experts on seaweeds from Thailand, in this workshop to which I am sure they will provide valuable information on the development of seaweed culture and utilisation in the region.

The regional study on taxonomy, ecology and processing of economically important red seaweeds was developed as a recommendation of the seaweed training workshop in April 1992. NACA was requested by FAO to carry out a research programme on the culture of red seaweeds, review country reports covering the taxonomy, ecology, and processing of agar and recommend follow-up action at both national and regional levels at this present workshop. It is our sincere conviction that, despite the current marketing problems, the future of seaweed culture, utilisation and the use of seaweed extracts is enormous. The uses of agar, carrageenan and other emulsifiers are well known. More products for medicinal, pharmaceutical, cosmetic, industrial and agricultural uses are on the way. Potential uses of seaweed as feeds for aquaculture are being developed. Marine biotechnology in seaweed tissue culture including genetic engineering is already on the list for future research. These efforts, of course should also address potential markets in actual implementation.

In anticipation of the outcome of the workshop, I wish you all success in your deliberations. I look forward to your conclusions and recommendations for future activities in order to promote seaweed culture in the region to its fullest extent.
SPEECH

Mr. Alex Brayle,
Director, Scientific and Technological Centre,
French Embassy, Bangkok.

I am pleased to be with you this morning for the Opening Ceremony of the Final Workshop of the Regional Study on Commercially Important Red Seaweeds. I am glad that this project is supported by the French Government Trust Funds with the Food and Agriculture Organization (FAO). It is indeed gratifying to see that the funds provided for the project has enabled the Network of Aquaculture Centres in Asia-Pacific (NACA) and its participating countries in the region to complete an important aspect of the study on red seaweeds.

The present study focuses on *Gracilaria* species, which is cultured in several Asian countries, and which has the scope to expand its production and yield of the very valuable phycocolloid, agar, which has applications in different fields of foods, Pharmaceuticals and the emerging field of biotechnology. I understand that very important contributions have been made by the participating countries in this study on the ecology, taxonomy and processing (agar yield and quality) of *Gracilaria*.

It is also very interesting to see that the study by the national participants has been guided by the regional and inter-regional resource persons/experts notably from Thailand (where the two Regional Referral Centres for the study are located), China and the Philippines, countries which are in the forefront of seaweed production and utilisation in the world.

Advanced technology and high applications of phycocolloids are available in the developed countries and France especially has considerable expertise and industry in this field. I am particularly pleased that French experts from IFREMER, University Rene Descartes of Paris and University of Lille, are here to interact and guide the activities of the workshop and advise on the outputs and evolving suitable follow-up activities.

I am especially pleased to see the co-operative efforts, goodwill and enthusiasm of the participating countries, the regional and international organisations and the organising groups at Bangkok in this get-together for development of a major aquatic resource in the region.

I am certain that in the next few days you will have very important and valuable deliberations and I look forward to the outcome of the workshop and specific recommendations for the follow-up activities.

I wish you a very successful and rewarding workshop.

Thank you.
Annex I-3d

WELCOME SPEECH

Dr. Banchong Tiensongrusmee,
NACA Co-ordinator, Bangkok, Thailand.

Ladies and Gentlemen: It is indeed a great pleasure for me to have this opportunity to welcome all of you in this Final Workshop of the Regional Study on the Taxonomy, Ecology and Processing of Commercially Important Red Seaweeds. On behalf of the Network of Aquaculture Centres in Asia-Pacific, I would like to express my gratitude to the Royal Thai Government through the Department of Fisheries, Ministry of Agriculture and Co-operatives for facilitating the preparations and providing many of the valuable resources needed to conduct this activity. As you know, the funding for this Regional Seaweed study came from the French Government Trust Fund with the Food and Agriculture Organization (FAO). On behalf of the participating Governments and the NACA Organization, I wish to express my deep appreciation to FAO for continuing to provide funding as well as technical support to this Project as well as activities in other areas. I also thank the French Government and IFREMER for the funding and technical support for the Project.

I wish to thank the participants from eleven countries in the regions, namely: Bangladesh; China; India; Indonesia; Iran; Malaysia; Myanmar; Philippines; Sri Lanka; Thailand; and Vietnam for their commitment to this regional activity. I wish also to thank the resource persons, particularly Prof. Gavino C. Trono of the Marine Science Institute, College of Science, University of the Philippines, the Philippines; Dr. Annette Alfsen, Director of Research, CNRS, Universite Rene Descartes; Dr. Olivier Barbaroux of IFREMER; Dr. Christophe Destombe, CNRS, University of Lille, France; and Dr. Chen Jiaxin, Director, Yellow Sea Fisheries Research Institute, Qingdao, China, (our former associate in the Sea Farming Development Project with NACA), for sharing their expertise with NACA in this study, despite their undoubtedly crowded academic and scientific commitments. Our special thanks are due to Prof. Khanjanapaj Lewmanomont of the Fisheries Faculty of Kasetsart University and Assistant Prof. Suwalee Chandkrachang, of the Biopolymer Research Unit of Srinakarinvirot University, Thailand, who headed the respective Regional Referral Centres in taxonomy and processing of *Gracilaria* for the study.

I am very pleased to welcome the participation in this morning's opening activities of the representatives of FAO, the French Embassy, and various divisions of the Department of Fisheries, Thailand as well as the officials of Kasetsart and Srinakarinvirot University. Your collective presence is a most encouraging sign to the NACA Organization which, as you are aware, is seeking the further co-operation of various organisations to support and implement the regional programme for aquaculture development. This workshop is the final activity of the Regional Study on the Commercially Important Red Seaweeds which was taken up as a priority topic under the seaweed development programme and is one of the projects under the large regional programme of NACA that adopts a holistic approach to aquaculture development.

This study was started with a training-worship in Bangkok in April 1992. The project is a clear example of the close and strong collaboration among various national sectors including the academe and regional as well as international agencies in a regional activity for benefit of countries in the region. I would like to express our heartfelt appreciation to all concerned and our wish for a close and continuing relationship.
I look forward to the recommendations and guidelines from the workshop for facilitating further success in seaweed production in countries in the region as well as appropriate mechanisms for supporting the future activities. NACA would be most keen to collaborate further with the participating countries and various regional and international organisations concerned in this respect.

It is my pleasant duty to extend to you a warm welcome and to wish all the participants a most fruitful and successful workshop.
WORKSHOP PROGRAMME

Tuesday, 24 January 1995,
Morning Programme.

1. Registration
   0830 - 0900   Arrival of Guests and Registration of Participants.
   0900 - 0915   Assembly at NIFI Meeting Hall.

2. Opening ceremony
   0920   Welcome address
           Dr Kitjar Jaiyen, Deputy Director General,
           Department of Fisheries, Thailand.
   0930   Speeches
           Dr Veravat Hongskul, Regional Fisheries Officer, FAO RAPA.
           Mr Alex Brayle, Director, Scientific and Technological Centre, French
           Embassy, Bangkok.
           Dr. Banchong Tiensongrusmee, Co-ordinator, NACA.
   0940   Adoption of the Workshop Agenda

3. Description of scope and objectives of the workshop
   0945   Dr M. N. Kutty, Senior Aquaculturist (Research and Training), NACA.

4. Presentation of Country Reports
   1000-1230   Bangladesh.
               China.
               India.
               Indonesia.
               Afternoon Session.

5. Presentation of Country Reports (continued)
   1400-1545   Iran.
               Malaysia.
               Myanmar.
               Philippines.
               Sri Lanka.
               Thailand.
               Vietnam.

6. Discussion of country reports
   1600-1730

   Wednesday, 25 January 1995,
   Morning Session.

7. Presentation of Regional Overviews and Resource Papers
   Afternoon Session.
8. Discussion of the Overview and Resource Papers
   1400-1530

9. Final Discussions and Recommendations
   
   Thursday, 26 February 1994,
   Whole Day.

10. Field Trip
    
    Friday, 27 February 1994,
    Morning Session.

11. Adoption of Workshop Report
    0930 – 1200    Summary report of the Workshop will be presented for adoption.

12. Closing Ceremony
    1200-1205    Vote of Thanks.
Annex II-1

BANGLADESH

Manmatha Nath Sarker,
Marine Fisheries Survey Management and Development Project,
Cox’s Bazar, Bangladesh.

ABSTRACT

Bi-monthly sampling of seaweed from the coastal area of Bangladesh was carried out during July, September and November 1992; January, March and May, 1993; and July and September 1994. Gracilaria spp. was not found during the study. Data regarding the ecological conditions of the coastal areas are presented. Seawater temperatures varied from 25-27°C, salinities from 21-35 ppt and turbidity from 30-68 cm during the period of the study. Amongst the 19 groups of seaweed collected, most were found only from November to March. The most abundant species were Asparagopsis taxiformis, Hypnea spp. and Sargassum spp. Agar extraction from A. taxiformis showed that the lowest yields (4.23% ± 0.70) were obtained during January and highest (12.7% ± 0.53) were obtained during March. The report recommends the introduction of Gracilaria spp. from neighbouring countries for the specific purpose of improving water quality in shrimp farms and also to manage and exploit resources like Hypnea spp. and Sargassum spp.

1. INTRODUCTION

Priority problems in Bangladesh were identified as the taxonomy, ecology and analysis of Hypnea spp. of red seaweeds, but as agreed at the workshop, most participating countries would concentrate on Gracilaria spp. According to the Action Plan of the workshop (Annex I-1), bi-monthly sampling of seaweeds in the St. Martins island and Teknaf beach areas of Bangladesh where seaweed is available, was carried out. Altogether, 6 samplings were carried out on: July 17, September 13 and November 12 of 1992; and January 10, March 09 and May 06 of 1993. Two more collections were carried out in July and September of 1994. No Gracilaria was found in any of the samplings so it was not possible to send herbarium or colloid samples to the referral centres at Kasetsart University, Faculty of Fisheries and Biopolymer Research Unit of Srinakariniwirot University, respectively. The ecological data collected from the seaweed growing areas (shown in Figure 1) are presented in Table 1.

Table 1: Ecological data from seaweed growing area at St. Martins Island, Bangladesh.

<table>
<thead>
<tr>
<th>Date</th>
<th>Time</th>
<th>Tide</th>
<th>Water Temp (°C)</th>
<th>Salinity (Ppt)</th>
<th>Turbidity (cm)</th>
<th>Season</th>
<th>Weather</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>17/7/92</td>
<td>17.00</td>
<td>low</td>
<td>25.00</td>
<td>21.00</td>
<td>30.00</td>
<td>Rainy</td>
<td>Rainy</td>
<td>Seaweeds absent</td>
</tr>
<tr>
<td>13/9/92</td>
<td>16.00</td>
<td>low</td>
<td>25.00</td>
<td>26.00</td>
<td>45.00</td>
<td>Autumn</td>
<td>Sunny</td>
<td>Seaweeds absent</td>
</tr>
<tr>
<td>12/11/92</td>
<td>16.00</td>
<td>low</td>
<td>27.00</td>
<td>34.00</td>
<td>52.40</td>
<td>Winter</td>
<td>Sunny</td>
<td>Seaweeds available</td>
</tr>
<tr>
<td>10/1/93</td>
<td>16.00</td>
<td>low</td>
<td>25.00</td>
<td>34.00</td>
<td>67.18</td>
<td>Winter</td>
<td>Sunny</td>
<td>Seaweeds available</td>
</tr>
<tr>
<td>9/3/93</td>
<td>16.00</td>
<td>low</td>
<td>27.82</td>
<td>35.0</td>
<td>67.98</td>
<td>Summer</td>
<td>Rainy</td>
<td>Seaweeds available</td>
</tr>
<tr>
<td>6/5/93</td>
<td>15.30</td>
<td>low</td>
<td>25.60</td>
<td>31.0</td>
<td>55.85</td>
<td>Summer</td>
<td>Rainy</td>
<td>No seaweeds found</td>
</tr>
<tr>
<td>24/7/94</td>
<td>16.00</td>
<td>low</td>
<td>25.00</td>
<td>21.00</td>
<td>30.00</td>
<td>Rainy</td>
<td>Rainy</td>
<td>No seaweeds found</td>
</tr>
<tr>
<td>20/9/94</td>
<td>15.30</td>
<td>low</td>
<td>26.00</td>
<td>25.00</td>
<td>45.00</td>
<td>Autumn</td>
<td>Sunny</td>
<td>No seaweeds found</td>
</tr>
</tbody>
</table>
Figure 1: The seaweed growing area around St. Martin's Islands, Bangladesh.
2. OBSERVATIONS AND CONCLUSIONS

Bangladesh is situated in the north-eastern part of the Bay of Bengal. The country is blessed with a network of rivers which continually discharge a large amount of silt to the estuarine environment. Most of the time, the coastal waters are turbid and turbidity is higher in the rainy season. As a result, some plant and animals of oceanic origin cannot exist in such waters. A thorough, all year round investigation was conducted in the seaweed growing area but no *Gracilaria* spp. was found. The reason behind the lack of *Gracilaria* spp. has not yet been ascertained. The list of seaweeds found is presented in Table 2.

Among the seaweeds available, red seaweed of the genus *Hypnea* spp. and brown seaweed of the genus *Sargassum* spp. were dominant. It has been estimated that about 200 tonnes of dried *Hypnea* spp. and another 1,000 tonnes of dried *Sargassum* spp. is available in Bangladesh, but there are presently no commercial uses for these species.

Table 2: List of the seaweed resources of Bangladesh.

<table>
<thead>
<tr>
<th>SLNo.</th>
<th>Scientific Name</th>
<th>Type</th>
<th>Abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td><em>Actinotrichia fragilis</em></td>
<td>R.S.</td>
<td>+</td>
</tr>
<tr>
<td>02</td>
<td><em>Asparagopsis taxiformis</em></td>
<td>R.S.</td>
<td>+++</td>
</tr>
<tr>
<td>03</td>
<td><em>Calliblepharis</em> spp.</td>
<td>R.S.</td>
<td>+</td>
</tr>
<tr>
<td>04</td>
<td><em>Caulerpa</em> spp.</td>
<td>G.S.</td>
<td>++</td>
</tr>
<tr>
<td>05</td>
<td><em>Ceramium</em> spp.</td>
<td>R.S.</td>
<td>+</td>
</tr>
<tr>
<td>06</td>
<td><em>Chysymenia</em> spp.</td>
<td>R.S.</td>
<td>+</td>
</tr>
<tr>
<td>07</td>
<td><em>Colpomenia sinuosa</em></td>
<td>R.S.</td>
<td>++</td>
</tr>
<tr>
<td>08</td>
<td><em>Cthonoplastis</em> spp.</td>
<td>R.S.</td>
<td>++</td>
</tr>
<tr>
<td>09</td>
<td><em>Dictyota</em> spp.</td>
<td>R.S.</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td><em>Galaxaura</em> spp.</td>
<td>R.S.</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td><em>Halyemenia</em> spp.</td>
<td>R.S.</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td><em>Hydroclathrus clathratus</em></td>
<td>B.S.</td>
<td>++</td>
</tr>
<tr>
<td>13</td>
<td><em>Hypnea</em> spp.</td>
<td>R.S.</td>
<td>+++</td>
</tr>
<tr>
<td>14</td>
<td><em>Liagora</em> spp.</td>
<td>R.S.</td>
<td>+</td>
</tr>
<tr>
<td>15</td>
<td><em>Lobophora variegata</em></td>
<td>B.S.</td>
<td>+</td>
</tr>
<tr>
<td>16</td>
<td><em>Padina</em> spp.</td>
<td>B.S.</td>
<td>+</td>
</tr>
<tr>
<td>17</td>
<td><em>Sargassum</em> spp.</td>
<td>B.S.</td>
<td>+++</td>
</tr>
<tr>
<td>18</td>
<td><em>Scinaia complanta</em></td>
<td>R.S.</td>
<td>+</td>
</tr>
<tr>
<td>19</td>
<td><em>Vanvoorstea coccina</em></td>
<td>R.S.</td>
<td>+</td>
</tr>
</tbody>
</table>

R.S. = Red seaweeds + Nominally available
B.S. = Brown seaweeds ++ Moderately available
G.S. = Green seaweeds +++ Abundantly available

Studies were conducted to find out the agar content of one red seaweed, *Asparagopsis taxiformis*. The results of agar extraction of *A. taxiformis* are presented in Table 3, with the percentage of agar calculated in different months. It should be mentioned that the species *A. taxiformis* was not available during the months of November and December.

The lowest percentage of extracted agar (4.23 + 0.70) from a dry sample was recorded in January, 1993 and the highest percentage of agar (12.70 + 0.53) from a dry sample was recorded in March 1993. From the minimum value, agar content in A. *taxiformis* increased gradually and finally in March 1993, it reached its maximum value. Agar contents were lower in the months of January and February and higher in the month of March, 1993.

**Table 3:** Percentage of agar contents in *A. taxiformis* in different months during the period of investigation.

<table>
<thead>
<tr>
<th>Observation No.</th>
<th>November (%)</th>
<th>December (%)</th>
<th>January (%)</th>
<th>February (%)</th>
<th>March (%)</th>
<th>April (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>4.30</td>
<td>9.30</td>
<td>13.35</td>
<td>--</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
<td>3.34</td>
<td>10.50</td>
<td>12.73</td>
<td>--</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>-</td>
<td>5.08</td>
<td>9.68</td>
<td>12.03</td>
<td>--</td>
</tr>
<tr>
<td>Mean</td>
<td>-</td>
<td>-</td>
<td>4.23</td>
<td>9.83</td>
<td>12.70</td>
<td>--</td>
</tr>
<tr>
<td>SD</td>
<td>+/-0.73</td>
<td>+/-0.50</td>
<td>+/-0.53</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3. **RECOMMENDATIONS**

On the basis of a review of historical data and the present study, the following work should be considered in Bangladesh, in co-operation with NACA/ IFREMER, Kasetsart University and Srinikaronvirot University, Bangkok, Thailand:

1. Research into the possibility of culturing *Gradlaria* spp. in Bangladesh using introductions from the contiguous coastal areas in Myanmar.

2. Research into the extraction of phyco-colloids from the available seaweed resources, such as *Hypnea* spp. and *Sargassum* spp.

3. Research into the potential of *Hypnea* spp. and *Sargassum* spp. for culture.
ABSTRACT

In 1993, China produced 450 tonnes of agar, 260 tonnes of which came from *Gracilaria* spp. More than half of the agar produced is consumed locally. *Gracilaria* spp. is cultured in Hainan, Guangdong and Fujian, with Hainan as the major producer, 28 species of *Gracilaria* have been reported.

The present study concentrated on *G. lemaneiformis*, *G. asiatica* and *G. tenuistipitata*. As far as possible, with the limited time available, the ecological conditions (mainly salinity and temperature) of the various species (including varieties in some cases, as in *G. tenuistipitata*) were correlated with the extracted agar yield and quality. The agar contents were high and gel strength was good (<500-608 g/cm²). Different methods of extracting agar were compared. No species was specifically prioritised for culture, but the three species described are already being cultured. Further study was recommended.

1. INTRODUCTION

Due to its fast growth and high agar content, *Gracilaria* has been one of the main species of seaweed used for extracting agar in the world for the last sixty years. At present, agar is widely utilised in a variety of industries, including food production, pharmacological, chemical and light industries as well as scientific research. The demand for agar is increasing and the agar properties of *Gracilaria* spp. are very significant for the culture and processing industries.

A number of agarophytes have been used as traditional Chinese medicines for cooling and relieving internal heat for over a thousand years. However, the agar industry in China was initiated only in the 1950’s, with wild *Gelidi um* spp. as the main material and *Gracilaria* spp. as a subsidiary material. With the accelerating pace of advances in culture techniques for *Porphyra hainanensis* and *Gracilaria* spp., however, these two species have been the main agarophytes in China since 1980. The quantity of agar produced reached approximately 450 tonnes in 1993, including 80 tonnes of *Gelidi um* spp. agar, 110 tonnes of *P. hainanensis* agar and 260 tonnes of *Gracilaria* spp. agar (Table 1).

Table 1: Out put, price and export quantity of agar in China in 1993.

<table>
<thead>
<tr>
<th>Seaweed of agar</th>
<th>Output of agar (tonne)</th>
<th>Price (US $/tonne)</th>
<th>Quantity of export (tonne)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Geliatum</em> spp.</td>
<td>80</td>
<td>13000-16000</td>
<td></td>
</tr>
<tr>
<td><em>Gracilaria</em> spp.</td>
<td>260</td>
<td>14000-17000</td>
<td>90</td>
</tr>
<tr>
<td><em>Porphyra hainanensis</em></td>
<td>110</td>
<td>15000-21000</td>
<td></td>
</tr>
</tbody>
</table>

More than half of the total output was consumed by the domestic market, mainly for the production of jellies, candies, stabilisers of granulated orange squash, dispersive pastes, drugs, smooth laxatives and culture medias in bacteriology. The rest was exported to
western Europe and other south-east Asian countries in the region, such as Japan, Malaysia and Taiwan. The prices of the agar product varied depending on seaweed species, product quality and demand in the agar market. The price of agar from *P. hainanensis* with a high agarose content and gel strength, is generally higher than that of the other two. In order to improve the quality of *G. tenuistipitata* var. *liui* agar, *Gracilaria* spp. and *P. hainanensis* are mixed in a proper ratio before the agar is extracted. Most of the agar yielding plants are distributed over the coastal areas of China, in areas such as Shanghai and the provinces of Shangdong, Fujian, Guangdong, Hainan and Guangdong. Most of the seaweed culture areas are private enterprises. About 35 agar processing plants in Fujian were set up between 1984 and 1987, but only about 10 plants remain now as a result of poor management, lack of circulating capital and free competition. In the northern part of China, the main raw material for agar is mainly *Gelidium* spp., but *Porphyra* spp. and *Gracilaria* spp. are the main species in Fujian, and *Gracilaria* is the main species in Hainan, Guangdong, Guangxi and Shanghai. In the last three years, the output of agarophytes has failed to meet the demands of processing plants and some plants have had to import *Gracilaria* spp. from Vietnam, the Philippines and other south-east Asian countries.

Before 1980, the agar industry used wild agarophytes as its raw materials, but supply soon fell short of demand. During 1958-1963, some scientific research units studied the artificial culture of *G. asiatica*, *G. tenuistipitata* and *G. lemaneiformis* with floating raft and floating nets in the inter-tidal zone. Good results were obtained, but the researchers did not attach any importance to them. In 1977, *G. tenuistipitata* var. *liui*, which has a very high regenerative capacity and simple culture methods, was found in a low salinity area in Hainan province. *Gracilaria* culture has spread over Hainan, Guangdong, Guangxi and Fujian. The output of cultivated *Gracilaria* increased from 107 tonnes in 1982 to 3,340 tonnes in 1993 (Table 2). The yield per hectare was about 1.12-2.49 tonnes. The reason for the low yield was poor management and some of the *Gracilaria* was sold to Taiwan province.

<table>
<thead>
<tr>
<th>Year</th>
<th>Guangdong</th>
<th>Hainan</th>
<th>Fujian</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Culture area (ha)</td>
<td>Output (tonnes)</td>
<td>Culture area (ha)</td>
</tr>
<tr>
<td>1982</td>
<td>43</td>
<td>107</td>
<td></td>
</tr>
<tr>
<td>1984</td>
<td>123</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td>1986</td>
<td>330</td>
<td>695</td>
<td></td>
</tr>
<tr>
<td>1987</td>
<td>150</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>1993</td>
<td>233</td>
<td>420</td>
<td></td>
</tr>
</tbody>
</table>

From 1987-1990, there was a decline in the culture area of *Gracilaria* due to high profits from shrimp culture and real estate. Some shrimp farmers soon switched back, however, because of serious shrimp disease problems. The depression of the real estate market also meant that fewer seaweed farmers were selling their land. In 1993 and 1994, the price of *Gracilaria* seaweed increased by over 60% and it is believed that there will be an upsurge in *Gracilaria* culture in China in a couple of years time.

2. **TAXONOMY OF THE MAIN SPECIES OF GRACILARIA IN CHINA**

*Gracilaria* spp. is distributed all along the coast of China, particularly in southern China including Hainan, Guangdong, Guangxi and Fujian, where warm seawater, rich in
nutrients is very suitable for its growth. A total of 27 species (including two varieties) were described by Xia, (1975) in mainland China (Table 3). Among them, the species of wide distribution and suitable for culture were: G. tenuistipitata var. liui; G. asiatica; G. tenuistipitata; G. chouae, G. blodgettii; G. articulata; and G. chorda, but only G. tenuistipitata var. liui has been widely cultured, the rest are undeveloped. The reason for G. tenuistipitata var. liui culture is that this species is a perennial plant which is euryhaline and eurythermal, with vegetative propagation. Its seedlings are also readily available and it grows easily and fast (except for high summer temperatures) and has several good harvests a year. Another species of Gradlaria cultured for abalone forage in Dongshan Island, Fujian province, is characteristic of that of G. tenuistipitata var. liui, but it has no been properly identified yet.

Table 3: Species and distribution of Gracilaria sp. in China.

<table>
<thead>
<tr>
<th>No.</th>
<th>Species</th>
<th>Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.</td>
<td>G. articulata</td>
<td>Hainan</td>
</tr>
<tr>
<td>3.</td>
<td>G. asiatica var. asiatica</td>
<td>Widely distributed on mainland coast</td>
</tr>
<tr>
<td>4.</td>
<td>G. asiatica var. zhengii</td>
<td>Fujian, Guangdong</td>
</tr>
<tr>
<td>5.</td>
<td>G. bainiae</td>
<td>Hainan</td>
</tr>
<tr>
<td>6.</td>
<td>G. blodgettii</td>
<td>Hainan, Fujian, Guangdong</td>
</tr>
<tr>
<td>7.</td>
<td>G. chorda</td>
<td>Hainan</td>
</tr>
<tr>
<td>8.</td>
<td>G. chouae</td>
<td>Fujian</td>
</tr>
<tr>
<td>9.</td>
<td>G. coronopifolia</td>
<td>Taiwan, Haina</td>
</tr>
<tr>
<td>10.</td>
<td>G. cuneifolia</td>
<td></td>
</tr>
<tr>
<td>11.</td>
<td>G. eucheumoides</td>
<td>Hainan</td>
</tr>
<tr>
<td>12.</td>
<td>G. fanii</td>
<td></td>
</tr>
<tr>
<td>13.</td>
<td>G. firma</td>
<td>Guangdong</td>
</tr>
<tr>
<td>14.</td>
<td>G. gigas</td>
<td>Guangdong</td>
</tr>
<tr>
<td>15.</td>
<td>G. glomerata</td>
<td></td>
</tr>
<tr>
<td>16.</td>
<td>G. hainanensis</td>
<td>Hainan</td>
</tr>
<tr>
<td>17.</td>
<td>G. lemaneiformis</td>
<td>Shandong</td>
</tr>
<tr>
<td>18.</td>
<td>G. lingula</td>
<td>Xiamen city of Fujian</td>
</tr>
<tr>
<td>19.</td>
<td>G. longirostris</td>
<td></td>
</tr>
<tr>
<td>20.</td>
<td>G. megaspora</td>
<td>Fujian</td>
</tr>
<tr>
<td>21.</td>
<td>G. mixta</td>
<td></td>
</tr>
<tr>
<td>22.</td>
<td>G. salicomia</td>
<td>Hainan</td>
</tr>
<tr>
<td>23.</td>
<td>G. spinulosa</td>
<td>Taiwan, Hainan</td>
</tr>
<tr>
<td>24.</td>
<td>G. tenuistipitata</td>
<td>Guangdong, Guanxi, Hainan and Fujian</td>
</tr>
<tr>
<td>25.</td>
<td>G. tenuistipitata var. liui</td>
<td>Guangdong, Guangxi, Hainan Fujian</td>
</tr>
<tr>
<td>26.</td>
<td>G. textorii</td>
<td>Yellow sea</td>
</tr>
<tr>
<td>27.</td>
<td>G. yamamotoii</td>
<td></td>
</tr>
<tr>
<td>28.</td>
<td>Gracilaria sp.</td>
<td>Dongshan Island of Fujian province.</td>
</tr>
</tbody>
</table>

nb: No. 1-27 from Xia, 1985; Zhang and Xia, 1988; Abbott et al. 1991; Zhang and Xia, 1992; 1994; No. 28 not identified
3. **STATUS OF STUDIES ON GRACILARIA CULTURE AND ECOLOGY IN CHINA**

Many studies on the ecology and culture methods of the red algal genus *Gracilaria* have been reported since the 1950's. Some studies in this field over the last twelve years are as follows:

Li Weixin (1982), studied the ecological habits of *G. tenuistipitata* at Zhanjiang Bay. The results showed that sporelings existed all the year round, but the most luxuriant time was in April-June when the water temperature was 25-29 °C. The vegetative growth of sporelings almost stopped in July-September when the water temperature was 30-35 °C. However, the platelets in dormancy began to grow once again in October and became gametophytes and sporophytes in November, when the water temperature was 25-29 °C. They grew vigorously throughout the winter and attained the fastest growth rate the following spring. The average growth rate of the generative thalli was 0.5-0.7 cm per day. Spring was the generative season of gametophytes and sporophytes when the water temperature was 22-23 °C. They faded away in the hottest months from July to September when the water temperatures were 30-35 °C.

Liu (1989a), tested a new method spray cultivation for *G. tenuistipitata*. Intermittent spray culture was conducted in a system composed of a shed, a water pond and spraying apparatus. The production of *G. tenuistipitata* with this system increased by 5.18% as compared to that of the natural open sea. Moreover, natural predators in the open sea could be avoided and harvest was convenient. In addition, the thallus of *Gracilaria* sp. could make full use of sunlight, accelerating photosynthesis and promoting its growth. The water temperature could also be easily controlled.

In general, the output of *G. tenuistipitata* by pond scattering culture is low. Liu (1990) tested raft cultivation of seaweed in different layers of seawater and applied fertiliser to the alga in the pond. The results showed that alga near to the surface grew faster than those in deep water. He proposed that if the depth of water was over one meter, raft culture methods for *G. tenuistipitata* should be adopted with fish and shrimp cultivated in the same pond. Dip testing of the alga with a fertiliser of 2% urea showed that the output increased by 23% after 40 days cultivation as compared to the control group.

Zhang (1986, 1990), studied the relationship between the growth of *G. tenuistipitata* var. *liui*, illumination intensity and the specific gravity of seawater. The results showed that the range of suitable specific gravity of seawater was 1.005 to 1.015 and the optimum specific gravity was 1.010. When the specific gravity was more than 1.020, the growth of *G. tenuistipitata* var. *liui* decreased.

Branchlets of 1 to 3 cm in length also grew well in seawater of 1.010 specific gravity, the growth rate including the amount of branches per unit length. The growth rates of branchlets of 1 cm in length was better than that of those 3 cm in length. From the point of view of culture, the length of cutting branchlet should be more than 3 cm because the net length of growth was longer in 3 cm branchlets than in 1 cm. The depth of water was related to the temperature and transparency of sea water. In spring and winter when water transparency is 1.6 m, water depth should be kept around 20-40 cm (less than 50 cm) when the water temperature is 13.2-17.1 °C. In the summer, water depths of 60-80 cm should be maintained when water temperatures are 31-34 °C and water depths of 40-60 cm should be maintained in the autumn.

Plant growth hormones are used to adjust and control the growth of plants. They were used to culture *Gracilaria* spp. in China by Liu (1989b), where the algae were immersed
in seawater solutions of various concentrations of plant growth hormones for three hours, then cultivated by floating raft method for 20 days. During the experiment, the temperature of the seawater was 17-23°C, the specific gravity was 1.018 - 1.020, pH was about 8.2, and nitrate and phosphate concentrations were about 60 mg/m$^3$ and 40 mg/m$^3$, respectively. The results showed that the output of *G. tenuistipitata* var. *liui* increased by 12.1-83.6%, compared to the control group. Optimum concentrations of the various chemicals were as follows: Gibberllin, 70 ppm; 2,4-dichlorobenzoxyacetic acid, 7 ppm; Fengchansu, 0.7 ppm; Colchicine, 10-20 ppm; and a-naphthyl acetic acid, B-indol acetic acid and dipentadecyl carbinol, less than 10 ppm, 5 ppm and 300 ppm, respectively. Higher concentrations of plant growth hormones inhibited the growth of *G. tenuistipitata*.

Zeng (1990) took advantage of the vegetative propagation of *G. tenuistipitata* var. *liui* and the spore propagation of other *Gracilaria* spp., such as *G. asiatica*, *G. tenuistipitata* and *G. lemaneiformis*, and conducted a cross breeding experiment in laboratory. The results showed that the agar content of the hybrid was 8-13% lower than that of *G. tenuistipitata* var. *liui* and 10-12% lower than that of *G. tenuistipitata*. The salinity required for growth was 15-17 ppt which was the same as that of *G. tenuistipitata* var. *liui*. The preliminary conclusion was that the hybrid could be cultivated in brackish water by vegetative propagation and it is a potential new culture species.

Ren and Chen (1986) studied the effects of temperature on the growth of *G. asiatica*. The results showed that the suitable temperature for gametophytes and sporophytes was 25°C and 15°C, respectively. The upper temperature limit was about 30°C and the lower temperature limit less than 5°C. *Gracilaria* spp. matured when temperatures were in the range of 10-25°C. The higher the temperature, the earlier the alga matured. Seedlings mainly came from the disc-like holdfast of old branches in the natural sea. The decrepit algae were replenished by new buds sprouted from new spores. In addition, *G. asiatica* has two growth periods in Qingdao, i.e., spring and autumn.

To date, studies on sources of seedlings by spore propagation have made little progress in China and are still in the experimental stage. There were systematic studies on the rearing of seedlings and culture in the open sea by Tseng et al (1987). In addition, Liu (1990) studied the intensive rearing of seedlings for *G. tenuistipitata* in the intertidal area.

4. **MORPHOLOGICAL AND ECOLOGICAL CHARACTERISTICS OF SEVERAL COMMERCIALLY IMPORTANT GRACILARIA SPECIES IN SUMMER**

Various properties of *Gracilaria* spp. agar are closely related to the species of *Gracilaria* and ambient environmental conditions. In the last two decades, many studies have been carried out on the discrimination of taxa in the red algal genus *Gracilaria* (Xia, 1985; Chang and Xia, 1976; Zhang and Xia, 1985; 1988; 1992; 1994; Abbott et al 1991). The ecology of *Gracilaria* spp. varies with species and region, e.g. the growing season of *G. asiatica* in the southern part of China is from November to the following April, but in the northern part of China, its growing season is from June to November. Studies on the relationship between various properties of agar and ecological conditions have not been carried out in mainland China.

4.1 **Materials and methods**

1. Specimens of *Gracilaria* spp. were collected from: Rongshan (June 11, July 13, August 16, 1994); Diancheng (June 15, August 18, 1994); Qianton (June 26, 1994); Beihai salt farm (June 13, 1994); Shanyao (June 26, July 31, September
2. Determination of seawater temperature: The temperatures of the surface and bottom layers were determined with a WNY-150 specific digit thermometer. The mean was then calculated.

3. Determination of salinity: The specific gravity of seawater, both surface and bottom layers, was determined using a specific gravity meter of seawater. The mean was calculated and converted into salinity using the relevant table of temperature, specific gravity and salinity.

4. Specimen slides: Specimens for mounting on slides were fixed in a solution of 5% neutral formalin, 5% Glycerine and 3% NaCl. Slides were prepared by Lin Hong, Qingdao Oceanographic University.

5. Identification of species: Conducted by Prof. Lu Baoren, Institute of Oceanology, Academic Sinica.

4.2 Results


   **Locality:** Tai Ping Comer, Qingdao City, Shangdong Province.
   **Date of Collection:** June 1st and August 27th, 1994.

   **Description:**

   Thalli erect, cylindrical, cartilaginous, 10-30 cm in height, solitary or caespitose, with many branches of 0.5-2 mm in diameter growing from large, flat and fresh red disc-like holdfast. Branches irregular, simple, elongated, sometimes with numerous short poliferous branchlets, secund or alternate, branch apex attenuate and without constriction at base. Thalli purplish-red when fresh; dark purplish-red, purplish brown or slightly green, when dried.

   Frond in transverse section consisting of large parenchymatous cells, medulla surrounded by two-three layers of cortical cell which are small and roundish, outermost layer pigmented. Transition of cell from medulla to cortex, abrupt. Spermatangial pattern superficial. Cystocarps protrude prominently, hemispherical or spherical, slightly or non-rostrate, slightly constricted at base. Gonimoblast composed of many small parenchymatous cells. Pericarp consisting of 10-13 layers of cells. Thallus grew on intertidal rock or in rock pools, lush in June when seawater temperature was 23-26°C and salinity was 2.86-3.10 % (June 1st, 1994). In August, however, the upper part of the alga rotted away and the rest had many cystocarps in branches. Some also existed in the seedlings (Figure 1).
2. *Gracitaria asiatica.*

   Locality: Taiping Comer, Qingdao City, Shandong Province
   Date of Collection: June 1st and July 24th, 1994.

   **Description:**
   Thallus erect, cylindrical, solitary or caespitose, growing from a small disc-like holdfast, 5-30 cm in height with 1-4 orders of branches. Branches irregularly secund, dichotomous or alternate, slightly constricted or non constricted at base; branches elongated, 0.5-2.0 mm in diameter, with or without many short or long branchlets, gradually tapering toward apices. Thallus purplish brown when fresh, sometimes slightly green or yellow. Dark brown or light green when dried. Frond in transverse section consisting of medulla of large, irregularly roundish parenchymatous cells, 75-250 \( \mu m \) in diameter, 10-32.5 \( \mu m \) in wall, surrounded by 3-5 layers of cortex. Transition of cells form medulla to cortex, gradual.

   Cystocarps globose or sub-globose, prominently protruding, nonrostrate or slightly rostrate, without or with slight constriction at base. Pericarps consist of 7-13 layers of cells, of which the outmost layer of cells are elongated. Spermatangia pattern pocket-like.

   Thallus grew on intertidal rocks, in rock pools or buried in sand. Cystocarps matured enormously in Qingdao in July (Figures 2 and 3).

3. *Gracilaria tenuistipitata* Chang and Xia.

   Locality: Shanyao salt farm Quanzhou City, Fujian Province
   Date of Collection: June 26th, July 31st, September 3rd, 1994

   **Description:**
   Thallus terete, cylindrical, cartilaginous, solitary or caespitose, growing from a small disc-like holdfast, 5-30 cm tall, 0.2-1 mm in diameter, slender near base. Branches
simple, elongated, dichotomous or alternate, non-constricted at base. Thalli purplish-brown, greenish-brown or purplish-red when fresh.

Frond in transverse section consisting of medulla of large parenchymatous cells at the centre, 120-300 μm in diameter surrounded by 1-3 layers of small cortical cells. Transition of medulla to cortex, abrupt. Tetrasporangia ovoid surrounded by elongated cortical cells. Cystocarps prominently protrude, globes, rostrate, constricted at base. Pericarp consisting of 8-10 layers of cells of which the outmost layer cells are round or ovoid, with a distinct cell wall. Spermatangia in surface, pit-like pattern or textorii-like. Algae grew on muddy substrate in the bottom of a water canal in a salt farm.

There was no *Gracilaria* sp. growing in the middle of the water canal channel due to rapid flows. Algae grew slowly and some branches had cystocarp when the water temperature was 31.9 °C and salinity was 28.5 ppt on June 26th, 1994. By July 31st, however, most of the algae had died or the upper part of the alga rotted away. Some branches turned white when the water temperature was 31°C and salinity was 32.2 ppt. On September 3rd 1994, part of the algae was buried in mud but had normal growth in the upper part when the water temperature was 29°C and salinity was 23 ppt. The algae was not exposed to air at low tide (Figures 4, 5 and 6).

4. **Gracilaria tenuistipitata** var. *liui*.

   **Locality:** Qiantou, Rongshan, Beihai salt farm, Diancheng
   **Date of collection:** June 26, 1994 (Qiantou, Quanzhou City)

   **Description:**
   Thalli slender, cylindrical, with numerous short to long flagella forming lateral branchlets of 0.18-0.5 mm in diameter. Branching mostly from percurrent axes, alternate or irregular, without holdfast, the thalli are usually detached and tumble about in fairly large masses. Branches slightly constricted or non-constricted at base. Branchlet apex apiculate, sometimes alternate. Conceptacles are hardly ever found. Thalli colour when fresh depends on the growing environment: meat-red; purplish-red; light-yellow or purplish-brown, usually occur as light yellow in low salinity.

   Frond in transverse section consisting of medulla of several large parenchymatous cells at the centre, 175-670 μm in diameter, surrounded by 1-2 layers of small cortical cells. Transition of cells from medulla to cortex abrupt (Figures 7 and 8).

5. **Gracilaria** sp.

   **Locality:** Qiujia, Dongshan Island, Fujian Province
   **Date of collection:** July 17th, 1994

   **Description:**
   Thalli terete, cylindrical, cartilaginous, easily broken, solitary, without holdfast at base, 10-40 cm in height, with numerous alternate, secund or irregularly elongated branches, somewhat chrysanthemum-like or fastigate-like in general outline. Branches slightly constricted at the base. Branchlets gradually taper toward the tip, ending in bifurcate apices. Thalli greenish, yellowish, green or olive-brown when fresh; and dark brown, purplish-brown or pink when dried.

   Frond in transverse section consisting of medulla of large, irregularly parenchymatous cells with angle, 140-400 pm in size, surrounded by 1-2 layers of spare arrangement of cortical cells. Transition from medulla to cortex, abrupt. Conceptacles hardly ever found.
The algae grew in shrimp ponds with salinites of 23-29 ppt. The alga was lush on July 17th, 1994 when the water temperature was up to 38°C and salinity was 24.2-28.3 ppt. The thalli colour was purplish-brown at a depth of 80-120 cm and yellowish-green at a depth of 40-60 cm. However, the upper part of the algae rotted away in a depth of 30-60 cm when the water temperature was 28.5 °C and salinity was 21.3 ppt on August 20th, 1994. Perhaps it was affected by the continuous high temperature.

5. YIELD AND PROPERTIES OF GRACILARIA AGAR

The properties and yield of Gracilaria agar vary with species, growing regions and extraction methods. There are no unified testing methods for agar content and properties of Gracilaria spp. in China. In the studies by Shi et al (1988), agarophytes were soaked in 32% NaOH solution at room temperature for five days and then washed until neutral. The agar was finally extracted with 0.1% of sodium hexametaphosphate solution. The yield of agar extracted by sodium hexametaphosphate was higher than that extracted directly by water. In the dilute alkali method of extraction, Gracilaria was treated using 2-6% of NaOH solution at 90°C for 60-90 minutes and then agar under 1 kg/cm² was extracted.
Figure 2: *G. asiatica*, collected from Tai Ping Corner, Quingdao. Thallus cross-section of main branch.

Figure 3: *G. asiatica*, collected from Tai Ping Corner, Quingdao. Thallus cross-section of cystocarp.
Figure 4: \( G. \textit{tenuistipitata} \) collected from Shanyao, Quanzhou (26th June 1994).
Figure 5:  *G. tenuistipitata* collected from Shanyao, Quanzhou (26th June 1994). Transverse section of main branch and cystocarp.

Figure 6:  *G. tenuistipitata* collected from Shanyao, Quanzhou (26th June 1994). Transverse section of main cystocarp.
Figure 7:  *G. tenuistipitata* var. *liui* collected from Qiantou, Quanzhou City, (26th June 1994).

Figure 8:  *G. tenuistipitata* var. *liui* collected from Qiantou, Quanzhou City, (26th June 1994). Transverse section of main stem.
Two new methods of agar extraction are the concentrated alkali-bleaching-organic acid-buffer solution treatment method (Lian, 1987) and the dilute alkali-alcohol solution treatment method. The purpose of this study was to evaluate the relationship between ecological conditions, agar extraction methods and agar properties.

5.1 Materials and methods

i. Extraction of agar

a) 5% NaOH treatment (DAT):
30.0 g of algae was weighed out and placed in 450 ml of 5% boiling NaOH solution, the temperature was kept at 95 ± 3°C for one hour. The alga was filtered and washed with tap water and then neutralised with 0.1 mol/L HC1. The alga was cut into small pieces, put into 750 ml of a boiling solution of 0.1% sodium hexametaphosphate and covered with tin foil with some holes. The solution was heated to boiling on an electric stove and then the breaker was placed in a pressure steriliser for 90 minutes to extract agar at a pressure of 1 ± 0.3 kg/cm₂. The breaker was then placed on an electric stove, heated and stirred for 30 minutes. The solution was filtered through nylon gauze of 200 mesh and 250 mesh separately. The residue was extracted once again with 200 ml distilled water at the same pressure for 1 hour and the filtrate combined with the first one (which had been kept at 80°C to avoid gelling), transferred into strips and frozen at -7±1°C for 40-48 hours. The frozen gel was thawed with tap water and washed with 200 ml of distilled water once and finally blown dry with electric fan.

b) 25% NaOH treatment (CAT), as above.

c) 5% NaOH-bleaching-organic acid and buffer solution treatment (BOBT):
50.0g of algae was put into 750 ml boiling solution of 5% NaOH and heated to 105±3°C for 20 minutes. The alga was then filtered, washed with tap water and neutralised with dilute HC1. The alga was then immersed in 750 ml of 0.08% active chlorine (NaCl solution) for 10 minutes, which was adjusted to pH 6 with about 18 ml of 2N HC1. The alga was washed with 750 ml tap water three times, immersed in organic acid for 20 minutes and washed with tap water until the pH was about 6. The alga was then immersed in sodium acetic acid buffer solution for 20 minutes, filtered and chopped at 10,000 rpm for 30 seconds. 1,000 ml of 0.1% sodium hexametaphosphate solution was added to a beaker with chopped alga, placed in an electric oven and kept simmering for 20 minutes. The rest of the procedure was as shown in the DAT method.

ii. Clean anhydrous weight (CAW%)
20.0 g of alga was weighed out and washed with tap water to remove the impurities including mud, calcareous encrustation, sand, salt and other species of algae. It was placed on a tray and blown dry with an electric fan. The alga was then dried in an oven at 105±3°C for 4 hours and the % CAW value was calculated.

iii. Moisture determination of algae and agar
2-3 g (O.000lg) of alga powder or agar was weighed into a weighing bottle and dried in an oven at 105±3°C for 4 hours. After cooling in a desiccator, it was weighed and the % moisture was calculated.

iv. Ash content of algae and agar
After determination of moisture content, the alga or agar was put into a weighed crucible which had been ignited and cooled in a desiccator and weighed soon after attaining
room temperature. The crucible was placed in a muffle furnace at not greater than 450 °C and the temperature was gradually increased to 525 °C. The sample was incinerated for about 4-6 hours until a constant weight was obtained. If it remained dark after cooling 1 ml H$_2$O$_2$ was added to the sample and it was evaporated in an electric oven and then incinerated. The sample was cooled in a desiccator and weighed soon after room temperature was attained and the % ash content was calculated.

v. Determination of gel strength of agar

1.5 g of dried base of agar was weighed and put into a 250 ml flask. 100 ml of distilled water was added and the sample was soaked for 12 hours at room temperature. The mixture was then heated in a reflux condenser until it dissolved completely. 25 ml of the resulting solution was poured into three or four 50 ml beakers separately and gelled at room temperature for 1 hour. The samples were then kept at 30±0.1°C constant temperature bath for 12 hours. The gel strength was measured on a Rowerbal weighing machine which added increasing loads until the gel ruptured, the weight was read as the gel broke. The mean strength of the three or four samples is taken as the gel strength.

vi. Melting temperature of agar gel

50 ml of 1.5% agar solution was poured into a test tube (25 x 200 mm) to 10 cm in height. A thermometer was inserted through a rubber stopper into the centre of the solution and the solution was left to congeal at room temperature for 12 hours. Two steel balls (0.3 mm) were placed in the gel, one 5 mm under the gel surface, another in the centre of the gel. After this, the test tube was immersed in a water bath of 60°C for 10 minutes so that the meniscus of the gel was 4 cm below that of the water. The water was then heated at a rate of about 1 °C/min until it reached 80°C, the temperature was then increased at a rate of about 0.5°C/min. The temperature was recorded when the ball dropped down through the solution. The average of both surface and centre temperatures was calculated.

vii. Sulphate content of algal material; and agar

0.1 g (0.000lg) of agar or alga was weighed out and put into a test tube 15 x 150 mm with a stopper. 10 ml of IN HC1 was added and the sample was left immersed for 12 hours at room temperature before it was hydrolysed in an oven at 107±3°C for 6 hours and 12 hours respectively for agar and algae. After cooling, the hydrolyse was neutralised with 5 mol/1 NH4OH until the pH was 6-7. 0.2 g of activated carbon was added and the solution was stirred for a while with a vortex mixer. The solution was filtered with No. 102 qualitative filter paper into a 50 ml volumetric flask and diluted to the mark. 10 ml of the filtrate was pipetted into a 25 ml of beaker and 1-2 ml of standard potassium sulphate solution and 10 ml of distilled water were added. To this solution, 1 ml of 6 mol /1 HC1 and 5 ml of 70% sorbitol were added, the solution was stirred with a magnet mixer for 1 minute, left to stand for 5 minutes and then stirred for 15 seconds. The absorbance of the suspension was read in a 721-spectrophotometer at 470 nm. A blank of distilled water was used as a reference.

5.2 Results and Discussions (See Tables 4-6)

i. Effect on properties of agar by agar extracting methods

a) Yield

Table 4 shows that the average yield of No. 1 and No. 2 samples extracted by DAT was 47.6% which was less than that by CAT (58.2%), shown in Table 5. The alkali solution showed "sol" phenomenon when cooled and the agar contained in the alga ran off.
view of this, the CAT method should be used to get a higher yield of agar from *G. lemaneiformis* and *G. asiatica*.

When *Gradlaria* spp. of Qiujia, (*G. tenuistipitata var liui* and *G. tenuistipitata*) was treated using DAT and CAT methods (Tables 4 and 5), the average yield of *Gracilaria* spp. agar was 51.3% and 50.6% for DAT and CAT respectively. In sample 5A, the yield of agar was 49.7% for DAT and 44.4% for CAT i.e., the agar yield extracted by CAT was less than that extracted by DAT. From the point of view of industrial production, the DAT method should be used to treat these seaweeds due to low consumption of alkali. The yields of samples number 2, 3 and 6 extracted by BOBT were higher than those extracted by CAT and DAT methods.

b) Ash content

The agar extracted by BOBT has good solubility and lustre. The ash content was also far less than that of similar material and those agars extracted by DAT and CAT methods. The ash of agar extracted with CAT and DAT was similar to, or higher than, that of similar material,. Sample 3A alga and those agars extracted by DAT and BOBT had 5.46, 10.1 and 1.65% ash contents, respectively. For sample 6A, 11.82, 9.72 and 3.84% ash contents were measured for alga, DAT agar and CAT agar, respectively. The low ash contents of agar extracted by BOBT suggests that most of the mineral must be removed after treatment with bleach and organic acid.

c) Gel Strength

The gel strength of agars extracted by the CAT method were all slightly less than those extracted by the DAT method. The gel strength of agars extracted by BOBT varied irregularly, e.g. the gel strength of sample 2A was 620g/cm² for BOBT and 682 and 578/cm² for DAT and CAT, respectively. Sample 8A had a gel strength of 551g/cm² for BOBT and 360g/cm² for DAT. Generally speaking, molecules of agar can be decomposed by acid and bleach, which results in low gel strength. However, it may be inferred that the effect on agar molecules of acid and bleach can be counteracted by the reduction in minerals as a result of this treatment.

d) Melting Temperature

The variation in melting temperature of agar extracted with DAT was similar to that of gel strength in samples No. 3 and No. 5. For instance, the melting point of sample No. 3 agar was 92.5 °C for June, 95 °C for July and 103 °C for September. The relevant gel strengths were 482, 634 and 1001 g/cm². The variation in gel strength of sample No. 5 was 93.5 °C for June, 99.2 °C for July and 97.2 °C for August. The gel strengths for the same months were 363, 720 and 440 g/cm², respectively. Irregular melting temperatures were found for sample nos. 1, 2 and 6. The lowest melting points were from samples 3A and 7A agar extracted with BOBT and were 80°C and 83°C separately.
Table 4: Relationship between the ecological conditions of *Gracilaria* spp. and properties of agar extracted by DAT.

<table>
<thead>
<tr>
<th>Locality</th>
<th>Species</th>
<th>Date (D/M/Y)</th>
<th>Salinity (%)</th>
<th>Temp (°C)</th>
<th>SO\textsubscript{4} (%)</th>
<th>Alga Ash (%)</th>
<th>Yield (%)</th>
<th>H\textsubscript{2}O (%)</th>
<th>Ash (%)</th>
<th>5% NaOH (P g/cm\textsuperscript{2})</th>
<th>Mpt. (°C)</th>
<th>SO\textsubscript{4} (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Qingdao</td>
<td><em>G. lemaneiformis</em> (1A)</td>
<td>01.06.94</td>
<td>2.98</td>
<td>23-26</td>
<td>2.27</td>
<td>9.09</td>
<td>46.4</td>
<td>18.7</td>
<td>7.65</td>
<td>848</td>
<td>93.5</td>
<td>1.56</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(IB)</td>
<td>27.08.94</td>
<td>3.26</td>
<td>5.04</td>
<td>47.6</td>
<td>19.2</td>
<td>6.91</td>
<td>91.0</td>
<td>872</td>
<td>1.64</td>
<td></td>
</tr>
<tr>
<td>Qingdao</td>
<td><em>G. asiatica</em> (2A)</td>
<td>01.06.94</td>
<td>2.98</td>
<td>23-26</td>
<td>3.42</td>
<td>12.0</td>
<td>44.1</td>
<td>19.9</td>
<td>7.45</td>
<td>682</td>
<td>90.0</td>
<td>1.43</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(2B)</td>
<td>24.07.94</td>
<td>5.30</td>
<td>6.96</td>
<td>52.3</td>
<td>20.1</td>
<td>8.85</td>
<td>92.0</td>
<td>534</td>
<td>1.62</td>
<td></td>
</tr>
<tr>
<td>Shanyao</td>
<td><em>G. tenuistipitata</em> (3A)</td>
<td>26.06.94</td>
<td>2.85</td>
<td>30.3</td>
<td>2.96</td>
<td>5.46</td>
<td>38.2</td>
<td>20.0</td>
<td>10.1</td>
<td>482</td>
<td>92.5</td>
<td>1.60</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(3B)</td>
<td>31.07.94</td>
<td>3.23</td>
<td>31.3</td>
<td>2.70</td>
<td>6.29</td>
<td>44.7</td>
<td>16.6</td>
<td>634</td>
<td>95.0</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(3C)</td>
<td>03.09.94</td>
<td>2.30</td>
<td>29.6</td>
<td>2.98</td>
<td>4.87</td>
<td>35.0</td>
<td>18.6</td>
<td>1001</td>
<td>103.2</td>
<td>0.776</td>
</tr>
<tr>
<td>Qiujia</td>
<td><em>Gracilaria</em> sp (4B)</td>
<td>17.07.94</td>
<td>2.83</td>
<td>38.0</td>
<td>4.38</td>
<td>5.48</td>
<td>48.7</td>
<td>19.6</td>
<td>10.2</td>
<td>609</td>
<td>96.8</td>
<td>1.65</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(4C)</td>
<td>17.07.94</td>
<td>2.42</td>
<td>38.0</td>
<td>3.23</td>
<td>6.76</td>
<td>57.5</td>
<td>19.8</td>
<td>576</td>
<td>93.0</td>
<td>1.44</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(4D)</td>
<td>20.08.94</td>
<td>2.31</td>
<td>28.5</td>
<td>3.30</td>
<td>6.29</td>
<td>47.6</td>
<td>19.7</td>
<td>549</td>
<td>94.0</td>
<td>1.24</td>
</tr>
<tr>
<td>Rongshan</td>
<td><em>G. tenuistipitata</em> var. <em>liui</em> (5A)</td>
<td>11.06.94</td>
<td>0.503</td>
<td>28.6</td>
<td>2.83</td>
<td>5.96</td>
<td>49.7</td>
<td>20.0</td>
<td>9.26</td>
<td>363</td>
<td>93.5</td>
<td>2.19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(5B)</td>
<td>13.07.94</td>
<td>0.967</td>
<td>29.0</td>
<td>3.98</td>
<td>6.00</td>
<td>38.0</td>
<td>20.4</td>
<td>720</td>
<td>99.2</td>
<td>1.14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(5C)</td>
<td>16.08.94</td>
<td>0.798</td>
<td>30.1</td>
<td>3.54</td>
<td>6.47</td>
<td>33.3</td>
<td>20.0</td>
<td>446</td>
<td>97.2</td>
<td>1.85</td>
</tr>
<tr>
<td>Dianheng</td>
<td><em>G. tenuistipitata</em> var. <em>liui</em> (6A)</td>
<td>15.06.94</td>
<td>2.04</td>
<td>29.0</td>
<td>4.00</td>
<td>11.8</td>
<td>40.8</td>
<td>20.4</td>
<td>9.72</td>
<td>543</td>
<td>92.0</td>
<td>1.83</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(6B)</td>
<td>18.08.94</td>
<td>0.00</td>
<td>27.2</td>
<td>3.50</td>
<td>4.85</td>
<td>36.6</td>
<td>20.8</td>
<td>153</td>
<td>93.8</td>
<td>1.53</td>
</tr>
<tr>
<td>Qiantou</td>
<td><em>G. tenuistipitata</em> var. <em>liui</em> (7A)</td>
<td>26.06.94</td>
<td>1.17</td>
<td>34.7</td>
<td>4.93</td>
<td>6.55</td>
<td>44.9</td>
<td>19.4</td>
<td>8.64</td>
<td>373</td>
<td>92.8</td>
<td>0.994</td>
</tr>
<tr>
<td>Beihai</td>
<td><em>G. tenuistipitata</em> var. <em>liui</em> (8A)</td>
<td>13.06.94</td>
<td>2.21</td>
<td>34.7</td>
<td>2.20</td>
<td>9.6</td>
<td>42.8</td>
<td>20.9</td>
<td>6.22</td>
<td>360</td>
<td>94.5</td>
<td>1.48</td>
</tr>
</tbody>
</table>

Editorial Note: The agar yield and ash content are high, possibly owing to the extraction technique of adding 0.1% sodium hexametaphosphate during extraction. This may have also caused the elevation of gel strength and melting point. The quality of the extracted agar may be different from the international specification for commercial agars.
Table 5: Relationship between the ecological conditions of *Gracilaria* spp. and properties of agar extracted with CAT.

<table>
<thead>
<tr>
<th>Locality</th>
<th>Species</th>
<th>Date (D/M/Y)</th>
<th>Salinity (%)</th>
<th>Temp (°C)</th>
<th>Alga SO₄ (%)</th>
<th>Alga Ash (%)</th>
<th>Yield (%)</th>
<th>Ash (%)</th>
<th>25% NaOH (Pg/cm²)</th>
<th>Mpt (°C)</th>
<th>SO₄ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Qingdao</td>
<td><em>G. lemaneiformis</em></td>
<td>01.06.94</td>
<td>2.98</td>
<td>23-26</td>
<td>2.27</td>
<td>9.09</td>
<td>57.0</td>
<td>7.20</td>
<td>820</td>
<td>89.4</td>
<td>1.40</td>
</tr>
<tr>
<td></td>
<td><em>G. asiatica</em></td>
<td>01.06.94</td>
<td>2.98</td>
<td>23-26</td>
<td>3.42</td>
<td>12.0</td>
<td>59.3</td>
<td>6.77</td>
<td>578</td>
<td>86.2</td>
<td>1.51</td>
</tr>
<tr>
<td>Qiujia</td>
<td><em>Gradlaria sp</em></td>
<td>17.07.94</td>
<td>2.83</td>
<td>37-39</td>
<td>4.38</td>
<td>5.48</td>
<td>56.7</td>
<td>8.98</td>
<td>556</td>
<td>89.0</td>
<td>1.07</td>
</tr>
<tr>
<td>Rongshan</td>
<td><em>G. tenuistipitata</em> var. liui</td>
<td>11.06.94</td>
<td>0.503</td>
<td>28.2-29.0</td>
<td>2.83</td>
<td>5.96</td>
<td>44.4</td>
<td>9.55</td>
<td>355</td>
<td>94.9</td>
<td>1.88</td>
</tr>
</tbody>
</table>

Table 6: Relationship between the ecological conditions of *Gracilaria* spp. and properties of agar extracted by BOBT.

<table>
<thead>
<tr>
<th>Locality</th>
<th>Species</th>
<th>Date (D/M/Y)</th>
<th>Salinity (%)</th>
<th>Temp (°C)</th>
<th>Alga SO₄ (%)</th>
<th>Alga Ash (%)</th>
<th>Yield (%)</th>
<th>5% NaOH-H₂O</th>
<th>25% NaOH-Bleacher-Acid-Buffer P (g/cm²)</th>
<th>Mpt (°C)</th>
<th>SO₄ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Qingdao</td>
<td><em>G. asiatica</em></td>
<td>01.06.94</td>
<td>2.98</td>
<td>24.5</td>
<td>3.42</td>
<td>12.0</td>
<td>24.1</td>
<td>20.6</td>
<td>620</td>
<td>93.0</td>
<td>1.50</td>
</tr>
<tr>
<td></td>
<td><em>G. tenuistipitata</em></td>
<td>26.06.94</td>
<td>2.85</td>
<td>31.1</td>
<td>2.96</td>
<td>5.46</td>
<td>21.2</td>
<td>1.85</td>
<td>748</td>
<td>90.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>var. liui</td>
<td>(6A)</td>
<td>15.06.94</td>
<td>2.04</td>
<td>4.00</td>
<td>11.8</td>
<td>48.2</td>
<td>21.5</td>
<td>457</td>
<td>94.0</td>
<td>1.45</td>
</tr>
<tr>
<td>Qiantou</td>
<td><em>G. tenuistipitata</em> var. liui</td>
<td>(7A)</td>
<td>26.06.94</td>
<td>1.17</td>
<td>4.93</td>
<td>6.55</td>
<td>44.9</td>
<td>21.5</td>
<td>277</td>
<td>83.0</td>
<td>0.635</td>
</tr>
<tr>
<td>Beihai</td>
<td><em>G. tenuistipitata</em> var. liui</td>
<td>(8A)</td>
<td>13.06.94</td>
<td>2.21</td>
<td>2.20</td>
<td>9.60</td>
<td>29.7</td>
<td>21.6</td>
<td>551</td>
<td>91.0</td>
<td>0.596</td>
</tr>
</tbody>
</table>
e) Sulphate Content

Alkaline treatment can change the L-galactose 6-sulphate of agarpectin into 3,6-anhydro-l-galactose and thus reduce the sulphate content of the agar. The sulphate content of the algae was 2.2-5.3 %, with an average content of 3.46% (Tables 4-6). The sulphate content of the algae dropped to 0.6-2.19%, with an average content of 1.35% after different alkali treatments. The average sulphate content of agar extracted by DAT, CAT and BOBT were 1.46%, 1.37% and 0.99%, respectively. Moreover, the sulphate content of samples 3A, 7A and 8A agar, extracted by the BOBT method almost approached that of agarose, that is, BOBT was superior to two other methods (DAT and CAT).

ii. Effect of ecological conditions on agar properties

a) Salinity-Gel Strength: (Tables 4-6)

Samples 4B and 4C collected at the same time had different salinity gel strengths which were 609 and 576g/cm\(^2\) when salinity was 28.3 and 24.2%. Sample No. 5 had gel strengths of 363, 720 and 446g/cm\(^2\) when salinities were 4.38-5.08, 9.04-10.3 and 7.33-8.62%, respectively. Sample No. 6 had gel strengths of 543 and 153g/cm\(^2\) when the salinity of seawater was 20.4% and 0.0% i.e., the gel strength of agar extracted by DAT increased with increases in environmental salinity.

The gel strengths of wild G. lemaneiformis, G. asiatica and G. tenuistipitata and cultivated Gracilaria. sp, were all higher than 450g/cm\(^2\). The highest gel strength was 1,001 g/cm\(^2\) for sample 3C and this had nothing to do with alkali treatment methods and collection date. It is evident that they can be used to produce high quality agar.

From this investigation, it can be inferred that algae growing in less than 0.6% of salinity can be used to produce low gel strength of agar. It is necessary to conduct further research to ascertain the optimum ecological conditions in order to obtain high yield and quality of agar materials.

b) Salinity -Ash content

The variations of ash content of algae and relevant agar were consistent with those of environmental salinity. The difference in environmental salinity of samples 6A and 6B was 20.4% and the differences of ash content of algae and relevant agar was 6.97 and 5.85%, respectively. As for samples 5A and 5B, the differences of salinity, alga ash and agar ash were 5.92%, 0.04% and 1.62%, respectively. In spite of the low ash content in samples 6B compared with 6A, its gel strength was less than that of 6A. The main reason was that it had rained for about two months resulting in low salinity which affected biosynthesis of the molecular of agar in alga.

6. CONCLUSIONS

1. The yield and properties of agar varied with the extraction method used. Therefore, the quality of algae cannot be judged by using only one kind of extraction method.

2. G. asiatica and G. lemaneiformis should be treated with concentrated alkalis not with dilute alkali solution, except when adding other chemical reagents.

3. When the algae were treated by BOBT and the agar extracted, the yield and properties of agar were superior to that produced by the DAT and CAT methods.
4. From the point of view of the yield and gel strength of agar, *Gracilaria* sp. of Qiujia, *G. tenuistipitata*, *G. asiatica* and *G. lemaneiformis* were good agar materials.

5. *G. tenuistipitata* var. *liui* cannot be used to produce agar with high gel strength, particularly when growing in environmental salinities of less than 0.6%.

6. *Gracilaria* sp. of Qiujia relies on vegetative propagation with fast growth rates, high agar content and quality. Its culture is worth studying and spreading.

7 **EXISTING PROBLEMS AND RECOMMENDATIONS**

1. The ecological conditions of *Gracilaria* spp., such as seawater temperature, salinity, illumination intensity and nutritive sources directly affect the yield and properties of agar. Therefore, a systematic investigation should be carried out to obtain precise results.

2. It was reported that the lush season of *Gracilaria* spp. is November to the following April in southern China, but the collected samples for this research were grown in the off season, *i.e.*, June to September. It is necessary to study materials from other seasons.

3. NACA should standardise the methods of agar quality determination again, *e.g.*, yield of agar and sulphate content. In addition, differences of experimental instruments and operation in turn affect research results. For this reason, NACA should further fund and supply relevant instruments, such as gel strength measuring instruments, pocket salinity meters, pocket pH meters and turbidimeters for researchers in each country.

**REFERENCES**


ABSTRACT

Studies were made on the yield and physical properties of agar from *Gracilaria corticata* var. *corticata*, *G. corticata* var. *cylindrica*, *G. crassa* and *G. edulis*, growing in four localities near Mandapam. The seaweeds were collected from Pudmadam, Pamban, Rameswaram and Thonithurai, respectively. The quality of agar in terms of gel strength was highest in *G. edulis*, but the yield of agar from this species was slightly less than that of *G. corticata* var *corticata* and *G. corticata* var. *cylindrica*, which may be due to the repeated commercial harvesting of *G. edulis* in the study area during recent years. There were no marked variations in the data collected on environmental and hydrological parameters from the four study areas, as all of them are located in the vicinity of Mandapam.

Thirty two species of *Gracilaria* have already been reported from Indian waters and their occurrence in different parts of the Indian coasts are given. The description and ecology of four species of *Gracilaria* (*G. edulis, G. corticata* var. *corticata, G. corticata* var. *cylindrica*, and *G. crassa*) which were investigated as part of the present study are also given. Investigations made on seasonal variations in growth, spore output, agar processing, yield and physical properties of agar on *Gracilaria* sp., *Gelidiella acerosa*, *Gracilariaposis sjostedtii* by different workers, are reviewed. The work carried out on experimental cultivation of *Gracilaria* spp. in different environments using various culture techniques are summarised. It is recommended that *G. edulis* should be selected for commercial scale cultivation in India, because of its high yield and good quality agar.

1. **INTRODUCTION**

Among the red seaweeds only some species, belonging to the genera *Gelidium, Pterocladiina Gelidiella* and *Gracilaria*, are used for commercial production of agar in various countries. However, in many countries of the Asia-Pacific region, the taxonomic status of these species is not properly known. This has often created confusion when comparing information and data provided by different countries on red seaweed production, culture methods, ecological parameters and phycocollloid properties. In addition, several other red seaweeds occurring in this region form an important source of raw material for the extraction of phycocolloids, but little is known about their correct taxonomic status and ecological requirements for culture. In the absence of information on the productivity, biochemical characteristics and chloroplastic genome pattern of the different strains and species, it is difficult to develop a viable culture technology for the lesser known species that would be economically efficient and sustainable. Studies on the *Gracilaria* spp. of India are currently undertaken to evaluate their agar yielding capacity and study their ecological requirements for commercial farming.

2. **ECOLOGY AND TAXONOMY OF GRACILARIA**

Boergesen (1933, 1934, 1937 a,b and 1938), reported 11 species and one variety of *Gracilaria* from different localities in India. Krishnamurthy and Joshi (1970), listed 12 species and two forms of *Gracilaria*. Umamaheswara Rao (1972), described 17 species
and two varieties of *Gracilaria*. Krishnamurthy and Rajendran (1986) described 4 new species of *Gracilaria* from Tamil Nadu.

**Figure 1:** Distribution of *Gracilaria* spp. in different parts of the Indian coast.

| 1. Gulf of Kutch | 12. Tuticorin |
| 2. Okha | 13. Kilakkarai |
| 3. Dwarka | 14. Pudumadam |
| 4. Gopnath | 15. Maniapam |
| 7. Karwar | 18. Visakhapatnam |
| 8. Quilon | 19. Lakshadweep |
| 9. Vizhinjam | 20. Andamans |
| 10. Kanyakumari | 21. Indian Ocean |
| 11. Tiruchendur |

Oza and Tewari (1990), reported *G. eucheumoides* from the Indian Coast. According to Krishnamurthy (1991), *Gracilaria* is represented by 32 species in the Indian region, of which 31 are found in different parts of the Bay of Bengal. A list of all species with places of occurrence is given in Table 1 and the places are shown in Figure 1.

Krishnamurthy (1989), gave the criteria for the classification of *Gracilaria* spp. Thomas (1977), reported the seasonal variations in growth, yield and physical properties of agar in *Gracilaria verrucosa*. Kaliaperumal et al. (1986) studied the growth, phenoiology and spore shedding of *Gracilaria arcuata* var. *arcuata* and *G. corticata* var. *cylindrica* occurring at Kilakkarai. Chennubhotla et al. (1986a) studied the growth, reproduction and spore output of *G. foliifera* and *Gracilariopsis sjoestedtii*. Chennubhotla et al. (1986b) studied the changes in growth and phycocollloid content of *Gelidiella acerosa* and *Gracilaria edulis*.

For the present study, four species of *Gracilaria* were collected from different localities near Mandapam every month for a period of five months from May to September 1994. *G. edulis* was collected from Thonithurai, where the plants grow attached to pebbles and
stones at a depth of 0.5 m. *G. corticata* var. *corticata* was collected from the intertidal sandy rocks at Pudumadam.
Table 1: Distribution of *Gracilaria* spp. in different parts of the Indian coast.

<table>
<thead>
<tr>
<th>Species Description</th>
<th>Gulf of Kutch</th>
<th>Okha</th>
<th>Dwarka</th>
<th>Guwahati</th>
<th>Bombay</th>
<th>Goa</th>
<th>Darwara</th>
<th>Quilon</th>
<th>Vizhinjam</th>
<th>Trivandrum</th>
<th>Tuticorin</th>
<th>Kanyakumari</th>
<th>Pudumadam</th>
<th>Madras</th>
<th>Mahabalipuram</th>
<th>Visakhapatnam</th>
<th>Lakshadweep</th>
<th>Andaman</th>
<th>Indian Ocean</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. <em>Gracilaria arcuata</em> var. arcuata (Zan.) Umamaheswara Rao</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2. <em>G. arcuata</em> var. attenuata (Zan.) Umamaheswara Rao</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3. <em>G. armata</em> (C.Ag.) J. Ag.</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4. <em>G. vurca-pastoris</em> (Gmel.) Silva</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5. <em>G. cacalia</em> (J. Ag.) Dawson</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6. <em>G. canalaiculata</em> (Kuetz.) Sonder</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8. <em>G. corticata</em> var. corticata J. Ag.</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9. <em>G. corticata</em> var. cylindrica (J. Ag.) Umamaheswara Rao</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10. <em>G. crassa</em> (Harvey) J. Ag.</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>11. <em>G. cylindrica</em> Boergersen</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>12. <em>G. debilis</em> (Forssk.) Boevers.</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>15. <em>G. edulis</em> (Gmel.) Silva</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>16. <em>G. eucheumoides</em> Harvey</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>17. <em>G. fergusonii</em> J.Ag.</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>18. <em>G. foliifera</em> (Forssk.) Boerhessen</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>19. <em>G. foliifera</em> f. aeruginosa Borgesen</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>20. <em>G. foliifera</em> f. granatea Boergesen</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>21. <em>G. indica</em> Umamageswara Rao</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>22. <em>G. kanyakumariensis</em> Umamaheswara Rao</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>23. <em>G. kilakaraensis</em> Krishnamurthy and Rajendran</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>24. <em>G. mammarensis</em> Umamaheswara Rao</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>25. <em>G. miliardetii</em> (Montagne) J. Ag.</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>27. <em>G. opuntia</em> (Svedelius) Durairatnam</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>28. <em>G. pudumadamensis</em> Krishnamurthy and Rajendran</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>29. <em>G. pygmaea</em> Boergesien</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>30. <em>G. textori</em> (Suringer) J. Ag.</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>31. <em>G. tuticorinensis</em> Krishnamurthy and Rajendran</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>32. <em>G. verrucosa</em> (Hudson) Papenfuss</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
G. corticata var. cylindrica was collected from the intertidal sandy rocks at Pamban. Plants of G. crassa were collected in the intertidal zone attached to the coral stones at Rameswaram. A map showing the collection places is given in Figure 2.

The description and ecology of the four species are given below.

1. *Gracilaria edulis* (Gmelin) Silva.

Plants erect and grow up to 20 cm or more. Brownish-red or greenish in colour, alternately to irregularly branched, branches slightly constricted at the base. Grows abundantly in seagrass beds in shallow lagoons formed between the shore and fringing coral reefs. It is also attached to small stones and shells in sandy and muddy areas. This agarophyte occurs throughout the year in harvestable quantities.


Plants stand 15 to 20 cm tall; brownish-red in colour, regularly dichotomously branched with thick and cartilagenous fronds, margins entire and rarely proliferous. Grows attached to rocks and stones in the intertidal area. Found throughout the year and occurs in harvestable quantities.
3. **Gracilaria corticata var. cylindrica** (J. Agardh) Umamaheswara Rao

Plants 6 to 10 cm. tall or more, attached by discs arising from the basal parts of fronds, dark red to yellowish-red in colour often with variegated spots on fronds. Fronds dichotomously, alternately and irregularly branched, flat or compressed at lower parts, subterete to cylindrical and closely branched above with pointed or spinous apices. Grows on rocks protected from wave action in the sub-littoral region and also in rock pools of the intertidal region. This seaweed occurs throughout the year but not in harvestable quantities.

4. **Gracilaria crassa** (Harvey) J. Agardh.

Plants brownish-red or greenish in colour. Forms dense cushions on the substratum with dichotomously and irregularly branched fronds. Branches are up to 4 mm. in diameter, cylindrical and sometimes constricted at the base forming club shaped segments or oblong articulations. It grows throughout the year on rocks and dead coral pieces as large tufts in the intertidal and subtidal regions. It is available in harvestable quantities.

Environmental data on depth of occurrence, atmospheric temperature, salinity and turbidity were recorded at the collection areas. The plants were examined for their reproductive stages and epiphytes. Other aspects like grazing exposure and maximum and mean values obtained for water depth, atmospheric temperature and salinity of sea water are given in Table 2.

<table>
<thead>
<tr>
<th>Seaweed</th>
<th>Locality</th>
<th>Water depth (cm)</th>
<th>Air Temperature (°C)</th>
<th>Salinity (ppt)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Min</td>
<td>Max</td>
<td>Mean</td>
</tr>
<tr>
<td>G. edulis</td>
<td>Thonithurai</td>
<td>25</td>
<td>45</td>
<td>35.5</td>
</tr>
<tr>
<td>G. corticata var. corticata</td>
<td>Pudumadam</td>
<td>30</td>
<td>35</td>
<td>31</td>
</tr>
<tr>
<td>G. corticata var. cylindrica</td>
<td>Pamban</td>
<td>30</td>
<td>35</td>
<td>31.0</td>
</tr>
<tr>
<td>G. crassa</td>
<td>Rameswaram</td>
<td>30</td>
<td>35</td>
<td>34.0</td>
</tr>
</tbody>
</table>

3. **PROCESSING OF GRACILARIA SPECIES**

During and after the Second World War, attempts were made to extract agar from Indian seaweeds and different techniques were used to purify the agar gel. Bose *et al.* (1943) leached the whole weed for 18 hours before extraction and the gel was maintained at 60°C to remove suspended impurities. Starch present in the gel was removed by treating with 0.2% acetic acid for 1 hour and then washing the gel in water. Karunakar *et al.* (1948) employed a bacterial method for gel purification. Chakraborty (1945) used freezing techniques to remove the suspended material. Mahonty (1956) found that heating under pressure at 230 °F was necessary for the removal of impurities in the gel of *G. verrucosa*. Thivy (1952;1960), made detailed investigations on extraction of agar from different species of *Gracilaria* and *Gelidiella acerosa* and on physical properties of the agar obtained from them. A cottage industry method for the manufacture of pure agar form *Gracilaria edulis* (=*G. lichenoides*) was developed in Central Marine Fisheries Research Institute (Thivy 1960). In this method, the impurities are removed from the seaweed before extraction. The leaching process minimised the cost of production and
the yield from pulverised weed was also higher than that obtained by the earlier methods. Details of this method is given in Appendix 1. Kappanna and Visweswara Rao (1963) suggested that the quality of agar could be improved by freezing and thawing. In pilot plant trials, Visweswara Rao et al. (1965) soaked the pulverised weed overnight in fresh water before wet grinding and extracting the agar. Details of this method is given in Appendix 2. To eliminate the cost of freezing, Desai (1967) suggested using 90% industrial alcohol for the flocculation of agar from filtrate.

Thomas and Krishnamurthy (1976), studied the yield and quality of agar in the cultivated plants of Gracilaria edulis. It is evident from the results that the best yield of agar was from plants harvested after 3 months growth. Umamaheswara Rao (1978), studied the yield and gel strength of agar extracted from G. corticata from the Visakhapatnam area. Oza (1978), studied the seasonal variations in gel strength, gelling and melting temperature of agar from G. corticata occurring at the Veraval coast. Chennubhotla et al. 1978, studied properties of agar from Gelidiella acerosa, Gracilaria edulis, G. corticata and G. foliifera occurring in the Mandapam area. Kaliaperumal et al. (1990), studied the agar content of Gelidiella acerosa and Gracilaria sp. from the south Tamil Nadu coast.

In the present study, samples were dried and bleached with several washings in fresh water. 20 g of clean sample was immersed in 5% NaOH and heated at 90°C for one hour. The seaweed was washed and acidified with 1 ml HC1. Fresh water was then added (1:30) and the material was boiled at 90°C for one hour. The dissolved seaweed was filtered through a cloth bag with a pressing tool and allowed to set at room temperature. The gel was kept in the freezer for two days. The frozen gel was thawed in tap water and then dried in an oven at 50-55°C to obtain agar-agar.

The present study on the yield of agar, gel strength and melting temperature was carried out on G. edulis initially by collecting samples bi-monthly during the period from June 1992 to April 1993. Later, monthly samples were collected from May to September 1994. Similar studies were made on G. corticata var. corticata, G. corticata var. cylindrica and G. crassa from the samples collected on a monthly basis for 5 months from May to September 1994. The results obtained are given in Table. 3.

Table 3: Data obtained on the yield and quality of agar from Gracilaria spp. in India.

<table>
<thead>
<tr>
<th>Seaweed</th>
<th>Locality</th>
<th>Agar Yield (%)</th>
<th>Month of Yield</th>
<th>Salinity (ppt)</th>
<th>Melting Point (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Min</td>
<td>Max</td>
<td>Mean</td>
<td>Min</td>
</tr>
<tr>
<td>G. edulis</td>
<td>Thonithurai</td>
<td>10.6</td>
<td>25.6</td>
<td>17.1</td>
<td>Jun</td>
</tr>
<tr>
<td>G. corticata</td>
<td>Pudumadam</td>
<td>15.8</td>
<td>30.8</td>
<td>21.5</td>
<td>Jun</td>
</tr>
<tr>
<td>var. corticata</td>
<td>Pamban</td>
<td>14.8</td>
<td>25.2</td>
<td>20.8</td>
<td>Aug</td>
</tr>
<tr>
<td>G. corticata</td>
<td>Pamban</td>
<td>14.8</td>
<td>25.2</td>
<td>20.8</td>
<td>Aug</td>
</tr>
<tr>
<td>var. cylindrica</td>
<td>G. Rameswaram</td>
<td>5.1</td>
<td>7.8</td>
<td>6.0</td>
<td>Aug</td>
</tr>
<tr>
<td>G. crassa</td>
<td>Rameswaram</td>
<td>5.1</td>
<td>7.8</td>
<td>6.0</td>
<td>Aug</td>
</tr>
</tbody>
</table>

4. SEAWEED CULTURE

In India, seaweeds used as raw material in the seaweed industry have been harvested from natural beds along Tamil Nadu and Gujarat coasts since 1966. There are about 21 agar industries and 25 algin industries in India (Silas and Kalimuthu 1987). As many seaweed processing industries are emerging, there is an increasing demand for
materials, particularly agarophytes, which the existing resources cannot meet. Hence, culture of seaweed has been attempted by the Central Marine Fisheries Research Institute, the Central Salt and Marine Chemical Research Institute and the National Institute of Oceanography and other research organisations. In the Central Marine Fisheries Research Institute, Umamaheswara Rao (1973) conducted culture experiments on *Gracilaria edulis* and *G. corticata*. Regeneration in both plants was found to be high, the plants growing to harvestable size within 3 to 4 months. Field experiments were conducted in the open-shore environment in the Gulf of Mannar (Umamaheswara Rao, 1974) using two coir nets of 4 x 2 m size. An average yield of 4.4 kg/m² fresh seaweed was obtained from the seed material of 0.31 kg/m² area of the coir net after 80 days growth.

Experiments were also carried out for cultivating *G. edulis* in the inshore waters of Gulf of Mannar, in submerged free floating condition (Chennubhotla *et al.* 1978). Culture frames of 2 x 2 m in size with 1.42 kg seed material were tied loosely to the poles fixed in the nearshore waters at 1 m depth. The fresh weight of the material harvested after 45 days growth was 1.985 kg/m² against the seed material of 0.355 kg.

Seaweed cultivation in inshore waters is beset with problems such as sedimentation, as well as grazing by fish. To overcome these constraints, cultivation of *G. edulis* was attempted in slightly deeper water of 3-4 m depth where sedimentation is less. Three plastic rope nets of 5 x 2 m in size were used and the seed material was tied at the mesh intersections using nylon twine. The nets were suspended at different levels with the help of plastic buoys and granite sinkers. Three such nets were introduced with seed material of 0.665 kg/m² at midwater level in 4 m depth and the yield after 90 days was 1617 gm/m² (Chennubhotla *et al.* 1987). Detailed studies were made on the influence of environmental parameters on the culture of *G. edulis* at Mandpam (Kaliaperumal *et al.* 1992). Attempts were also made to culture *G. edulis* at Minicoy Island (Lakshadweep) by transporting the seed material from Mandapam and Kavaratti Island (Lakshadweep). A very encouraging result of a 30 fold increase in biomass was obtained after 60 days growth. *G. edulis* could be very effectively cultured in the lagoons of Lakshadweep islands during pre-monsoon (March-June) and post-monsoon (October to February ) seasons (Kaliaperumal *et al.* 1992a and Chennubhotla *et al.* 1992a and b).

At the Central Salt and Marine Chemical Research Institute, Raju and Thomas (1971) cultured *G. edulis* by longline method in a sandy lagoon at Krusadi Island. Three harvests were made at the end of 5, 8 and 10.5 months after planting and the total harvest during the year was about 3.5 kg from a 1 m length of rope. Krishnamurthy *et al.* (1975) carried out *G. edulis* cultivation at Krusadai Island lagoon using coir ropes. In 5 months, the plants attained a length of about 30 cm and the average weight of the plant was about 300 g. The harvest was made by clipping the plants close to the rope and the remnants were left on the rope for further growth. Two more harvests were made at intervals of 10 weeks, thus giving three harvests in a period of 10 months.

Attempts were made to culture *Gracilaria* spp. from spores. Krishnamurthy *et al.* (1969) raised the germlings of *Gracilaria edulis* and *G. corticata* on a nylon fabric from carpospores under laboratory conditions. They were then transferred to the sea. After 4 months, the young plants appeared and took another 4 months to attain maturity and develop reproductive structures. *G edulis* was cultivated successfully also by Reeta Jayasankar and Kaliaperumal (1991) and Reeta Jayasankar (1992) using tetraspores and carpospores. The spores from mature plants were liberated and settled on circular cement blocks, coir ropes, nylon ropes, plastic strip and coral stones and cultured to germlings in the laboratory for 17 days in Conway & Walne's medium at a temperature of
23-25 °C, light intensity of 1000 lux and a photoperiod of 16:8 LD cycle. Thereafter they were transferred to the sea. The young plants grew from the germlings after one month of transplantation and they took another 4 to 5 months to reach harvestable sized plants.

5. DISCUSSION AND CONCLUSIONS

There are no marked variations in the data collected with respect to environmental and hydrological parameters from the four study areas as all of them are located near to each other in the vicinity of Mandapam. The present study made on the yield and physical properties of agar from *Gracilaria corticata* var. *corticata*, *G. corticata* var. *cylindrica*, *G. crassa* and *G. edulis* from the Mandapam area indicates that the quality of agar (gel strength) obtained from *G. edulis* is higher than the other three species, although the yield of agar is slightly less than that in *G. corticata* var. *corticata* and *G. corticata* var. *cylindrica* (Table 3). The slightly low yield of agar obtained from *G. edulis* may be due to the vegetation being subject to repeated commercial harvesting from the study areas during recent years.

<table>
<thead>
<tr>
<th>Species</th>
<th>Yield (%)</th>
<th>Gel strength (g/cm²)</th>
<th>Gelling temp. (°C)</th>
<th>Melting temp (°C)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>G. arcuata</td>
<td>52.2</td>
<td>67</td>
<td>47-50</td>
<td>89-99</td>
<td>Kaliaperumal <em>et al.</em> 1992b</td>
</tr>
<tr>
<td>var. arcuata</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G. corticata</td>
<td>38</td>
<td>20</td>
<td>44</td>
<td>68</td>
<td>Thivy, 1952</td>
</tr>
<tr>
<td>var. corticata</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mandapam</td>
<td>42.8</td>
<td>22</td>
<td>40-49</td>
<td>49-60</td>
<td>Chennubholti <em>et al.</em> 1979</td>
</tr>
<tr>
<td>Tiruchendur</td>
<td>44</td>
<td>19</td>
<td>33</td>
<td>51</td>
<td>Subba Rao <em>et al.</em> 1977</td>
</tr>
<tr>
<td>Tiruchendur</td>
<td>27.2</td>
<td>9</td>
<td>33</td>
<td>70</td>
<td>Kaliaperumal <em>et al.</em> 1990</td>
</tr>
<tr>
<td>Manapad</td>
<td>21.5</td>
<td>9</td>
<td>36</td>
<td>72</td>
<td>Kaliaperumal <em>et al.</em> 1990</td>
</tr>
<tr>
<td>Visakhapatna</td>
<td>44.6</td>
<td>134</td>
<td></td>
<td></td>
<td>Umamaheswara Rao, 1978</td>
</tr>
<tr>
<td>Veraval</td>
<td>22.5</td>
<td>27</td>
<td>40-42</td>
<td>60-62</td>
<td>Oza, 1978</td>
</tr>
<tr>
<td>G. corticata</td>
<td>48.6</td>
<td>67</td>
<td>45-52</td>
<td>90-99</td>
<td>Kaliaperumal <em>et al.</em> 1992b</td>
</tr>
<tr>
<td>var. cylindrica</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kilakkarai</td>
<td>37.9</td>
<td>15</td>
<td>45</td>
<td>74</td>
<td>Kaliaperumal <em>et al.</em> 1990</td>
</tr>
<tr>
<td>G. crassa</td>
<td>23</td>
<td>140</td>
<td>48</td>
<td>84</td>
<td>Thivy, 1952</td>
</tr>
<tr>
<td>Karaichalli Is.</td>
<td>18</td>
<td>11</td>
<td>13</td>
<td>86</td>
<td>Kaliaperumal <em>et al.</em> 1990</td>
</tr>
<tr>
<td>(Tuticorin)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G. edulis</td>
<td>43</td>
<td>120</td>
<td>45</td>
<td>84</td>
<td>Thivy, 1952</td>
</tr>
<tr>
<td>Mandapam</td>
<td>55</td>
<td>63</td>
<td>48</td>
<td>65</td>
<td>Chennubholti <em>et al.</em> 1977</td>
</tr>
<tr>
<td>Rameswaram</td>
<td>49.2</td>
<td>111</td>
<td>41-57</td>
<td>46-69</td>
<td>Chennubhotla <em>et al.</em> 1979</td>
</tr>
<tr>
<td>Krusadils.</td>
<td>45</td>
<td>139</td>
<td>44-50</td>
<td>61-78</td>
<td>Chennubholti <em>et al.</em> 1979</td>
</tr>
<tr>
<td>Hare Is.</td>
<td>43.8</td>
<td>9</td>
<td>39</td>
<td>64</td>
<td>Kaliaperumal <em>et al.</em> 1990</td>
</tr>
<tr>
<td>(Tuticorin)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G. fergusonii</td>
<td>35</td>
<td>19</td>
<td>22</td>
<td>38</td>
<td>Subba Rao <em>et al.</em> 1977</td>
</tr>
<tr>
<td>G. foliifera</td>
<td>12</td>
<td>15</td>
<td>40</td>
<td>-</td>
<td>Thivy, 1952</td>
</tr>
</tbody>
</table>
In general, the present observations agree with the earlier findings on the yield and physical properties of commonly occurring *Gracilaria* spp in the Mandapam region and other parts of the Indian coast (Table 4).

Variations in the yield and quality of agar within a species may be due to its geographic location (Figures 1 and 2) and seasonal variation in the growth and size of the plants at the time of collection. It is concluded that *G. edulis* should be selected for commercial scale culture because of the high yield and quality of agar obtained from it.

6. **RECOMMENDATIONS**

All of the Indian agar industry currently uses *G. edulis* as a raw material for the production of food grade agar while *Gelidiella acerosa* is used for the manufacture of bacteriological grade agar. Since 1980, many agar industries have developed in India and there is paucity of raw materials of *G. acerosa* and *G. edulis* for the production of agar. As a result, some agar industries have tried *G. crassa* and *G. foliifera* for producing agar whenever plants of *G. edulis* were not available in harvestable quantity. However, due to the poor yield and quality of agar from these two species most have stopped using them (Kalimuthu et al. 1990). Annually 318 to 982 tonnes (dry wt) of *G. edulis* are commercially exploited from natural seaweed beds on the Tamil Nadu coast. This amount is not sufficient to meet the raw material requirements of the agar industry. The whole requirement of raw materials for the Indian agar industry is met only by the seaweeds harvested from the natural beds of the Tamil Nadu coast.

In order to augment production and have a continuous supply of raw materials for the agar industry, large scale cultivation of *G. edulis* had to be undertaken by preserving the natural beds as a source of seed material. Studies made on the growth behaviour of different species of *Gracilaria* in nature and experimental culture showed that *G. edulis* is a fast growing species and suitable for culture conditions. The technology developed for commercial scale cultivation of *G. edulis* by the Central Marine Fisheries Research Institute (Chennubhotla and Kaliaperumal, 1983) using coir rope net methods may be adopted for large scale cultivation in India. The yield and quality of seaweed could be improved by selecting fast growing and high agar yielding strains and by adopting breeding and other modern scientific techniques of crop improvement.

At present, cultivation of seaweeds such as *Gracilaria, Gelidiella, Hypnea* and *Acanthophora* (red algae) *Sargassum, Turbinaria, Cystoseira* and *Hormophysa* (brown algae) *Ulva, Enteromorpha* and *Caulerpa* (green algae) is carried out only on an experimental scale by the Central Marine Fisheries Research Institute, the Central Salt Marine Chemical Research Institute and other research organisations at different field
environments using various culture techniques. No attempt was made at commercial scale cultivation of any of these species. The seaweed suppliers and users should come forward first to undertake large-scale cultivation of seaweeds availing the financial assistance provided by banks and other funding agencies connected with rural development programmes. Once they demonstrate successful harvests, fish farmers and private entrepreneurs will automatically come forward to take up seaweed culture on a large scale. Seaweed cultivation on a commercial scale would not only augment a continuous supply of raw material to the seaweed industry, but will also improve the economic status of people living in the coastal areas.

Detailed studies on the ecology, growth and agar content of all other *Gracilaria* spp. growing in Indian waters have to be undertaken with a view to identifying the higher agar yielding species among them. Experimental cultivation of those species by vegetative propagation and spore method must be attempted in different field environments using various culture techniques in order to develop suitable technologies for commercial scale cultivation. This will lead to the increased production of *Gracilaria* spp. in the country and help to meet the needs of the Indian agar industry.

**REFERENCES**


Chakraborty, D. 1945. Agar-agar manufacture from *Gracilaria confervoides*. Journal of the Proceedings of the Institute of Chemists (India)., (India) 17:188.


Karunakar, P. D., Raju, M. S. and Varadarajan, S., 1948. Manufacture of agar-agar from


Appendix I

CMFRI COTTAGE INDUSTRY METHOD FOR THE EXTRACTION OF AGAR FROM

GRACILARIA SPECIES.

Gracilaria

Bleached weed 100g
(wet alkaline and concentrate)

↓

Cleaning by abrasion in a stone mortar to remove sand and other traces of foreign matter

↓

Soaking the weed in 6-8 litres of soft water for 24 hours

↓

Wet grinding the weed in a stone mortar into pulp using soft water

↓

Leave the pulp in 6-7 litres of soft water for 24 hours

↓

Separation of pulp by filtering through cloth

↓

Dry Seaweed Powder → Extraction ← Sedimentation of suspended impurities in the solution

at 90°C in 3 litres of soft water for 1-2 hours

↓

Filtering the hot seaweed with 2-3 layers of cloth

↓

Cooling the agar solution at room temperature

↓

Shredding the agar and sun drying the gel strips on plastic netting

↓

AGAR - AGAR
METHOD FOR AGAR EXTRACTION ON A COMMERCIAL SCALE
(VISWESWARA RAO et al. 1965).

Pulverised Seaweed
(8.5 kg raw material)
↓
Wash with fresh water
↓
Soak overnight in fresh water
↓
Wet grinding for 30 minutes in edge runner on
pestle-motor type grinder
↓
← Wash with soft water
Seaweed Pulp
↓
Extraction with 100 litres of water for 2 hours in a double-
jacketed open-pan evaporator with circulating steam. Adjust pH to 6
using 4-500 ml of N Sulphuric acid.
↓
Filtering
(in a double jacketed vacuum filter)
↓
Cooling the filtrate at room temperature
↓
Shredding the agar gel in gel chopper
↓
Freezing the agar for 24 hours (in ice plant)
↓
Thawing (at room temperature)
↓
Drying
(in air oven with hot air circulation)
↓
AGAR-AGAR (10 kg yield)
INDONESIA

by

Suci Antoro, National Seafarming Development Centre, PO Box 74, Telukbetung, Indonesia

and

Sutimantoro, Laboratory and Fish Processing Development, Directorate General of Fisheries, Jl. Harsono RM No. 3 Ragunan, Jakarta 12550, Indonesia.

ABSTRACT

Five species of Gracilaria, namely: G. edulis; G. lemaneiformis; G. salicornia; G. eucheumoides; and Gracilaria sp., were studied. G. edulis and G. lemaneiformis occurred most commonly and also give good agar yields (8-17%) with good agar quality (gel strength: 565-880 g/cm²). Most species had a low gelling temperature, which is an important characteristic of agar to be used as a microbiological medium. The study recommends G. edulis and G. lemaneiformis, which have robust thallus and high biomass production (estimated on a per square metre basis), for culture in both pond and field areas.

1. INTRODUCTION

Indonesia has a very long coastline (81,000 km) and occupies a 5,000 km band of ocean between the Pacific and Indian oceans. This area is the heart of the Indo-west Pacific biogeographic region which has the world's most diverse biota. Briggs cited by Frankenberg (1986), said that the diversity of the Indo-West Pacific shelf fauna and flora far exceeded that of other tropical regions. The Siboga Expedition (1888-1889), reported not less than 555 seaweed species as part of its findings (Weber van Bosse as cited by Soegiarto et al., 1978). Among Indonesia's algal flora, 55 species have been utilised by coastal people mostly as food and sometimes for medicinal treatments (Zeneveld cited by Soegiarto and Sulistijo, 1986). Presently, most of Indonesia's seaweed production serves as raw material for the manufacture of industrial products, such as agar and carrageenan. In 1993, Indonesia exported 14,367 tonnes of dried seaweed valued at US $ 7,429,000 (Indonesia Fisheries Statistics, 1994).

The world-wide increasing demand for seaweed and seaweed products has been the main factor which has encouraged the development of production technologies of economic species of seaweed. The production of carrageenophyte in Indonesia comes mainly from artificial cultivation, but the greater part of agarophyte production comes from direct harvest of natural stocks. The agarophytes which are harvested are mainly from the genera Gracilaria, Gelidium and Hypnea. At present, pond culture of Gracilaria is still being attempted.

The major constraint to Gracilaria cultivation in Indonesia is that there is no complete database available about the taxonomy, ecology and agar content on each species of the genus. Trono (1990) recommended that the species targeted for culture should have the following properties:

- It must be a fast growing species which can be propagated using vegetative propagules (cuttings) or spores and can produce large amount of biomass within relatively short cropping periods.
- It must have a high content of good quality of agar.
Taking these restrictions into consideration, the available species can be screened and comparative studies on their productivity may be conducted to determine the best species to select. As the properties of agar differ among species, it is very important that the correct name be applied. Thus the taxonomy of the species *Gracilaria* should be clarified to stimulate the culture of *Gracilaria* in Indonesia.

2. **TAXONOMY AND ECOLOGY OF *GRACILARIA***

Earliest taxonomic and ecological data of *Gracilaria* spp. dates from the Siboga Expedition (1888-1889). Of the 31 sheet herbarium specimens belonging to the *Gracilariaceae*, six species were identified, namely: *Polycavernosa fastigiata*, *P. vanbossae*, *P. unrillei*, *Gracilaria arcuata*, *G. arcuata* var. *snackeyi* and *G. blodgettii* (Abbott, 1988). Soerjodenoto (1962), based on Valerie May and Zeneveld descriptions, reported three species from Indonesia: *Gracilaria confervoides*, *G. lichenoides* and *G. taenioides*.

Presently, studies on the taxonomy, ecology and processing (agar yield and quality) of *Gracilaria* spp. in Indonesia are conducted under the seaweed development programme, one of the projects under the large regional aquaculture development and environment programme of NACA. This study focuses on two provinces, Lampung and West Java (Figure 1).

**Figure 1:** Map of sampling site.
A: Grupuk    B: Cukuh Balak;    C: Bojonegara;    D: Pameungpeuk.
2.1 Materials and methods

For the purposes of this study, fresh materials were examined from four locations namely Cukuh Balak and Grupuk in Lampung Province, and Bojonegara and Pameungpeuk in West Java Province. Nine herbarium specimens were collected and marked as SW 01, SW 02 from Grupuk, SW 03, SW 04, SW 05, SW 06 from Cukuh Balak, SW 08, SW 09 from Pameungpeuk and SW10 from Bojonegara. Liquid specimens were preserved with 5% formalin in seawater and examined. Ecological parameters such as salinity, temperature, transparency, substratum and season, were also recorded. Sections were cut by hand, stained with 1% aniline blue, mounted in 25% glucose syrup and examined under a light microscope.

2.2 Results

Among the nine specimens collected, five species were identified: *Gracilaria edulis*, *G. lemaneiformis*, *G. salicornia*, *G. eucheumoides*, and *Gracilaria sp.*

2.3 Species descriptions

1. **Gracilaria edulis** (Gmelin) Silva (Figure 2).

   Thallus growing from a disk-like holdfast with prostrate rhizome forming a tuft or cluster of 8-25 cm tall, dark green to yellowish in colour, branching di- or tri-chotomous, main axis 1-1.5 mm, branches 0.5-1.0 mm in diameter with wide angle furcation. Lower branch intervals much longer than the last two orders, branch cylindrical, lower branch about 1 mm in thickness and getting thinner to 0.5 mm for terminal segments with attenuate apices. Fronds in transverse section consisting of roundish thin walled medulla cells, 100-250 um in diameter, 1-2 rows of small cortical cells, transition from medulla to cortex, abrupt. Cystocarp globose 0.5-1.0 mm in diameter with rostrate tips and constriction at the base.

   Grows on rocks or muddy surfaces in sandy-mud areas, sheltered or not sheltered, intertidal zone, clear water and not exposed to air during low tide. Distributed around Grupuk and Cukuh Balak Lampung and Bojonegara West Java along the Java sea coast.

2. **Gracilaria lemaneiformis** (Bory) Weber-van Bosse (Figure 3).

   Thallus solitary or caespitose, dark green to brownish in colour, up to 40 cm tall with few to several long branches. Branches 0.5-1.5 mm in diameter, branching irregular, mostly from lower portion, branches simple, 2-5 branchlets frequently occur from a single branch apex. Fronds in transverse section of medulla of large thin walled cells, 130-260 um in diameter, two layers of cortex, transition of cell from medulla to cortex, abrupt. Cystocarp spherical 0.7-1 mm, slightly or non rostrate, constriction at the base.

   Grows on sand or in muddy areas, intertidal zone, clear water, sheltered area, not exposed to air during low tide. Found in the Grupuk, Lampung area.

3. **Gracilaria eucheumoides** Harvey (Figure 4A).

   Thallus cartilaginous, greenish-brown to purple in colour when fresh, forming prostrate clumps and attached to the substratum by hapters originating from the ventral side of the flattened branches. The branching pattern is very irregular and the branches are compressed measuring 2.5-7 mm across, provided with coarse teeth along their margins, attached by discoid holdfast.
Figure 2: *Gracilaria edulis* (Gmelin) Silva.

A. Thallus

B. External view of cystocarp.

C. Cross-section of main axis
Figure 3: *Gracilaria lemaneiformis* (Bory) Weber-van Bosse.

A. Thallus

B. External view of cystocarp

C. Cross section of main axis
Grows on rocks in the intertidal zone, in clear water exposed to moderate to strong wave and currents during low tide not exposed to air. Found in Cukuh Balak, Lampung and along the coast of Semangka Bay.

4. **Gracilaria salicornia** (C. Agardh) Dawson (Figure 4.B).

Thallus grow creeping or erect from a disc on subtidal shell, or rock, 5-15 cm in length, 3-8 cm broad, cylindrical throughout, very brittle when fresh, light orange to yellow-green in colour; main axes absent or distinct in lower portion. Branches irregular, some strongly constricted throughout, branches at nodes, dichotomously or some trichotomous and some without regular constriction. The basal portion of some thalli are less consciously constricted than those in the upper portion, some branches from root-like discs on the apices of ramuli or on margins, by which fronds attach to the substratum.

Grows on rocks, shell or sand surface in the intertidal zone, clear water, exposed area. During low tide partially exposed to air. Found at Cukug Balak, Lampung and Pameungpeuk West Java, along the coast of the Indian ocean.

5. **Gracilaria sp.** (Figure 5).

Thallus rigid, 4-12 cm tall, compressed, with many orders of short densely branched intervals; branching pattern dichotomy, 0.5-1 mm in diameters, dark red to brownish in colour. Fronds in transverse section consist of large thin walled medulla, 100-250 um in diameter, 1-2 layers of small cortical cells; transition from cortex to medulla abrupt.

Grows on rocks or shell in clear water in the intertidal zone, exposed to wave and currents, during low tide not exposed to air. Found in Cukuh Balak, Lampung and Pameungpeuk West Java along the coast of the Indian ocean.

3. PROCESSING (AGAR YIELD AND QUALITY) OF **GRACILARIA**

As described above, the species targeted for culture must have a high content of good quality agar. Unfortunately, no complete information exists on the agar content of various seaweeds and their properties in Indonesia. There are, however, three publications covering this subject. Harlim (1986), extracted agar from *G. crassa* and *G. eucheumoides* which were collected from the coast of 10 islands of the Spermonde (Table 1).

<table>
<thead>
<tr>
<th>Locations</th>
<th><em>Gracilaria crassa</em></th>
<th><em>G. eucheumoides</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Lae-Lae and Gusung Islands</td>
<td>15%</td>
<td>27%</td>
</tr>
<tr>
<td>Samalona Islands</td>
<td>20%</td>
<td>30%</td>
</tr>
<tr>
<td>Kodemgareng Keke Island</td>
<td>22%</td>
<td>32%</td>
</tr>
</tbody>
</table>

Soegiarto and Soelistijo (1986), reported the organic compositions of *Gracilaria* sp. and *G. confervoides* and the nutritional value of *G. gigas* from Bali Island (Table 2). Finally, Anggadireja (1990) studied the quality of *Gracilaria* spp. from 31 locations (Table 3). In this study, agar yield and quality was analysed from 5 species, namely: *Gracilaria edulis*, *G. lemaneiformis*, *G. salicornia*, *G. eucheumoides* and *Gracilaria* sp.
Figure 4A: *Gracilaria eucheumoides* Harvey.

![Gracilaria eucheumoides](image)

Figure 4B: *Gracilaria salicornia* (C. Agardh) Dawson.

![Gracilaria salicornia](image)
Figure 5: *Gracilaria* sp. (A, B, C show main thalli; D shows a cross section of the main axis.)
Table 2: Organic composition of *Gracilaria* sp., *G. confervoides* and *G. gigas*.

<table>
<thead>
<tr>
<th>Locations</th>
<th><em>Gracilaria</em> spp.</th>
<th><em>G. confervoides</em></th>
<th><em>G. gigas</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>19.01</td>
<td>24.95</td>
<td>12.90</td>
</tr>
<tr>
<td>Protein (6.25 N)</td>
<td>4.17</td>
<td>3.14</td>
<td>7.30</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>42.59</td>
<td>37.52</td>
<td>0.09</td>
</tr>
<tr>
<td>Fats</td>
<td>9.54</td>
<td>0.52</td>
<td>4.94</td>
</tr>
<tr>
<td>Crude Fibre</td>
<td>10.51</td>
<td>9.14</td>
<td>2.50</td>
</tr>
<tr>
<td>Ash</td>
<td>14.18</td>
<td>15.77</td>
<td>12.54</td>
</tr>
<tr>
<td>Thiamin (mg/100 g)</td>
<td>-</td>
<td>-</td>
<td>0.02</td>
</tr>
<tr>
<td>Riboflavin (mg/100 g)</td>
<td>-</td>
<td>-</td>
<td>4.00</td>
</tr>
<tr>
<td>Vitamin C (mg/100 g)</td>
<td>-</td>
<td>-</td>
<td>12.00</td>
</tr>
<tr>
<td>Agar (%)</td>
<td>-</td>
<td>-</td>
<td>47.34</td>
</tr>
<tr>
<td>Minerals, Ca (ppm)</td>
<td>-</td>
<td>-</td>
<td>29.920</td>
</tr>
<tr>
<td>Fe (ppm)</td>
<td>-</td>
<td>-</td>
<td>0.701</td>
</tr>
<tr>
<td>Cu (ppm)</td>
<td>-</td>
<td>-</td>
<td>3.581</td>
</tr>
<tr>
<td>Pb (ppm)</td>
<td>-</td>
<td>-</td>
<td>0.190</td>
</tr>
</tbody>
</table>

Table 3: Variation in the quality of some Indonesian *Gracilaria*.

<table>
<thead>
<tr>
<th>Location</th>
<th>Yield (%)</th>
<th>Gel strength (g/cm²)</th>
<th>Gelling/Melting Temp.(°C)</th>
<th>Dried Product (tonne/yr)</th>
<th>Harvest Season</th>
</tr>
</thead>
<tbody>
<tr>
<td>Teroa, Bali</td>
<td>8.7</td>
<td>170</td>
<td>25/96</td>
<td>8.0</td>
<td>Aug - Jan</td>
</tr>
<tr>
<td>Geger, Bali</td>
<td>9.4</td>
<td>210</td>
<td>29/76</td>
<td>9.5</td>
<td>Aug - Jan</td>
</tr>
<tr>
<td>Paciran, East Java</td>
<td>18.7</td>
<td>880</td>
<td>41/88</td>
<td>80.0 (FA)</td>
<td></td>
</tr>
<tr>
<td>Sekotong, Lombok</td>
<td>19.2</td>
<td>890</td>
<td>40/92</td>
<td>60.0</td>
<td>Jul - Feb</td>
</tr>
<tr>
<td>Lembar, Lombok</td>
<td>17.3</td>
<td>825</td>
<td>39/90</td>
<td>50.0</td>
<td>Jul - Feb</td>
</tr>
<tr>
<td>Lbn. Haji, Lombok</td>
<td>15.4</td>
<td>770</td>
<td>36/87</td>
<td>40.0</td>
<td>Sept - Feb</td>
</tr>
<tr>
<td>Tg. Gontor, Sumbawa</td>
<td>18.2</td>
<td>900</td>
<td>39/91</td>
<td>60.0</td>
<td>Sept-Mar</td>
</tr>
<tr>
<td>Lbn. Lalar, Sumbawa</td>
<td>18.2</td>
<td>920</td>
<td>41/91</td>
<td>120.0</td>
<td>Sept-Feb</td>
</tr>
<tr>
<td>Sumbawa Besar</td>
<td>16.6</td>
<td>740</td>
<td>35/87</td>
<td>40.0</td>
<td>Jun-Feb</td>
</tr>
<tr>
<td>TLSateh, Sumbawa</td>
<td>18.4</td>
<td>690</td>
<td>34/87</td>
<td>60.0</td>
<td>Jun-Feb</td>
</tr>
<tr>
<td>Dompu, Sumbaw</td>
<td>20.6</td>
<td>720</td>
<td>36/88</td>
<td>50.0</td>
<td>Sept - Jim</td>
</tr>
<tr>
<td>Plb.Ratu, West Java</td>
<td>16.9</td>
<td>570</td>
<td>36/88</td>
<td>30.0</td>
<td>Jul - Dec</td>
</tr>
<tr>
<td>Lb. Batten, W. Java</td>
<td>17.1</td>
<td>710</td>
<td>34/83</td>
<td>35.0</td>
<td>Aug - Dec</td>
</tr>
<tr>
<td>Malimping, W. Java</td>
<td>16.7</td>
<td>770</td>
<td>41/87</td>
<td>40.0</td>
<td>Aug - Dec</td>
</tr>
<tr>
<td>Pameungpeuk, W. Java</td>
<td>15.4</td>
<td>480</td>
<td>33/81</td>
<td>35.0</td>
<td>Oct-Mar</td>
</tr>
<tr>
<td>Baron, Yogyakarta</td>
<td>14.9</td>
<td>390</td>
<td>32/81</td>
<td>25.0</td>
<td>Nov - Apr</td>
</tr>
<tr>
<td>Pacitan, East Jaba</td>
<td>15.8</td>
<td>440</td>
<td>33/83</td>
<td>15.0</td>
<td>Jun-Dec</td>
</tr>
<tr>
<td>Warambadi, Sumba</td>
<td>18.2</td>
<td>660</td>
<td>37/87</td>
<td>25.0</td>
<td>Apr - Nov</td>
</tr>
<tr>
<td>P. Sawu</td>
<td>17.1</td>
<td>690</td>
<td>35/89</td>
<td>45.0</td>
<td>Jul-Jan</td>
</tr>
<tr>
<td>P. Besar, Flores</td>
<td>12.3</td>
<td>310</td>
<td>31/82</td>
<td>15.0</td>
<td>Sept - Jan</td>
</tr>
<tr>
<td>Tongga, P. Rote</td>
<td>17.4</td>
<td>720</td>
<td>36/89</td>
<td>40.0</td>
<td>Jul-Jan</td>
</tr>
<tr>
<td>Tg. Pilu, P. Rote</td>
<td>13.3</td>
<td>290</td>
<td>32/86</td>
<td>10.0</td>
<td>Aug - Jan</td>
</tr>
<tr>
<td>Tg. Bunga, Sulawesi</td>
<td>18.1</td>
<td>710</td>
<td>39/89</td>
<td>35.0 (FA)</td>
<td></td>
</tr>
<tr>
<td>Sibatua, Sulawesi</td>
<td>17.3</td>
<td>810</td>
<td>40/91</td>
<td>200.0 (FA)</td>
<td></td>
</tr>
<tr>
<td>Maros, Sulawesi</td>
<td>14.4</td>
<td>510</td>
<td>35/87</td>
<td>20.0</td>
<td>Jul - Dec</td>
</tr>
<tr>
<td>Maumju, Sulawesi</td>
<td>17.5</td>
<td>965</td>
<td>41/92</td>
<td>(FA)</td>
<td></td>
</tr>
<tr>
<td>Takalar, Sulawesi</td>
<td>18.7</td>
<td>1090</td>
<td>40/92</td>
<td>60.0 (FA, WS)</td>
<td></td>
</tr>
<tr>
<td>Bone, Sulawesi</td>
<td>17.1</td>
<td>790</td>
<td>40/88</td>
<td>50.0 (FA, WS)</td>
<td></td>
</tr>
</tbody>
</table>

Notes: Moisture content of all samples less than 20%  
FA= farming area; WS = wild stock;
4.1 Materials and methods

Dried material of seaweeds from fresh specimens were collected from the same 4 locations as the specimens for taxonomic and ecological study. Not less than 1 kg (wet weight) of each species was collected for analysis. The sequence of processes involved in the extraction of agar from seaweed was adopted from Chandrkrachang (1992), meanwhile methods for analysis of agar yield and quality were adopted from Laboratory Methods for Qualification of Red Seaweed and their Polysaccharides (Anon, 1992). Nine properties of seaweeds and seaweed products were recorded from 5 species, namely: wet weight (g), dry weight (g), clean anhydrous weight (CAW), moisture content (%), ash content (%), agar yield (%), gel strength, sulphate content (%) and gelling and melting temperature (°C).

4.2 Results

The quantity and quality of agar varied according to species and the locations of the collected sample, as shown in Table 4.

Table 4: Analytical results of agar quantity and quality.

<table>
<thead>
<tr>
<th>Species Code</th>
<th>SW01</th>
<th>SW02</th>
<th>SW03</th>
<th>SW04</th>
<th>SW05</th>
<th>SW06</th>
<th>SW08</th>
<th>SW09</th>
<th>SW10</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Agar yield</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>and properties (kg/m²)*</td>
<td>0.41</td>
<td>0.65</td>
<td>0.60</td>
<td>0.37</td>
<td>0.36</td>
<td>0.08</td>
<td>0.15</td>
<td>0.12</td>
<td>0.52</td>
</tr>
<tr>
<td><strong>Biomass production (kg)</strong></td>
<td>4.82</td>
<td>5.12</td>
<td>9.75</td>
<td>12.6</td>
<td>22.0</td>
<td>2.5</td>
<td>4.15</td>
<td>4.5</td>
<td>5.1</td>
</tr>
<tr>
<td><strong>Water (%)</strong></td>
<td>12.53</td>
<td>15.74</td>
<td>29.02</td>
<td>11.35</td>
<td>9.99</td>
<td>10.66</td>
<td>35.27</td>
<td>14.0</td>
<td>17.23</td>
</tr>
<tr>
<td><strong>Ash (%)</strong></td>
<td>35.76</td>
<td>24.08</td>
<td>29.90</td>
<td>35.90</td>
<td>47.37</td>
<td>31.87</td>
<td>10.46</td>
<td>18.78</td>
<td>14.51</td>
</tr>
<tr>
<td><strong>Sulphate (%)</strong></td>
<td>0.9</td>
<td>0.15</td>
<td>0.52</td>
<td>0.60</td>
<td>0.84</td>
<td>0.49</td>
<td>0.69</td>
<td>1.04</td>
<td>0.92</td>
</tr>
<tr>
<td><strong>CAW (%)</strong></td>
<td>42.19</td>
<td>63.04</td>
<td>41.61</td>
<td>52.66</td>
<td>37.88</td>
<td>53.18</td>
<td>32.88</td>
<td>69.16</td>
<td>70.36</td>
</tr>
<tr>
<td><strong>Agar Yield (%)</strong></td>
<td>8.13</td>
<td>9.96</td>
<td>13.61</td>
<td>8.18</td>
<td>5.47</td>
<td>16.99</td>
<td>11.07</td>
<td>12.12</td>
<td>15.77</td>
</tr>
<tr>
<td><strong>Gel strength (g/cm²)</strong></td>
<td>610</td>
<td>880</td>
<td>840</td>
<td>780</td>
<td>580</td>
<td>850</td>
<td>625</td>
<td>550</td>
<td>575</td>
</tr>
<tr>
<td><strong>Melting temperature (°C)</strong></td>
<td>68</td>
<td>85</td>
<td>90</td>
<td>86</td>
<td>75</td>
<td>82</td>
<td>72</td>
<td>89</td>
<td>79</td>
</tr>
<tr>
<td><strong>Gelling temperature (°C)</strong></td>
<td>36</td>
<td>34</td>
<td>39</td>
<td>28</td>
<td>32</td>
<td>29</td>
<td>31</td>
<td>28</td>
<td>32</td>
</tr>
</tbody>
</table>

Locations

| G | G | CB | CB | CB | CB | P | P | B |

* wet weight

5. DISCUSSION AND CONCLUSIONS

In addition to determining species, other factors which should be considered in the selection of species for culture include: production of biomass per square meter (Table 4), ecological parameters, and the quantity and quality of agar content. The taxonomic and ecological data recorded, indicated that *Gracilaria edulis* and *G. lemaneiformis* have robust thalli and the highest production of biomass. *G. edulis* is widely distributed in various areas from shallow areas in Grupuk and Bojonegara with sandy-muddy substrates, to unprotected area in Cukuh Balak with coral or rock substrates. *G. lemaneiformis* is only found in Grupuk with sandy-muddy substrates. Other species, have lower biomass production and are only found in unprotected areas with coral or rock substrates.

As shown in Table 4, the quantity and quality of agar from the 5 species collected, varied
according to species and the locations of sample collection. Two species had higher agar contents than the other species, *G. eucheumoides* from Cukuh Balak (16.99%), and *G. edulis* from Bojonegara (15.77%) and Cukuh Balak (13.61%). Results of the analyses of agar properties, indicated that *G. lemaneiformis* from Grupuk had the highest gel strength (880 g/cm²), followed by *G. eucheumoides* from Cukuh Balak (850 g/cm²) and then *G. edulis* from Cukuh Balak (840 g/cm²). Gel strength is one of the factors that is used to determine agar quality (Dawes, 1981).

The utilisation of highly productive species or strains with high quality and quantity of agar is essential for the successful farming of *Gradlaria*. On the basis of the results of this study and Trono's description of the factors that should be considered in the selection of species for culture, the species selected must:

- have a large amount of biomass (indicated by large and robust thalli);
- have a high content of good quality agar; and
- be found in areas characterised by calm water, high nutrient levels and shallow areas with sandy-muddy substrates;

This study concluded that *G. edulis* and *G. lemaneiformis* from Grupuk Lampung Province had good potential for artificial cultivation in pond or field areas, compared with the other species examined.

6. RECOMMENDATIONS

Bearing in mind that *Gradlaria* spp. are economically important seaweeds, the following recommendations are made:

- *Gradlaria edulis* and *Gracilaria lemaneiformis* showed greatest potential for artificial cultivation in both pond or field areas, compared with the other species studied.
- A follow-up study should be held on the taxonomy, ecology and processing of seaweeds, especially *Gracilaria* spp., by the Indonesian Government around Indonesian territories to gather complete and accurate data as a basis for planning and establishing seaweed farms and the seaweed processing industry.

REFERENCES


ABSTRACT

This study was undertaken to determine suitable species of *Gracilaria* for culture in Malaysia. Specimens were collected from five locations throughout the West Coast of Peninsular Malaysia. Physical parameters for each location were noted. The samples were brought back to the laboratory for preparation of the herbarium specimens and analysis of agar yields. Agar extraction was carried out using the hot water technique. The results showed that *Gracilaria changii* has the following desirable characteristics for cultivation: a high yield of good quality agar; abundant in many locations; euryhaline (22-32 ppt); a rapid growth rate; and easily cultured either using vegetative cuttings or spores. It was therefore recommended that *Gracilaria changii* be given the highest priority for culture in Malaysia.

1. INTRODUCTION

Seaweeds, either collected from the wild or cultured, are a very important resource for utilisation. They also play an important role in maintaining the well being of the aquatic environment, especially in buffering the impacts of organic pollution. In addition, seaweeds also contain a number of important compounds such as carrageenan, agar, alginic acid, manitol and iodine, which may be commercially extracted. Seaweeds can be consumed directly as food, or used as an additive in food products and other industrial products, as well as being utilised as fodder for animals.

Research in seaweed farming in Malaysia was initiated with limited success in 1983 (Faazaz, 1986). Since then, research efforts have been focused on the culture of *Gracilaria* in brackishwater ponds in Peninsular Malaysia. On the other hand, *Eucheuma* farming has been proven to be successful and is known to be expanding in East Malaysia.

At present there is no commercial production of *Gracilaria* in Malaysia, however, there is a small scale agar extraction factory (namely Algae Bio Tech) based in Selangor, which uses imported raw materials from Thailand. This factory processes 70 kg of dry *Gracilaria* spp. daily, producing 8 kg agar per day which is sold at RM 72.00/kg to wholesalers. In Sabah, *Eucheuma* spp. farms cover a total area of more than 1,000 hectares with a production of about 100 tonnes (dry weight) of *Eucheuma* spp. per month, which is mostly exported to Sweden. No processing is done in Malaysia.

Agar is imported into Malaysia in 4 main forms, namely: agar strips; bacteriological agar; agar desserts; and flavoured powder mixtures (Jahara and Phang, 1989). It was reported that as much as 90% of the agar imported for food is in the form of agar strips, while the remaining 10% is in the form of powdered or jelly desserts. Malaysia has a large domestic market for seaweed and agar products. In 1988, about RM 6.55 million worth of agar strips were imported into this country (Jahara and Phang 1989). The successful development of seaweed culture would therefore contribute towards foreign exchange earnings.
With an aim of promoting seaweed culture in Malaysia, several objectives have been identified, namely:

i. To obtain fanning technology which is economically viable on a commercial scale; and

ii. To use cultured seaweed in the treatment of effluents from aquaculture activities, especially from shrimp farms.

1 US $ is approximately equal to 2.5 RM

2. ECOLOGY AND TAXONOMY OF GRACILARIA

2.1 Literature review

The first records of marine algal taxonomy from the Asian region were from the collections made during the Pruessische Expedition nach Ost-Asien from 1860-1862 by Eduard von Martens. Since then, many reports have been published on this subject and based on these reports, a preliminary list has been completed for Malaysia (Phang, 1986).

In 1991, a new checklist was published by Phang and Wee. A total of 50 collection sites were recorded and 212 taxa listed. This total included: 5 families, 13 genera and 12 species of Cyanophyta; 13 families, 24 genera and 74 species of Chlorophyta; 19 families, 39 genera and 59 species of Rhodophyta; and 8 families, 13 genera and 46 species of Phaeophyta. Out of these, 25 species are considered common, while information on the remaining 187 is insufficient. Subsequently, a few other publications have been released. These papers listed an additional 7 taxa of Chlorophyta, 18 taxa of Rhodophyta and 5 taxa of Phaeophyta (Phang, 1994). In addition, based on the collections of the Institute of Advanced Studies, University of Malaya, 19 new taxa have been reported.

2.2 Materials and methods

Gracilaria spp. specimens were collected from five locations, namely: at the Middle Bank in Penang (A); Tanjung Dawai (B), Ban Merbok (C) and Teluk Bayu (D) in Kedah and Pulau Carey (E) in Selangor (Figure 1). The samples were brought back to the laboratory in plastic bags for the preparation of the herbarium specimen and agar analysis. Physical factors, such as salinity, turbidity and temperature, were noted at each location.

Each location had its own unique characteristics and a brief description is given below:

A. Middle Bank, Penang. Located between Penang island and the mainland, the lagoon has a sandy loam bottom, is sheltered from strong wind and wave action and is exposed during low tides. Gracilaria spp. was found attached to the hairy roots of seagrass, plastic and othersolid materials in the area.

B. Tanjung Dawai, Kedah. This area forms the estuary of the Merbok River. It is sheltered from wind and wave action and is submerged during low tide. Gracilaria spp. was found attached to the netting materials and other floating substances offish cages.

C. Ban Merbok, Kedah. A shrimp pond with a muddy bottom.

D. Teluk Bayu, Kedah. Upper region of the Merbok river with a brackishwater habitat, sheltered from strong wind and wave action and submerged during low tide. The area is affected by low salinities during the rainy season. Gracilaria spp. was found attached to net cages and floating substances of the fish cages.
Figure 1: Map indicating collection sites A, B, C, D and E.
E. Pulau Carey, Selangor: A mangrove forest, sheltered from strong wind and wave action and exposed during low tide with low light intensity. *Gracilaria* spp. was found attached to the aerial root of mangrove trees or dead branches which have fallen off onto the ground.

2.3 Results

The results of the experiments are summarised in Table 1. The samples collected were identified as *Gradlaria changii*, *Gracilaria fastigiata*, *Gracilaria* sp. 1 and *Hypnea* sp. *Gracilaria* sp. 1, which looked very similar to *G. changii*, was identified as *Gracilaria changii* initially, but after growing this species in the pond, the morphological characteristics were found to be far different. The species was then termed *Gracilaria* sp. 1, but the exact taxonomy of this species needs further confirmation.

All of the species were collected from sheltered and nutrient rich areas with a salinity range of between 22 to 32 ppt. They were found to be dominant in these locations. The sample, MSW 005, which was identified as *Hypnea* sp. and was found growing together with cultured *Gradlaria* sp. in the ponds. It has a very fast growth rate (about 4 times as fast as *Gracilaria* sp.) and was found to grow well even on muddy bottoms.

3. PROCESSING (AGAR YIELD AND QUALITY) OF GRACILARIA SPECIES

3.1 Literature review

Faazaz (1986) reported that *Gracilaria cylindrica* (now *Gracilaria changii*) produced good quality agar which is suitable for agar production. *Gracilaria changii* produced more than 45% agar (Ramli, 1989) without alkali treatment and between 33 to 37.5% with alkali treatment. Kasim and Ismail (1983) found that *Gracilaria changii* yielded 16.4% agar without alkali treatment and 24.5% with pre alkali treatment. Pre alkali treatment was found to increase the gel strength of agar from 547.7 gm/cm² to 844.8 gm/cm².

3.2 Materials and methods

The collected *Gracilaria* spp. samples were cleaned with sea water and weighed. The samples were then dried in the sun and re-weighed. The dried samples were soaked in fresh water for one to two hours and then sun dried. The clean anhydrous weight (CAW) of the samples was then calculated. The samples were kept in plastic bag until it was time for extraction.

A 100 g sample of dried *Gracilaria* spp. was soaked in freshwater for 30 minutes before agar extraction. After dripping, the sample was boiled with 4 litres of freshwater for 1 hour in a stainless steel container. The mixture was screened through 2 layers of cotton and nylon cloth mesh with the aid of coconut presses. The agar solution was then collected in an aluminium tray and kept aside to cool and form a gel. The trays were then kept in the refrigerator for two to three hours to harden the gel, which was then cut into 2-3 cm thick pieces and kept in the deep freeze for another 24 to 48 hours. The gel was exposed to air for thawing and dried with sunlight thereafter. The dried agar was weighed and the percentage yield was calculated.

3.3 Results

The results of this study are summarised in Table 1. Agar yield of *Gracilaria changii* varied from 22.1% to 41.7%. *Gracilaria fastigiata* yielded 15.8% of agar while *Hypnea* sp. yielded 13.5% of carrageenan, using the same procedure for agar extraction (not in acid condition).
Table 1: Data on the taxonomy, ecology and processing of collected seaweeds.

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Species</th>
<th>Locality</th>
<th>Date</th>
<th>Salinity (%)</th>
<th>Turbidity (cm)</th>
<th>Temp. (°C)</th>
<th>Wet/dry</th>
<th>% Agar</th>
<th>Note: Tidal exposure, epiphytes, grazing.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSW 001</td>
<td><em>Gracilaria changii</em></td>
<td>Middle Bank Penang</td>
<td>15.5.92</td>
<td>30</td>
<td>20</td>
<td>28</td>
<td>11.1</td>
<td>41.7</td>
<td>Sheltered, Exposed, <em>Ulva</em> sp. <em>Siganus</em> spp.</td>
</tr>
<tr>
<td>MSW 002</td>
<td><em>G. changii</em></td>
<td>Tg. Dawal, Kedah</td>
<td>28.5.92</td>
<td>32</td>
<td>90</td>
<td>30</td>
<td>15.6</td>
<td>39</td>
<td>Sheltered, Not Exposed <em>Siganus</em> spp.</td>
</tr>
<tr>
<td>MSW 003</td>
<td><em>G. changii</em></td>
<td>Tg. Dawai, Kedah</td>
<td>9.7.92</td>
<td>32</td>
<td>85</td>
<td>29</td>
<td>14.7</td>
<td>35.8</td>
<td>Sheltered, Not Exposed <em>Enteromorpha</em></td>
</tr>
<tr>
<td>MSW 004</td>
<td><em>G. changii</em></td>
<td>Merbok Kedah</td>
<td>9.9.92</td>
<td>28</td>
<td>115</td>
<td>29</td>
<td>16.1</td>
<td>30.5</td>
<td>Sheltered, Not Exposed <em>Enteromorpha</em></td>
</tr>
<tr>
<td>MSW 005</td>
<td><em>Hypnea</em> sp.</td>
<td>Merbok Kedah</td>
<td>9.9.92</td>
<td>28</td>
<td>115</td>
<td>29</td>
<td>39.1</td>
<td>13.5</td>
<td>Sheltered, Not Exposed <em>Enteromorpha</em></td>
</tr>
<tr>
<td>MSW 007</td>
<td><em>G. fastigiata</em></td>
<td>Middle Bank Penang</td>
<td>24.4.93</td>
<td>28</td>
<td>20</td>
<td>28</td>
<td>12.4</td>
<td>15.8</td>
<td>Sheltered, Exposed <em>Ulva</em> sp. <em>Siganus</em> spp.</td>
</tr>
<tr>
<td>MSW 008</td>
<td><em>G. changii</em></td>
<td>Teluk Bayu, Kedah</td>
<td>14.5.93</td>
<td>25</td>
<td>75</td>
<td>30</td>
<td>10.6</td>
<td>25.4</td>
<td>Sheltered, Not Exposed <em>Enteromorpha</em>, <em>Siganus</em> spp</td>
</tr>
<tr>
<td>MSW 009</td>
<td><em>Gracilaria</em> .sp. 1</td>
<td>Pulau Carey Selangor</td>
<td>14.4.94</td>
<td>31</td>
<td>-</td>
<td>30</td>
<td>16.7</td>
<td>20.4</td>
<td>Sheltered, Exposed <em>Enteromorpha</em></td>
</tr>
<tr>
<td>MSW 010</td>
<td><em>Gracilaria</em> .sp. 1</td>
<td>Pulau Carey Selangor</td>
<td>27.7.94</td>
<td>30</td>
<td>-</td>
<td>29</td>
<td>16.4</td>
<td>24.4</td>
<td>Sheltered, Exposed <em>Enteromorpha</em></td>
</tr>
<tr>
<td>MSW 011</td>
<td><em>G. changii</em></td>
<td>Merbok Kedah</td>
<td>17.9.94</td>
<td>22</td>
<td>130</td>
<td>28</td>
<td>16</td>
<td>25.2</td>
<td>Sheltered, Not Exposed <em>Enteromorpha</em></td>
</tr>
<tr>
<td>MSW 012</td>
<td><em>G. changii</em></td>
<td>Merbok Kedah</td>
<td>8.10.94</td>
<td>25</td>
<td>125</td>
<td>29</td>
<td>15.4</td>
<td>22.1</td>
<td>Sheltered, Not Exposed <em>Enteromorpha</em></td>
</tr>
</tbody>
</table>
4. DISCUSSION AND CONCLUSIONS

The percentage yield of agar in *Gradlaria changii* varied with the locality and time of collection, which may be due to differences in environmental factors, such as salinity, turbidity, nutrients, temperature and seasons. Even though the percentage yields of agar varied, the values were still relatively high and economic for seaweed farming.

Agar quality was not analysed due to a lack of the necessary equipment but, based on a study by Kasim and Ismail (1993), this species produced high quality agar. Gel strength in this study was 547.7 g/cm$^2$, which is Grade 1 classification under the Japanese standard for processed agar quality (as quoted by Chandrkrachang and Chinadit, 1988 in Table 2). These authors also reported that its gel strength can be increased to 844.8 g/cm$^2$ if given pre alkali treatment. This would then be classified as special grade agar, based on the same criteria.

**Table 2: Processed agar quality standard set by the Japanese.**

<table>
<thead>
<tr>
<th>Agar Grade</th>
<th>Gel strength (g/cm$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Special Grade Agar</td>
<td>600 or more</td>
</tr>
<tr>
<td>Grade 1</td>
<td>350 or more</td>
</tr>
<tr>
<td>Grade 2</td>
<td>250 or more</td>
</tr>
<tr>
<td>Grade 3</td>
<td>150 or more</td>
</tr>
</tbody>
</table>

*Gracilaria changii* is found in many locations throughout the country, especially in nutrient-rich areas such as fish cage culture sites. This species is also easily grown in shrimp ponds either by using spore or vegetative cuttings as seedstock and has a fast growth rate.

Our preliminary research has shown that *Gracilaria changii* can produce more than 14 tonnes (dry weight)/hectare/year using vegetative cuttings as a seedstock. On the other hand, using spores as seed material, production can be increased to more than 19 tonnes (dry weight)/hectare/year. These studies were performed in brackishwater shrimp ponds and the production figures show that they are economically viable. Based on the above study, *Gracilaria changii* is undoubtedly the seaweed species that should be given the highest priority for culture in Malaysia.

This species has the following desirable characteristics for culture purposes:

- It has high agar yield and quality.
- It is abundant in many locations.
- It is euryhaline (22-32 ppt).
- It has a rapid growth rate.
- It is easily cultured either using vegetative cuttings or spores.

As for the other two alternative species, *Gracilaria fastigiata* and *Hypnea* sp., further studies should be conducted to confirm their culture requirements. During the study, we found several other species, such as *Gracilaria verrucosa*, but the quantity was insufficient for analysis. These species need to be propagated and cultured to increase their biomass for analysis.
5. RECOMMENDATIONS

- Further research should be geared towards producing *Gracilaria* spp. on a commercially viable scale.
- Problems of epiphytes such as *Enteromorpha*, often found in *Gracilaria* spp. culture ponds, must be overcome.
- Methods to overcome or minimise predatory problems (*Siganus* sp.) when *Gracilaria* spp. is cultured in open waters should be devised.
- Small commercial extraction plants should be developed (capacity of 50-200 tonnes dried seaweed per day) for agar production.
- Financial and technical support should be available for purchasing up-to-date equipment for seaweed research.
- There should be support from international experts.
- Funding of support staff would accelerate research projects.

REFERENCES


ABSTRACT
The taxonomic studies identified seven species, namely: *G. verrucosa* (Hudson); *G. edulis* (Gmelin) Silva; *G. crassa* (Harvey) J. Agardh; *G. folifera* (Forsskal) Boergesen; *G. millardetii* (Montagne) J. Agardh; *G. textorii* Suringer and *G. eucheumoides* Harvey. The first six of those listed were studied for agar yield and quality. Four species, *G. crassa*, *G. foliifera*, *G. verrucosa* and *G. edulis* were cultured in a small-scale experimental system. The results of the culture experiment showed *G. edulis* to be the most promising. The study recommends its culture in inland ponds and coastal waters. Identification of the species reported as "verrucosa" is not confirmed.

1. INTRODUCTION
The abundant seaweeds in Myanmar are considered an important natural resource. It has been reported that at least 35 species of alginophyte, 19 species of carageenophyte, 7 species of agarophyte and altogether 162 species of different kinds of seaweeds occur throughout the coastal areas of Myanmar. Amongst those species, some economic species have been utilised by people for many years. Red seaweeds, such as *Hypnea* spp., *Catenella* spp., *Solieria* spp. and *Gracilaria* spp. are well known in the market as a raw material for the production of phycocolloid. A great deal of research and experimental work has been carried out by Myanmar researchers to exploit and develop seaweed utilisation. In 1969 and the 1970s, coastal surveys, cultivation practices and extraction work on seaweeds was carried out.

According to taxonomic studies on the genus *Gracilaria*, seven species of *Gracilaria* have been identified in Myanmar. Since 1982, four out of the seven species have been tested for agar-extraction. In the present study, a total of six species of *Gracilaria* were studied for agar processing potential, emphasising the agar yield and quality of the different species of *Gracilaria* in Myanmar.

Eight species of useful seaweeds were experimentally cultured including four species of *Gracilaria*: *G. crassa*; *G. folifera*; *G. verrucosa*; and *G. edulis*. The Myanmar government is paying special attention to cultivating *Gracilaria* at the laboratory scale, with the intention of developing it to commercial scale. Important results and valuable experience have been obtained, some of which are reported in this paper. Previous research has produced useful results for the culture of *Gracilaria*, but all of these projects are still at the small scale research level.

2. TAXONOMIC STUDIES AND SOME ECOLOGICAL NOTES ON SEVEN SPECIES OF GRACILARIA IN MYANMAR

2.1 Background and current status
Common and widespread species of *Gracilaria* along the coastal waters of Myanmar
According to more detailed taxonomic studies on the genus Gracilaria (Hla Hla Cho, 1975; Kyi Shwe and Aung Myint, 1991), seven species have been identified:

1. *Gracilaria verrucosa* (Hudson) Papenfuss
2. *G. edulis* (Gmelin) Silva
3. *G. crassa* (Harvey) J. Agardh
4. *G. foliifera* (Forsskal) Boergesen
5. *G. millardetii* (Montagne) J. Agardh
6. *G. textorii* Suringar
7. *G. eucheumoides* Harvey

In this study, the above seven species of *Gracilaria* are referred to as a part of post-workshop activities under NACA-FAO/ Government Programme. The taxonomic studies and some ecological notes are also presented.

### 2.2 Materials and methods

The material used for detailed study was collected from intertidal regions (including drift) of the Rakhine and Taninthari coast including the Mergui Archipelago in Myanmar. After field collection, the materials were first preserved in 10% formalin in seawater, then either kept in formalin and seawater or 70% alcohol with glycerine and mounted on the herbarium sheets. The specimens were kept in the Phycological Herbarium of the Marine Science Department, Mawlamyaing University, Mawlamyaing and Applied Chemistry Research Department, Myanmar Scientific and Technological Research Department (MSTRD) Yangon, Myanmar. Distribution areas are given only for those species whose full herbarium records have been obtained.

Dried herbarium materials were found to be more suitable for cutting sections to prepare microscope slides. A fragment of the material was sectioned (25-50 um thick) under a dissecting microscope using a double edged razor blade. A fragment of the material was put on a piece of white paper and pressed lightly with a microscope slide as a guide for the razor blade. The object parallel to the end of the guide slide which was pressed gently with the left hand was cut transversely in continuous series. The flattened materials were more suitable for cutting longitudinal sections. The sections were stained
with 1% aqueous aniline blue, acidified by adding one to two drops of 0.1 N hydrochloric acid and washed with water. The excess water from the materials was drawn off with a piece of blotting paper from one side and 20%-50% Karo (corn syrup) with phenol was applied in series from the opposite side of the cover glass.

2.3 Summary

Seven species of *Gracilaria*, namely: *G. verrucosa*, *G. edulis*, *G. crassa*, *G. foliifera*, *G. millardetii*, *G. textorii* and *G. eucheumoides* are reported in the present study (Figures 1-9).

The form of the thalli varied being either cylindrical, compressed or flattened. *G. verrucosa* and *G. edulis* have cylindrical branches, *G. foliifera* is compressed or flattened and *G. millardetii*, *G. textorii* and *G. eucheumoides* are flattened throughout. A wide range of morphological variations were observed within the different species or even in a single species. Although the form of the plants differ greatly from one another, the anatomy of the thallus, shape of spermatangial cavities and the structure of the cystocarps are considered determining factors to differentiate one species from another.

The sexual plants are dioecious. Three forms of spermatangial cavities were observed. The depressions occur in *G. textorii*, cup-like cavities in *G. verrucosa* and deep conceptacles in *G. edulis*, *G. crassa*, *G. foliifera* and *G. millardetii*. Sex organs were not found in *G. eucheumoides*.

Two-celled carpogonial branch projects singly and outwardly. The supporting cell produces two to four vegetative filaments each with 1-3 cells. The fertilised carpogonium fuses with the adjacent cells forming a fusion cell. The fusion cell cuts off several gonimoblast initial cells outwardly and further developed into gonimoblast parenchyma. These produce the short-celled gonimoblast filaments. The peripheral layer of these cells transform into chains of carposporangia each with a conspicuous central body.

The gonimoblast parenchyma cells are small or large having dense or less protoplasmic contents and may or may not produce nutritive filaments. Cystocarps are globose or dome-shaped, rostrate or non-rostrate, ostiolate or non-ostiolate and scattered or aggregated on all sides of the cylindrical species or on surfaces of the flattened species. The cruciate tetrasporangia are scattered and sunken in the cortex surrounded by unmodified or modified cortex.

In some parts of the Myanmar coastline, economically important agarophytes occur abundantly, especially on the Rakhine coastal region. The plants studied so far do not necessarily represent all the species of the genus *Gracilaria* of the coastal regions of Myanmar. It is possible that more species may be discovered with further exploration in different localities at different times. The present study may not be sufficient to give a comprehensive account on the ecological notes on each species. Therefore further study on the species of *Gracilaria* in Myanmar will be required.
Figure 1: *Gracilaria verrucosa* (Hudson) Papenfuss. Cystocarpic plant showing very irregular and luxuriant branching.

Figure 2: *Gracilaria edulis* (Gmelin) Silva. Tetrasporic plants showing numerous curved, short branchlets spreading through the branches.
Figure 3: *Gracilaria edulis* (Gmelin) Silva.
Cystocarpic plant showing numerous short branchlets spreading through the branches.

Figure 4: *Gracilaria crassa* (Harvey) J. Agardh.
- A Male plant showing the irregular constrictions of some branches.
- B Tetrasporic plant showing the swollen part of the branches.
- C Cystocarpic plant showing the unconstricted thallus.
Figure 5: *Gracilaria foliifera* (Forsskal) Boergesen. Tetrasporic thalli showing the fastigiate branches and a few marginal proliferations at the upper parts of the branches.

Figure 6: *Gracilaria foliifera* (Forsskal) Boergesen. Cystocarpic plant showing subterete branches throughout the thallus except close to the base of the plant.
Figure 7:  *Gracilaria millardetii* (Montagne) J. Agardh.
A. Habit of sterile plants.
B. Cystocarpic plant showing cylindrical stripe near the base of the plant.

Figures 8:  *Gracilaria textorii* (Suringar) J. Agardh.
A. Cystocarpic plants of broad thalli showing proliferations.
B. Tetrasporic plants of narrow thalli with a few proliferations.
C. Broad thalli showing entire margins.
3. PROCESSING (AGAR YIELD AND QUALITY) OF GRACILARIA IN MYANMAR

3.1 Background and current status

Studies on the availability of agarophytes in Myanmar, methods of extraction, yield and the quality (gel strength, gelling and melting temperatures) of agar obtained have been carried out by the Department of Marine Science, Mawlamyaing University and the Central Research Organisation. Recently collected seaweeds were processed and the agar yield and quality were studied so that the results could be compared with those of previous studies. Altogether six species of *Gracilaria* were collected and their respective agar yield and quality was determined. A few connections between yield, quality and ecological notes were established. The results will partially fulfil the requirements of NACA's post-workshop activities and provide some data to the Myanmar Scientific and Technological Research Department for their objective of progressing to developmental research for agar production at an economic scale.

3.2 Materials and methods

i. Extraction of agar

100 g of sun-bleached *Gracilaria* spp. seaweed was washed with water and put into a 2.5 l stainless steel beaker adding 1 litre of 2-6% NaOH solution. After heating at 90°C for 1.5 hrs the contents were filtered and washed with water and several ml of 0.1 mol/L HCl was added to neutralise the excess of alkali. After filtering and washing, the seaweeds were then extracted with water at 100°C for 2 hours, which was finally filtered. The filtrate was transferred into a tray and allowed to congeal at room temperature. The gel was cut to short sticks and put into a refrigerator to freeze at about -10 °C for one day. The frozen gels were thawed with tap water and then sun-dried.
ii. **Determination of agar yield**

1-2 g. of dry seaweed and agar product were weighed separately and dried in an oven at 105°C for 2 hrs. After cooling in a desiccator the agar yield was calculated.

iii **Determination of moisture content**

2.5 g of seaweed powder or agar was weighed in a weighed bottle and dried in an oven at 105°C for 2 hrs. After cooling it was weighed and the moisture content was calculated.

iv. **Determination of ash content**

A crucible was treated with 3 mol/L HC1 solution and washed with water and then dried in a furnace at 500°C for 1/2ahr. The crucible was weighed after cooling in a desiccatcor. 2-5 g of seaweeds or agar were weighed into the weighed crucible and calcined in the furnace of 500 °C for 4 hrs. If it remained dark, 1 ml. of concentrated HNO₃ was added after cooling and further calcined until combustion was complete. The ash content was then calculated.

v. **Determination of gel strength of agar**

a) **Preparation of 1.5 % solution of agar:**

1.5 g of agar was weighed and put into a 250 ml beaker and 100 ml of water was added. The agar was soaked for two hours at room temperature and then heated (while covering the beaker) until it dissolved completely.

b) **Determination of gel strength with arm-balance type gel tester**

Twenty five (25 ml) of 1.5% agar solution was poured separately into four 50 ml beakers. After gelling at room temperature for at least two hours, the beaker with gel sample was placed on one hand of an arm-balance type gel tester, and a beaker with water was placed on the opposite balance arm. Adjustment was made by adding or subtracting water so as to have the gel and water beakers in an exactly balanced position. After zero setting the arm-balance, the stainless steel surface (1 cm x 1 cm) was placed on the surface of agar sample just to touch the uppermost layer of the gel and firmly clamped. Water was filled into the opposite beaker using a burette. The burette reading was equivalent to the weight in grams forced to the surface of the agar sample. The addition of water stopped as soon as the initial crack of agar was observed and the volume of water in the burette or weight in grams gives the gel strength in gm/cm².

vi. **Determination of gelling temperature of agar**

50 ml of 1.5% solution of agar is poured into a test tube (2.5 x 20 cm) to 10 cm height. A thermometer was inserted through a rubber stopper into the solution and the temperature was set at room temperature to congeal the solution slowly. At around 40 °c, the test tube was inclined to a 45° angle and the temperature when the surface of the solution stops moving, was observed.

vii. **Determination of melting temperature of agar**

The test-tube used above was put in cold water allowing it to congeal completely, and then heated in a water bath. At the same time a stainless steel ball (0.3 mm) was dropped down through another hole in the stopper onto the gel surface. The temperature that the ball dropped down through the solution was observed.
viii  Determination of the sulphate content of agar

A weighed sample (2-3 g) and 25 ml of concentrated nitric acid were boiled gently in a 400 ml tall-form beaker covered with a watch glass. When the evolution of brown fumes has ceased, 1 g of potassium chlorate was added in portions. If the residue was not white, the oxidation was repeated. Concentrated HC1 (25 ml) was then added and the solution evaporated to dryness to remove the last traces of nitric acid.

The residue was dissolved in water and filtered through paper (No. 40, 11 cm, ashless). The filtrate was diluted to 200 ml. Two drops of concentrated HC1 were added and the solution was heated to boiling in a covered beaker. 10 ml of 10% barium chloride solution was then added dropwise with stirring. The boiling was continued for a few minutes and the solution was kept in hot water for 6 hours. The precipitate of barium sulphate was filtered on a weighed porcelain filter crucible. The latter was heated 1 hour at 800°C, cooled in a dessicator and weighed.

It was essential to remove all nitric acid in the above procedure as barium nitrate may be precipitated with barium sulphate giving a high value. Any tendency of barium chloride to co-precipitate can be avoided by its slow addition to the hot solution. Chloric acid is decomposed during the digestion with HC1.

ix  Infrared spectroscopic analysis of agar

4-6 mg agar was weighed into a test tube and 2 ml of water was added. It was then heated to dissolve the colloid (agar). An appropriate amount of solution was transferred to a plastic cover and dried at 40-50°C to allow a film to form, this was then subject to Infra red spectrometric identification.

3.3 Results

Six species of Gracilartia in Myanmar were collected, processed and analysed for agar yield and quality. For the analysis, the laboratory methods for red seaweeds and their polysaccharides, recommended and provided by NACA, were mostly applied. Due to the lack of proper equipment and apparatus, in some cases (e.g. determination of gel strength or sulphate contents) other standardised procedures had to be employed.

The data in Table 1 shows a wide range of variations in results. These variations depend predominantly on the age or maturity and the degree of development of the seaweed. The results, however, do not indicate what species of Gracilartia gives the best results for agar production, all of the species gave agar of fair quality for food grade.

Table 1: Yield and quality of agar extracted from some Gracilaria species in Myanmar.

<table>
<thead>
<tr>
<th>No.</th>
<th>Gracilaria sp.</th>
<th>Agar Yield (%)</th>
<th>1.5% solution of agar</th>
<th>Sulphate content (%)</th>
<th>Ash Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Gel Temp (°C)</td>
<td>Melt Temp (°C)</td>
<td>Gel Strength gm/cm</td>
</tr>
<tr>
<td>1</td>
<td>G. verrucosa</td>
<td>9-22</td>
<td>27-40</td>
<td>52-70</td>
<td>62 - 120</td>
</tr>
<tr>
<td>2</td>
<td>G. edulis</td>
<td>10-22</td>
<td>27-39</td>
<td>52-73</td>
<td>60 - 120</td>
</tr>
<tr>
<td>3</td>
<td>G. crassa</td>
<td>12-21</td>
<td>28-40</td>
<td>55-73</td>
<td>70 - 120</td>
</tr>
<tr>
<td>4</td>
<td>G. foliifera</td>
<td>11-19</td>
<td>28-39</td>
<td>50-82</td>
<td>60-98</td>
</tr>
<tr>
<td>5</td>
<td>G. millardetii</td>
<td>10-19</td>
<td>29-39</td>
<td>50-80</td>
<td>60-92</td>
</tr>
<tr>
<td>6</td>
<td>G. textorii</td>
<td>11-19</td>
<td>27-39</td>
<td>52-73</td>
<td>60-110</td>
</tr>
</tbody>
</table>
4. EXPERIENCE OF GRACILARIA CULTURE IN MYANMAR (Figures 10-12)

Eight species of useful seaweeds were experimentally cultured including four species of *Gracilaria*: *G. edulis*; *G. verrucosa*; *G. crassa*; and *G. foliifera*. Basically, constant depth or constant level methods were applied depending on the species to be farmed. Different artificial substrata were used except in broadcasting methods, which was done in ponds. Ropes and bamboo were mostly used to form artificial substrata, constructed in a way that takes into consideration the optimum requirements of the plants.

Some seaweeds were cultured experimentally in the intertidal area, tidal creeks, the open sea and in ponds. Seed stocks used were selected from natural stocks. The initial plants should be free from weeds and unhealthy signs. The luxuriant strain with fresh and healthy thallus were the most suitable initial seed stocks used. Various kinds of culture methods including different systems constructed to hold the artificial substrata, were devised considering the following factors:

- Readily available materials were used in construction;
- Easily manipulated structures were designed;
- Environmental requirements of the cultured species were considered;
- The seaweed should be easily planted, weeded and harvested;
- High durability in seawater; and
- Lowest investment cost.

Large amounts of spore producing plants of *G. edulis* and *G. verrucosa* were obtained while these species were growing. *G. edulis* had shown good potential for culture. There were obvious changes into sexual plants and spores were produced which settled and germinated on the culture bed. The germlings grew rapidly to harvestable size. Three main problems were found to be the principal constraints to successful farming: seed availability; grazing by fish; and weeds and pests.

The culture experiments were carried out according to the following timescale:

- Construction and preparation of culture beds : November to January
- Planting, fixing the initial plants to the nets : January to February
- Weeding : February to March, (regular work)
- Harvesting: (March to September)
Figure 10: Myanmar coastline showing the distribution of different species of *Gracilaria* observed in the present study.
Farming season for *G. edulis* at the Maung-shwe-lay site was as follows:

- **February:** Start of growth period.
- **March to September:** Growing period for production.
- **September to November:** Deteriorating period.
- **November to February:** Resting period.

*G. edulis* grows through 8 months of the year on culture beds. The plants can be harvested once a month from April to September. The results show that the cultivation of *G. edulis* has commercial potential. Data collected from this experiment are shown in Table 2.
Table 2: Annual harvest of *G. edulis* from Maung-shwe-lay seaweed farming site.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Year</th>
<th>No of net line system</th>
<th>Wetwt (kg)</th>
<th>Drywt (kg)</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1980</td>
<td>25</td>
<td>243</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1981</td>
<td>40</td>
<td>567</td>
<td>81</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1982</td>
<td>50</td>
<td>2,835</td>
<td>405</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1983</td>
<td>50</td>
<td>3,965</td>
<td>567</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1984</td>
<td>90</td>
<td>1,134</td>
<td>167</td>
<td>Damage by storm</td>
</tr>
<tr>
<td>6</td>
<td>1985</td>
<td>90</td>
<td>2,268</td>
<td>324</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>1986</td>
<td>90</td>
<td>2,438</td>
<td>348</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>13,450</strong></td>
<td><strong>1,926</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 12: Some records of *G. edulis* culture experiments at Maung-shwe-lay, Rakhine Coast, Myanmar.

A. Initial plants on culture nets.
B. 30 day old plants on culture nets.
C. 80 day old plants on culture nets hauled up from the sea for management.
D. Maximum size of a farmed plant (80 days old).
E. Young plants from the germlings.
F. 80 day old plants from germlings on culture nets.
Our experiences in the experimental culture of the seaweeds had resulted in the following important considerations:

- The simplest design or system is usually the most appropriate one;
- Locally made large fibre-glass balls are available to use as floats on calm sea surface with wave action and currents;
- Single floating lines are the most suitable system to cultivate *G. edulis* in the open sea area;
- Sequential work activities have to be followed and carried out as scheduled;
- Knowledge on the biology of species such as scientific names, growing nature (habit and habitat) and ecological relationship are essential to successful cultivation;
- The local inhabitants must be convinced on the feasibility of farming. They should also be organised into a farmers co-operative;
- Problems of harvesting and drying the farm produce (seaweed) may become one of the problems for operation of commercial farms; and
- Seaweed farming projects for commercial scale culture is a long term activity.

5. DISCUSSION AND CONCLUSION

Detailed taxonomic studies on *Gracilaria* spp. in Myanmar and agar-extraction research work have shown some economic potential. Our research data show that all of the *Gracilaria* species gave a fair yield and quality as food grade agar.

*Gracilaria* species are one of the most common species of seaweeds in Myanmar. The economically important species, such as *G. vermicosa* and *G. edulis* are, however, restricted to estuarine areas. Best development of those two species occurs in the salinity range of 20 to 30 ppt and a temperature between 18 and 28°C. *G. foliifera* is characteristically different and thrives in a typically marine environment. Experience of *G. edulis* culture from 1978 to 1987 showed that maximum thallus development occurred during the month of February to September every year. The following conclusions may be formulated from our studies of *G. edulis*:

1. It has been noted that *G. edulis* is the most common species whose thallus development is best compared with other species. Because of this dominant characteristic, it can be concluded that Myanmar coastal waters are naturally suited for this species.
2. Growing habit is commonly restricted at the well-protruded tissues and it grows luxuriantly on small holdfast which attaches to any kind of hard substrate. It is important to consider the problem of site and selection of artificial substrata for use in farming.
3. The results of our experiments showed that *G. edulis* can grow successfully and its potential for large scale production is high. The results also showed that two different methods are applicable, inland pond culture and coastal open sea culture.
6. **RECOMMENDATIONS**

The objective of this study was to evaluate and pinpoint the suitability of specific species of *Gracilaria* for culture in Myanmar. Based on the study results, Myanmar will concentrate on studies relating to the species *Gracilaria edulis* as a post workshop activity.

The phycocolloid industry in Myanmar has been practised since 1974. Successful laboratory extraction in 1970 led to the implementation of commercial scale phycocolloid industries. This extraction technology was first exploited by the Government and later many private cottage industries began commercial operation. It partially fulfils the country's need for phycocolloid products.

Much experience has been gained by our previous research and farming experiments on *G. edulis* therefore problems and difficulties which will inevitably occur in culture may be anticipated and avoided. The following are a few points which should be considered when further production and expansion of seaweed culture are planned:

- There should be more co-operation and collaboration between people concerned with seaweed cultivation and agar production to develop a processing industry;
- Raw material procurement and processing of the seaweeds must be synchronised properly to gain maximum profit and reduce the cost of production. A continuous flow of well-grown harvested material to the processing plant site should be guaranteed and planned accordingly;
- Priority should be given to research work for commercial production;
- Budget allocation or investment should be sufficient to meet the goals of the project;
- For open sea culture, a well-planned and functioning hatchery and nursery pond for spore collection and seed stock should be established;
- For pond culture, our experience indicates that polyculture or mixed culture should be practised. Mixed culture with seaweed and prawn (*Penaeus monodon*) or brineshrimp (*Artemia spp.*), in coastal ponds have been getting a lot of attention in Myanmar recently;
- Agar processing from seasonally harvested farmed seaweeds should be carried out to determine which species gives an agar product of good quality with high yield;
- To correlate the most productive season and agar quality in order to obtain optimum conditions from an economic standpoint, seasonal crops should be systematically harvested and processed under constant parameters.

To upgrade agar quality, more research work on processing techniques and purification of products should be carried out. Furthermore, research work on advanced culture techniques and crossbreeding experiments leading to cultivating a high yield crop for economic size farming experiments should be planned. For that purpose, proper equipment, well-trained personnel as well as other supporting materials should be provided. This research work should be performed at a suitable coastal area by a group which must have support and co-operation from various sources within the country as well as abroad.
ACKNOWLEDGEMENTS

First and foremost the authors would like to express their gratitude to NACA-FAO Government Cooperative Programme Authorities who partially fulfil the development of Asia-Pacific region including Myanmar. Secondly to the Directorate General, Deputy Director General and superior departmental officials of Myanmar Scientific and Technological Research Department (MSTRD) who always encourage me to carry out this research work on the utilisation of seaweeds in Myanmar. Thirdly to U Aung Myint (Marine Biologist, IFE) and to U Soe Tun (Manager, DOF) for their intensive discussions and suggestions concerning seaweed culture experiences. Lastly to my commemorate researchers in Maung-shwe-lay farm and Applied Chemistry Research Department of MSTRD, particularly to Daw Mo Mo Than, Daw Mi Mi Soe and U Than Soe, for their intensive participation.

REFERENCES


ABSTRACT

This paper (Part I) discusses the taxonomy and ecology of 11 Gracilaria species, namely: G. arcuata; G. changii; G. eucheumoides; G. fastigiata; G. firma; G. gigas; G. heteroclada; G. lemaneiformis; G. manilaensis; G. salicorin; and G. tenuistipitata, identified from the materials collected in Philippine waters between June 1992 and December 1994. Collecting sites include several areas in eight provinces of the Philippines, namely: Cagayan, La Union, Cavitc and Sorsogon in Luzon Island; Aklan, Samar and Bohol in the Visayas region; and Sulu (Tawi-Tawi and Sitankai) in Southern Mindanao.

Of the 11 species reported, five (G. changii, G. fastigiata, G. firma, G. heteroclada and G. tenuistipitata) have been identified as potential species for culture or farming purposes, based on the quality of their phycocolloid as well as number of ecological considerations. Of these five, G. firma, G. heteroclada and G. tenuistipitata were considered priority culture species based primarily on their wide distribution in the country. This paper also includes a discussion of the present status of the country's seaweed industry and a brief review of previous related studies on Philippine Gracilaria spp.

1. INTRODUCTION

Seaweeds constitute the Philippines' third largest fishery export (after tuna and shrimp), contributing to national economy in terms of foreign exchange, as well as domestic revenue and employment. Seaweeds contribute 41% to overall aquaculture production, thereby topping the list of major cultured species; and 90% of the country's total mariculture production. The Philippines is the leading producer of raw (dried) Eucheuma spp. and semi-refined carrageenan, also called Philippine Natural Grade (PNG) carrageenan, contributing 70% of the world's supply. The country still ranks fourth among the largest seaweed-producing countries in the world and second (after Japan) in the production of red seaweeds.

One of the world's most important agarophytes is Gracilaria spp. which is also highly utilised (at least for the commercial species) in the Philippines. Although ranking only third in importance (after Eucheuma spp. and Caulerpa spp.) in terms of production, it has more potential than Caulerpa spp. in terms of utilisation or application. The utilisation of Gracilaria spp. is not limited to human food or animal feed. The seaweed can also be dried for export or domestic markets, or used as a raw material for local agar production. Agar is very popular in the Philippine market as processed agar bars, locally called "gulaman".

1.1 Statistics of production and value of seaweeds

The trend in seaweed production in the Philippines is generally still increasing. Table 1 shows the annual production data on cultured seaweeds (mainly Kappaphycus sp. and Eucheuma sp.) which account for 99.5% of the country's total seaweed production. The
remaining 0.5 % comes from the gathering of wild or natural stocks.

Table 1: Five-year production data on cultured seaweeds in the Philippines, 1989-1993.  
(Source: BAS, Fishery Statistics, 1993.)

<table>
<thead>
<tr>
<th>Year</th>
<th>Quantity (mt)</th>
<th>Value ('000 Peso$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1989</td>
<td>268,701</td>
<td>604,578</td>
</tr>
<tr>
<td>1990</td>
<td>291,176</td>
<td>796,077</td>
</tr>
<tr>
<td>1991</td>
<td>283,783</td>
<td>1,039,771</td>
</tr>
<tr>
<td>1992</td>
<td>349,505</td>
<td>1,526,896</td>
</tr>
<tr>
<td>1993</td>
<td>380,010</td>
<td>1,544,288</td>
</tr>
</tbody>
</table>

2 1 US $ = 25 Pesos approximately

The volume of seaweeds produced in 1989 increased in 1990 by 8.36% and the value by 31.67%. However, in 1991 seaweed production declined in volume by 2.5%, but increased in value by 30.6%. In 1992, there was a remarkable increase in production, both in quantity (23.2%) and value (46.85%). In 1993, there was again an increase in the volume (8.72%) and value (1.14%) of seaweed production.

The volume of Gracilaria spp. gathered from the wild stocks has fluctuated annually (Table 2), but the value of production has maintained an increasing trend.


<table>
<thead>
<tr>
<th>Year</th>
<th>Quantity (tonnes)</th>
<th>Value ('000 Pesos)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1983</td>
<td>371</td>
<td>-</td>
</tr>
<tr>
<td>1984</td>
<td>415</td>
<td>-</td>
</tr>
<tr>
<td>1985</td>
<td>655</td>
<td>-</td>
</tr>
<tr>
<td>1986</td>
<td>728</td>
<td>-</td>
</tr>
<tr>
<td>1987</td>
<td>434</td>
<td>-</td>
</tr>
<tr>
<td>1988</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1989</td>
<td>398</td>
<td>1,218</td>
</tr>
<tr>
<td>1990</td>
<td>359</td>
<td>1,865</td>
</tr>
<tr>
<td>1991</td>
<td>452</td>
<td>2,671</td>
</tr>
</tbody>
</table>

Production volume dropped by 9.8% in 1990 from the previous year's level. However, the value of production increased by 53.1%. In 1991, there was a remarkable increase of 25.9% in the quantity of Gracilaria spp. produced as well as the value, which increased by 43.2%. The largest volume of gathered Gracilaria spp. seaweeds came from the Southern Tagalog and the Central Visayas regions.

Dried Gracilaria spp. costs from three to five pesos per kilo, depending on the species and quality of the product. The retail value of processed Gracilaria bars or "gulaman" is Pesos 6.50-7.50 per bar (approximately weighing five grams) or Pesos 1,300-1,500 per kilogram (Guanio, 1993).

1.3 Local consumption, export and import

About 99% of total seaweed production is absorbed by local processors and export traders while only 1% is consumed locally as food. It is estimated that 75 % to 97 % of seaweed production is exported as dried seaweeds. The remaining 3 % to 25 % is utilised by local processors. Seaweed exports have been declining since 1990, as
shown in Table 3.

Table 3: Export data on dried seaweeds.

<table>
<thead>
<tr>
<th>Year</th>
<th>Quantity (tonnes)</th>
<th>Value ('000 Pesos)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1990</td>
<td>35,346</td>
<td>1,192,331</td>
</tr>
<tr>
<td>1991</td>
<td>26,830</td>
<td>581,950</td>
</tr>
<tr>
<td>1992</td>
<td>22,756</td>
<td>480,398</td>
</tr>
</tbody>
</table>

Denmark has absorbed most of the country's seaweed exports, about 26% to 33% of the total volume. Many other countries like France, the United Kingdom, U.S.A. and Korea also absorbed larger shares (11-17 %) compared to the other importing countries. The country's imports of dried seaweeds are minimal and declining, as shown in Table 4.

Table 4: Import of dried seaweeds to the Philippines.

<table>
<thead>
<tr>
<th>Year</th>
<th>Quantity (tonnes)</th>
<th>Value ('000 Pesos)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1989</td>
<td>723</td>
<td>1,297</td>
</tr>
<tr>
<td>1990</td>
<td>473</td>
<td>923</td>
</tr>
<tr>
<td>1991</td>
<td>143</td>
<td>377</td>
</tr>
</tbody>
</table>

The largest volume of imported dried seaweeds was supplied by Indonesia. In 1991, import from this country abruptly decreased as the Philippines started to import dried seaweeds from Taiwan.

2. GRACILARIA OF THE PHILIPPINES

2.1 Literature review

Among the first of the early reports on Philippine *Gracilaria* spp. (citing Abbott, 1994) were those of Montagne (1844) of *G. confervoides*, and Dickie (1876) of *G. dactyloides* and *G. eucheumoides* from the Challenger Expedition specimens. The report of Abbott (1985) which included 17 species and one variety, and that of Silva *et al.* (1987) with 23 species, one variety and one form, represent compilations of species from earlier literature on Philippine *Gracilaria* spp.


The literature review revealed that there have been many studies already conducted on Philippine *Gracilaria*, however, the identification of *Gracilaria* species has not been established until now. New names have been added and old names have been renamed so confusion exists in the recognition of species. Moreover, according to Yamamoto and Trono (1994), several of the species reported probably have not been identified correctly. Abbott (1994), stressed mat nearly all the *Gracilaria* spp. reported for the Philippines require a critical re-examination.
2.2 Materials and methods

Materials were collected from the wild or natural stocks of *Gracilaria* spp. seaweeds from eight different areas in the Philippines, namely: Cagayan, La Union, Cavite and Sorsogon in Luzon Island; Aklan in Panay Island in the Western Visayas region; Samar in the Eastern Visayas; Bohol in the Central Visayas; and Tawi-Tawi, Sulu Province, in Southern Mindanao (Figure 1). Once only collection of materials was done in most of these areas, except in Sorsogon and Samar.

Collection of samples and ecological data gathering, between June 1992 and December 1993, was done in Sorsogon, the site of the FAO/UNDP Seaweed Production Development Project (PHI/89/004) being implemented by the Bureau of Fisheries and Aquatic Resources (BFAR). The following areas were covered: Bagacay, Gubat; Layog, Barcelona; and Dancalan, Bulusan, along the eastern coast of Sorsogon Province; and Tughan, Juban, along Sorsogon Bay, designated as Stations 1 to 4, respectively, including Casiguran, Castilla, Prieto Diaz and Caditaan in Magallanes (Figure 2) which were also visited during the monitoring period.

In Western Samar, the site of last year's seaweed resources assessment project of the BFAR, collection of *Gracilaria* samples and ecological data gathering was carried out monthly, starting February until December 1994, in three adjoining bays, namely: Maqueda Bay, Villareal Bay and Laguinit Bay. Five stations were occupied in Maqueda Bay (San Sebastian, Hitaasan, Barubaybay, Pinabacdao and San Isidro), three stations in Villareal Bay (Mayongpayong Pt, San Rafael and Mahayag) and only one station in Laguinit Bay (San Roque) (Figure 3).

**Figure 1:** Philippine map showing the areas (provinces) from where the *Gracilaria* spp. materials were collected.
Figure 2: Map of Sorsogon Province showing the stations occupied for sampling/monitoring activities.
Figure 3: Map of Samar (Philippines) showing the location of the stations used for sampling activities in Maqueda Bay, Villareal Bay and Laguinit Bay.
Collected materials were sorted by species. A few of these were segregated as specimens for the herbarium collection and taxonomic studies; the rest were dried for processing and analysis. Sections were made to aid further taxonomic analysis.

2.3 Description of the species

Eleven species of *Gracilaria* identified from the materials collected are described here, with reference to previous studies particularly those of Trono (1993).

1. *Gracilaria arcuata* Zanardini (Figure 4).

Plants cartilaginous and greenish to purplish in colour. Thallus erect; branches terete or cylindrical throughout and slightly constricted at the base. Branching at the primary and secondary axes generally secund. Main branches arcuate, distal portion of branches cervicorn, ultimate branchlets coarse spinose.

Cortex consists of one to two layers of round to oval cells. Cell size transition from cortex to medulla very abrupt. Cystocarps hemispherical, not constricted at the base and scattered along the entire thallus. Pericarp consists of one layer of elongated cells at the surface; the inner layer of thin-walled cells arranged anticlinally. Base of gonimoblast made up of small irregularly shaped and flattened cells. Carpospores in chain.

2. *Gracilaria changii* Xia et Abbott (Figure 5).

Plants erect to spreading, attached to substratum by disc-like holdfast. Branching irregular. Main axis percurrent, branches cylindrical, lateral branches distinctly constricted and somewhat enlarged near the base, and tapering toward the apices. Cortex with two to three layers of cells; medulla with large, thin-walled parenchymatous cells; cell size transition from cortex to medulla very abrupt. Cystocarps hemispherical, some slightly rostrate, base not constricted. Gonimoblast with small cells; nutritive filaments few, pericarp thick, with three to six layers of oval outer cells and six to seven flattened inner cells. Spermatangial conceptacles *verrucosa* type to *Polycaevemosa* type.

*Figure 4:* *G. arcuata.*
3. **Gracilaria eucheumoides** Harvey (Figure 6).

Plant prostrate with creeping thick branches attached to hard substrate by hapers. Greenish-brown to purplish in colour. Hapers strongly developed at basal ventral portions of branches. Branching is irregular. Branches distinctly compressed, with the margins serrate or with coarse, short dentations. Cortex thick, from two to four layers of cells, medulla consists of thick-walled cells, cell size transition from medulla to cortex, gradual.

4. **Gracilaria fastigiata** (Chang et Xia) Chang et Xia (Figure 7).

Plants erect, usually green but sometimes dark green in colour; exposed plants lighter, dry ones brownish to purplish in colour. Thallus terete or cylindrical. Branching irregular to alternate. Main axes more robust than branches. Branches not constricted, but sometimes slightly tapered at the base, generally bifurcating and usually becoming finer and fastigate toward distal portions. Anastomosing or curving branches sometimes present, spinose branchlets commonly present. Cortex consists of one to three layers of cells; medullary cells thin-walled; cell size transition from cortex to medulla abrupt.

Cystocarps semi-globose to globose, slightly constricted at the base, rostrate, irregularly distributed along the thallus. Pericarp consists of 9 to 11 layers of cells; to three outermost layers anticlinally arranged, elongated to irregularly oval, becoming larger in the mid-layers with granular cell contents; two to three innermost layers flattened, small cells present in the bottom medial portion of pericarp.
Figure 6:  G. eucheumoides.

Figure 7:  G.fastigata.
Nutritive filaments prominent, branching, found near the base or below gonimoblast. Centre of gonimoblast composed of large cells with granular cell contents.

5. **Gracilaria firma** Zhang et Xia (Figure 8).

Plants erect, texture firm to leathery, yellowish brown or green in colour. Branches terete or cylindrical, distinctly constricted at the base. Branching irregular or alternate, sometimes unilateral. Axes and branches robust.

Cortical cells one to two layers, irregularly round to oval; medullary cells thin-walled; cell size transition from medulla to cortex gradual.

Cystocarps conical or semi-globose, not constricted at the base, somewhat embedded in thallus and rostrate. Pericarp consists of seven to 13 layers of cells thinning somewhat near the base; outermost layer of cells round to ovoid; middle layers flattened, ovoid or showing pit connections; innermost layer flattened or disintegrating. Nutritive filaments absent. Carpospores in chains; round, ovoid or tear-drop shaped. Centre of gonimoblast made up of densely packed small cells. Spermatangial conceptacles deep and oval in shape (*verrucosa* type).

6. **Gracilaria gigas** Harvey (Figure 9).

Plants erect, texture cartilaginous. Thallus axes coarse, thick and succulent. Branches few and cylindrical, with a short stipe showing slight constriction at the base, branch apices acute, sometimes blunt.

Cortex thick, with two to four layers of rounded cells. Cell size transition from medulla to cortex gradual.

Cystocarps prominent, semi-globose, slightly rostrate and constricted at the base. Pericarp consists of 13-15 layers of cells; outermost layer marked by elongated cells, followed by layers of anticlinally arranged cuboidal to elongated cells becoming smaller and loosely arranged towards the gonimoblast. Carpospores not forming chains, oval to obovate, with stellate central body. Nutritive filaments abundant between pericarp and gonimoblast.

7. **Gracilaria heteroclada** Zhang et Xia (Figure 10).

Plants erect, up to more than 60 cm tall, dark brown to purple, sometimes olive-green in colour, brittle when fresh. Thallus terete or cylindrical throughout. Branching irregularly alternate. Main branches percurrent with many spinose determinate branchlets; branches distinctly larger than the branchlets.

Cortex thin, consisting of one to two layers of irregularly spherical to oval cells. Medulla consists of large parenchymatous cells, surrounded by two to three layers of small roundish cells. Transition of cell size from medulla to cortex abrupt.

Cystocarps prominently protruding, hemispherical, nonrostrate to slightly rostrate, not constricted at the base. Centre of gonimoblast composed of small ovoid cells. Spermatangia superficial and continuous over surface of thallus (*chorda* type).
Figure 8. G. firma

Figure 9: G. gigas.
Figure 10:  G. heteroclada.

Figure 11:  G. lemaneiformis.
8. **Gracilaria lemaneiformis** (Bory) Weber-van Bosse (Figure 11).

Plants solitary or caespitose, with few to several long branches. Branching irregular, mostly from lower portion. Cortex consists of two layers of cells, outermost layer elongate; medullary cells large and thin-walled; transition in cell size from medulla to cortex abrupt.

Cystocarps spherical, slightly rostrate or nonrostrate, constricted at the base; pericarp 10-14 layers; gonimoblast cells consist of filaments of many small cells with obscure cell walls; carpospores rounded to ovoid; absorbing filaments absent. Spermatangial conceptacles superficial (*chorda* type).

9. **Gracilaria manilaensis** Yamamoto et Trono (Figure 12).

Plants fleshy to somewhat cartilaginous, purplish red or sometimes greenish in colour. Fronds caespitose; main axis cylindrical throughout, usually percurrent. Branching alternate, sometimes secund or irregular. Branches abundant, long, similar to main axis, with lateral branchlets; both branches and branchlets sharply constricted at the base.

Cortex consists of one to two layers of dense protoplasmic cells; outermost cells with primary pit connections; hair basal cells scattered. Medullary cells polygonal, increasing in size toward the centre; outer layer more or less compressed parallel to frond surface. Cell size transition from cortex to medulla abrupt.

Cystocarps globose; gonimoblast cells elongated; absorbing filaments present, penetrating into pericarp. Spermatangial conceptacles cup-shaped, roundish or oval (*yerrucosa* type).

**Figure 12:** *G. manilaensis*. 

![Figure 12: G. manilaensis.](image)
10. **Gracilaria salicornia** (C. Agardh) Dawson (Figure 13).

Plants semi-erect to erect, bright yellow to dark green in colour. Branching dichotomous to trichotomous, divaricately arranged. Branches distinctly divided into terete or cylindrical, subclavate to clavate segments which are swollen at the distal end and constricted at the base.

Cortical cells, two to three layers; gland cells numerous; cell size transition from medulla to cortex gradual.

Cystocarps prominent, globose, slightly rostrate, slightly constricted or not at the base; pericarp thick, 10-11 layers of cells; nutritive filaments present. Spermatangial conceptacles oval (yerrucosa type).

11. **Gracilaria tenuistipitata** var. **liui** Zhang et Xia (Figure 14).

Plants generally small, yellowish brown to black in colour. Thallus slender, somewhat tough and firm. Branching irregular, mostly from percurrent axes. Branches not constricted at the base, tapering to acute apices occasionally bifurcating.

Cortex consists of two to three layers of round to oval cells; transition in cell size from medulla to cortex gradual.

Female fertile plants usually small and less branching. Cystocarps prominent, often broader than the branch, and scattered irregularly along main branches, semi-globose to globose, rostrate, constricted at the base. Pericarp thin, outer two layers cuboidal, inner layers flattened to oval, showing pit connections. Absorbing filaments rare. Centre of gonimoblast with small cells with strong pit connections. Carpospores in chains. Spermatangial conceptacles consist of shallow cavities (textorii type).

In addition to these species, there are at least three more species which have not been identified but have been recognised from the materials collected as being separate species from those reported in this paper.

2.4 **Ecology of the species**

*Gracilaria* spp. is widely distributed throughout the Philippines. Different species have been reported to occur in various habitats in many areas of the country, however, very few species occur in abundance in one locality, and not too many localities or areas have an abundance of the *Gracilaria* spp. resource throughout the year.

*Gracilaria* spp. seaweeds are generally found in shallow water, from the lower intertidal zone to the upper subtidal areas. They thrive in almost all types of habitats - from mangrove areas and brackish waters of soft and mud substrates or firm sandy type of bottom to open sea areas of rocky-coraline substrates. A species may be found in several areas of these habitats but may also be restricted to a particular substratum. The type of bottom generally has a profound effect on their distribution. A soft substratum, for example, characteristically supports a rich growth of *G. heteroclada* and *G. tenuistipitata*. However other species, like *G. firma* or *G. fastigiata* prefer a firm type of bottom to which they can be attached by means of holdfasts.
Figure 13:  *G. salicornia*.

Figure 14:  *G. tenuistipitata* var. liui.
Among the other species that prefer a firm substrate are *G. arcuata*, *G. changii*, *G. eucheumoides*, *G. fastigiata*, *G. gigas* and *G. salicornia*. *G. manilaensis* and *G. lemaneiforms* prefer soft-bottom areas.

With respect to the salinity and water temperature requirements of *Gracilaria* spp., the data obtained from Sorsogon showed that variations in salinity were not very great at Station 1 compared to the other stations. Salinity values obtained, on average, ranged from about 25 ppt to 37 ppt. At Station 2, salinity dropped to as low as 2.0 ppt in December from a high salinity value of 35 ppt obtained in September. At Station 3, the salinity ranged from 5 ppt obtained in May to 36 ppt; while at Station 4, there was a tremendous drop in salinity from as high as 36 ppt to as low as 2.0 ppt.

Monthly fluctuations in water temperature were evident but the time of day that sampling was conducted could have made the difference. Nevertheless, a comparison of data obtained from different areas would indicate a uniform fluctuation pattern in all the stations. At Station 1, water temperature values ranged from 23.5 °C in February to 35 °C in June; Station 2, 25-36 °C; Station 3, 29-34 °C; and Station 4, 24-35 °C.

3. DISCUSSION AND CONCLUSIONS

Although the species identification of the *Gracilaria* materials collected is still subject to verification, the species which are suitable for culture or farming purposes have been tentatively identified based on the quality of their agar content and ecological considerations, such as bottom-type preference and salinity.

The following species have been considered with potential for culture or farming: *G. firma*, *G. changii*, *G. fastigiata*, *G. heteroclada* and *G. tenuistipitata*. The first three species can be farmed in the open sea or in bays and coves, while the last two species are more suitable for farming in brackishwater ponds. Of these five species, however, *G. firma*, *G. heteroclada* and *G. tenuistipitata* can be considered priority culture species, considering not only their ecological characteristics but also their wide availability in many areas of the country.

*G. firma*, with quite a high percentage of agar yield and gel strength as analysed can be cultured in areas with a wide range of salinity (from 27 ppt to 37 ppt), in semi-turbid to clear water, and can withstand moderate to strong water movement. *G. heteroclada* and *G. tenuistipitata*, which have also good quality agar content and high yield, have the advantage of tolerating a wide range of salinity (as low as 2 ppt to as high as 36 ppt) and turbid waters, and may be cultured in ponds or sheltered bays where there is less wave action.

Among the areas where collection of *Gracilaria* spp. was undertaken, only Sorsogon and Samar (Maqueda Bay) are quite good sources of the raw material throughout the year. Cagayan (Buguey Bay), Bohol (Inabanga) and Tawi-Tawi (Buan Island) are also good sources; however, *Gracilaria* spp. is reportedly seasonal in these areas.

4. RECOMMENDATIONS

Considering the problem of *Gracilaria* spp. identification, it is recommended that follow-up studies on taxonomy and ecology should still be conducted to be able to gather sufficient samples, especially of the male materials.

Production development or culture studies should be continued and possibly be expanded to include other potential seaweed farming areas, for the three priority species: *G. firma* in open sea; *G. heteroclada* and *G. tenuistipitata* in sheltered bays and ponds.
REFERENCES


Bureau of Fisheries and Aquatic Resources. 1983-87. Fisheries Statistics of the Philippines. BFAR, Dept Agric, Quezon City.


ABSTRACT

This paper (Part II) deals with the processing (analysis of agar content and quality) of *Gracilaria* spp in the Philippines.

Over two hundred samples were collected for this study from wild stocks, particularly from Sorsogon. The experiments consisted of pre-treatment with 5% alkali (NaOH) at 90°C for 3 hours, extraction of agar and determination of agar quality. Results showed that the average mean yield of agar ranged between 16.18%-24.67% with *G. tenuistipitata* having the least and *G. changii* the highest yield, respectively. Gel strength was determined using a 1.5% agar solution and was highest for the agar from *G. heteroclada* (892 g/cm²). Other species with high gel strength were *G. tenuistipitata* (726 g/cm²), *G. flrma* (606 g/cm ) and *G. changii* (583 g/cm²). *G. salicoma* (287 g/cm²), and *G. fastigiata* (250 g/cm²) showed the lowest gel strength. Melting temperatures ranged from a high of 92 °C in the agar from *G. flrma* to a low of 79 °C from *G. fastigiata*. Most of the species showed high quality agar which can be used for bacto-agar and agarose production.

1. INTRODUCTION

The objective of the present study was to evaluate the commercial potential of seaweeds resources, identify the species suitable for culture and to provide guidelines on *Gracilaria* spp. farming.

There are numerous species of seaweeds in Philippine waters and they have become an important aquatic resource in the country. The species which are traditionally used for food include: *Caulerpa lentillifera; Gelidiella acerosa; Gracilaria* sp; *Eucheuma denticullatum; Kappaphycus alvarezi; and Sargassum* sp. *Gracilaria* spp. is gathered from wild stocks or farmed, it is then dried for export to developed countries as raw materials. Processing plants for *Eucheuma* spp. and *Gracilaria* spp. were established in the country in the late 60’s, so processing for food product agar and carrageenan, semi-refined and refined carrageenan from *Euchuema* spp. and *Kappaphycus* spp. is now in existence. An estimated 70% of carrageenan world-wide is being produced today from *K. alvarezi* and *E. denticulatum*.

The Philippine species of *Gracilaria* spp. occurs naturally over the entire country. The chief sources of agar are *Gracilaria* spp., *Gelidiella* spp. and other related seaweeds (Santos and Doty, 1978). However, in recent years the demand for dried *Gracilaria* spp. raw material has increased both to meet local supply and to fulfil export demand. The natural stocks of the agarophytre species started to decline and are still declining. Agarophytes are higher priced than other colloid bearing seaweeds. The demand for agar exists in developed and developing countries for food industry, Pharmaceuticals, microbiological research and genetic engineering. Agarose, a purified agar extract, is the most expensive and has been utilised for medical diagnosis, cell and tissue cultures.
2. PROCESSING (AGAR YIELD AND QUALITY) OF GRACILARIA

Studies have been made on agar from Philippine seaweeds. *Gracilaria verrucosa* (synonymous with *G. confervoides*) and *Gelidiella* spp. were studied as a source of agar in the early 1950's by the Philippines Fisheries Commission (now Bureau of Fisheries and Aquatic Resources) and the National Institute of Science and Technology. Other species of *Gracilaria* studied were *G. eucheumoides*, *G. arcuata*, and *G. salicombia* (Santos and Doty, 1978); *G. coronopifolia*, *G. arcuata*, *G. edulis*, *G. eucheumoides*, *G. verrucosa*, *G. salicombia and Gracilaria* sp. A. (Hurtado-Poncc and Umezaki, 1988); *G.firma*, *G. fastigiata*, *G. cylindrica*, *G. salicornia and G. tenuistipitata*, (Santos, 1993;. de la Pena and de Jesus, 1993). Similar studies were undertaken at UP-MSI, Diliman, SEAFDEC, in Iloilo and other universities. Most of the results of the studies showed that species of *Gracilaria* have potential quality of agar useful for food and other specialised agar products. The production of high quality agar from *Gracilaria* spp. for local processing and for the international market is the brightest prospect for the Philippine agar industry. Since *Gracilaria* spp. is the major raw material for agarophytes, a knowledge and understanding of their taxonomy and processing would be of importance for commercial culture and industry.

2.1 Materials and methods

The *Gracilaria* spp. samples used for this study were collected from Sorsogon and different coastal sites in the Philippines. Several species were identified. The collected samples were cleaned, sorted, coded and then weighed fresh. The seaweeds were then dried under the sun.

i. **Moisture content**

20 g of dry seaweed samples were weighed and dried further in an oven at 105 °C to a constant weight.

ii. **Clean anhydrous weight (CAW)**

20 g of dry seaweed samples were weighed and soaked in 600 ml of water 3 times for 10 minutes. The water was drained off and the seaweed dried to constant weight at 60°C. The % CAW obtained is used for calculation of agar yield.

iii. **Alkali treatment**

Two 100 g samples of dried *Gracilaria* spp. were placed in 2 litre capacity stainless steel pots containing 1.2 litres of 5% alkali solution and heated on a water bath for 3 hours at 90°C.

iv. **Extraction of agar**

Pre-treated samples were washed with water until free of alkali and put back stainless pots containing 600 ml of water. The pH of the water and sample was adjusted to 6.0 with 1% acetic acid.

The samples were heated for 2 hours at 85-90°C in a water bath and blended. 75 g of filter aid was added to the blended sample and stirred for half an hour. The extract was then filtered while hot under pressure at 60-85 psi. Filtrate was collected in a rectangular aluminium pan and gelled at room temperature. The gel was cut into cubes and frozen overnight. The next day, the gel was thawed and washed with water until free of colour. Isopropanol was added to facilitate drying at 60°C. Percentage agar yield was then calculated. The dried agar was powdered for quality analysis.
v. Moisture content - Oven method (AOAC, 1975)

Moisture content was determined in triplicate by weighing 1 g of agar into a tared porcelain crucible which was placed in an oven at 105°C for 5 hours. The porcelain crucibles were transferred to a desiccator, cooled and re-weighed.

vi. Gel strength - FMC gel tester

A 1.5% solution of agar sample was heated in a water bath until dissolved and allowed to set for 2 hours at 20°C before measurement of the gel strength.

vii. Melting temperature

A 1.5% solution of agar sample was heated and dissolved in a water bath. The agar solution was transferred to a test tube and gelled for 1 hour at 20°C. The tube was then placed in a water bath at 60°C and a lead shot dropped inside. The temperature was noted when the lead shot dropped to the bottom of the tube.

vii. Sulphate-turbidimetric method

The agar sample was dissolved in magnesium nitrate and hydrochloric acid. The sulphate content was determined by turbidimetric method by spectrophotometer with absorbance of 425 nm

3. RESULTS AND DISCUSSION

More than two hundred Gracilaria samples were collected at different sites in the coastal areas of the Philippines particularly in Sorsogon. Table 1 shows the environmental requirements of the different species of Gracilaria studied (Taw, 1994). However, only the common species of Gracilaria were analysed for their physico-chemical qualities.

<table>
<thead>
<tr>
<th>Species</th>
<th>Salinity (PPt)</th>
<th>PH</th>
<th>Temp (°C)</th>
<th>Transparency</th>
<th>Environment</th>
<th>Tide (cm)</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>G. changii</td>
<td>33-35</td>
<td>7-8</td>
<td>27-33</td>
<td>clear</td>
<td>coraline</td>
<td>30-20</td>
<td>Open sea</td>
</tr>
<tr>
<td>G. firma</td>
<td>25-35</td>
<td>6-8</td>
<td>25-35</td>
<td>semi clear</td>
<td>sandy/mud</td>
<td>30-250</td>
<td>Bays, mangrove</td>
</tr>
<tr>
<td>G. fastigiata</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>channels</td>
</tr>
<tr>
<td>G. heteroclada</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>mangrove channels</td>
</tr>
<tr>
<td>G. heteroclada</td>
<td>10-25</td>
<td>6-8</td>
<td>25-33</td>
<td>turbid</td>
<td>muddy</td>
<td>30</td>
<td>Brackish water ponds</td>
</tr>
<tr>
<td>G. tenuistipitata</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

It was observed that Gracilaria species appeared in the intertidal zone at different times of the year depending on the location. Nevertheless, during the rainy season the thalli deteriorated and disintegrated, which may be related to changes in seawater temperatures. It was also found that during summer the thalli remains healthy even at above 35°C (Wang et al. 1984). This observation correlated with the properties of agar. The physico-chemical properties of agar were generally good during the summer months. Table 2 summarises the yield of agar from the ten species of Gracilaria studied with their corresponding yield, clean anhydrous weight (CAW), moisture content, gel strength, total sulphate, and melting temperature and pre-treatment at 5% NaOH. The CAW ranged between 35 to 56% and moisture content between 12-17% (Table 3).
Table 2: Mean values of physico-chemical analysis of agar extracted by 5% NaOH pretreatment of Gracilaria species in the Philippines

<table>
<thead>
<tr>
<th>Species</th>
<th>% CAW</th>
<th>% Moisture content</th>
<th>% Yield</th>
<th>Gel Strength (g/cm²)</th>
<th>Total Sulphate</th>
<th>Melting Temp. (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G. fastigiata</td>
<td>44.4</td>
<td>16.89</td>
<td>19.24</td>
<td>250</td>
<td>3.28</td>
<td>79</td>
</tr>
<tr>
<td>G. salicornia</td>
<td>41.8</td>
<td>12.06</td>
<td>20.07</td>
<td>287</td>
<td>2.42</td>
<td>80</td>
</tr>
<tr>
<td>G. changii</td>
<td>56.7</td>
<td>14.48</td>
<td>20.07</td>
<td>583</td>
<td>0.61</td>
<td>86</td>
</tr>
<tr>
<td>G. tenuistipitata</td>
<td>52.8</td>
<td>15.51</td>
<td>16.18</td>
<td>726</td>
<td>0.90</td>
<td>88</td>
</tr>
<tr>
<td>G. firma</td>
<td>54</td>
<td>16.11</td>
<td>17.61</td>
<td>606</td>
<td>0.22</td>
<td>89</td>
</tr>
<tr>
<td>G. heteroclada</td>
<td>48.5</td>
<td>17.39</td>
<td>20.05</td>
<td>892</td>
<td>0.78</td>
<td>92</td>
</tr>
</tbody>
</table>

The physical properties varied from species to species and, in addition, the monthly sampling, post harvest handling, seasonal variation and drying may contribute to the quality of dried Gracilaria spp. The average yield ranged from 16 to 24%, G. changii gave the highest yield of agar followed by G. salicornia, G. heteroclada, G. fastigiata, G. firma, and G. tenuistipitata. Other species analysed also gave high yields but this has to be confirmed by more sampling. The gel strength of agar ranged from 250-892 g/cm² with the agar from G. fastigiata and G. salicornia providing the lowest gel strength and the highest gel strength provided by G. heteroclada, G. tenuistipitata, G. changii, and G. firma at 92°C.

G. fastigiata gave the highest total sulphate content and lowest gel strength as compared to the other species. From the data gathered, it appeared that the higher the total sulphate content, the lower the gel strength (Santos and Doty, 1978). G. firma and G. changii showed the lowest total sulphate content of 0.22% and 0.61%, respectively. Agar quality from all species in the present study showed similar results to previous reports (Santos, 1992 and de la Pena and de Jesus, 1993). Our results showed that among the species tested, G. heteroclada, G. firma, G. changii, and G. tenuistipitata showed high gel strength and melting temperature. The results of agar properties showed that Gracilaria spp. samples collected during summer seasons generally gave high yield and gel strength, although it varies with the species, season and location. The properties of the different species of Gracilaria spp. tested, showed that the agar of some species could be used for specialised agar products in microbiological culture media or agarose and the species with soft gel strength could be utilised for food industry.

4. RECOMMENDATIONS

The results obtained at time of observation in 1992-1993 indicated that several samples of G. fastigiata, G. changii, G. firma, G. tenuistipitata, and G. heteroclada have an average yield of 16.18-24.67% and the gel strength as high as 892 g/cm². G. firma, G. changii, G. tenuistipitata, G. heteroclada, and G. fastigiata were found to have good quality and the environmental parameters for optimum growth were observed. G. changii grows in the open sea with coraline substrate, clear, transparency and salinity of 33-35 ppt and water temperatures between 27-33°C. G. firma, G. fastigiata, G. heteroclada and G. tenuistipitata were both found to adapt and grow in brackishwater ponds with muddy substrate and turbid transparency. They also tolerate low salinity. The species mentioned above are recommended for farming having the required quality and environmental tolerance suitable for the agar industry.
# Table 3: Collection sites, dates and quality of agar extracted from different *Gracilaria* species from the Philippines

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Place of Collection</th>
<th>Date of Collection</th>
<th>Wet weight (g)</th>
<th>Dry weight (g)</th>
<th>CAW (%)</th>
<th>Moisture (%)</th>
<th>Agar Yield (%)</th>
<th>Gel Strength (g/cm²)</th>
<th>Total Sulphate (%)</th>
<th>Melting Temp. (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G. fastigiata</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SW 0001</td>
<td>Bagacay, Gubat, Sorsogon</td>
<td>5/18/92</td>
<td>500</td>
<td>600</td>
<td>42</td>
<td>18.0</td>
<td>21.43</td>
<td>150</td>
<td>3.93</td>
<td>76</td>
</tr>
<tr>
<td>SW 0055</td>
<td>Layog, Barcelona, Sorsogon</td>
<td>6/18/92</td>
<td>20,050</td>
<td>2,467</td>
<td>44.5</td>
<td>15.81</td>
<td>20.9</td>
<td>106</td>
<td>5.2</td>
<td>75</td>
</tr>
<tr>
<td>SW 0094</td>
<td>Bagacay, Gubat, Sorsogon</td>
<td>12/12/92</td>
<td>8,000</td>
<td>766</td>
<td>44.5</td>
<td>14.65</td>
<td>21.70</td>
<td>335</td>
<td>4.07</td>
<td>75</td>
</tr>
<tr>
<td>SW 0115</td>
<td>Bagacay, Gubat, Sorsogon</td>
<td>2/3/93</td>
<td>8,500</td>
<td>922</td>
<td>45</td>
<td>16.22</td>
<td>12.50</td>
<td>670</td>
<td>1.66</td>
<td>90</td>
</tr>
<tr>
<td>SW 0119</td>
<td>Bagacay, Gubat, Sorsogon</td>
<td>3/16/93</td>
<td>2,200</td>
<td>250</td>
<td>46</td>
<td>19.44</td>
<td>23.00</td>
<td>370</td>
<td>2.97</td>
<td>82</td>
</tr>
<tr>
<td>SW 0125</td>
<td>Bagacay, Gubat, Sorsogon</td>
<td>4/14/93</td>
<td>6,500</td>
<td>800</td>
<td>43.5</td>
<td>17.33</td>
<td>26.89</td>
<td>136</td>
<td>4.38</td>
<td>85</td>
</tr>
<tr>
<td>SW 0134</td>
<td>Bagacay, Gubat, Sorsogon</td>
<td>5/19/93</td>
<td>2,200</td>
<td>250</td>
<td>46</td>
<td>19.44</td>
<td>23.00</td>
<td>370</td>
<td>3.75</td>
<td>80</td>
</tr>
<tr>
<td>G. salicornia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SW 0070</td>
<td>Casiguran, Sorsogon</td>
<td>10/29/92</td>
<td>4,766</td>
<td>592</td>
<td>47</td>
<td>9.22</td>
<td>18.5</td>
<td>329</td>
<td>1.86</td>
<td>79</td>
</tr>
<tr>
<td>SW 0181</td>
<td>Bongansaran, Gubat, Bor.</td>
<td>9/2/93</td>
<td>5,000</td>
<td>621</td>
<td>36.5</td>
<td>14.89</td>
<td>21.64</td>
<td>245</td>
<td>2.98</td>
<td>81</td>
</tr>
<tr>
<td>G. changii</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SW 0148</td>
<td>Malaweste, uguey, Cagayan</td>
<td>6/17/93</td>
<td>16,000</td>
<td>2,000</td>
<td>35</td>
<td>17.87</td>
<td>29.71</td>
<td>1033</td>
<td>0.51</td>
<td>90</td>
</tr>
<tr>
<td>SW 0155</td>
<td>Mabuhay, Bulusan</td>
<td>7/13/93</td>
<td>6,000</td>
<td>1,030</td>
<td>65.5</td>
<td>18.79</td>
<td>25</td>
<td>912</td>
<td>0.95</td>
<td>89</td>
</tr>
<tr>
<td>SW 0223</td>
<td>Bongansaran, Gubat, Sor.</td>
<td>11/16/93</td>
<td>4,750</td>
<td>327</td>
<td>46.5</td>
<td>13.69</td>
<td>26.24</td>
<td>408</td>
<td>0.98</td>
<td>89</td>
</tr>
<tr>
<td>SW 0020</td>
<td>Layog, Barcelona, Sorsogon</td>
<td>6/19/92</td>
<td>16,500</td>
<td>1,973</td>
<td>56</td>
<td>19.83</td>
<td>15.71</td>
<td>216</td>
<td>0.51</td>
<td>81</td>
</tr>
<tr>
<td>SW 0050</td>
<td>Mapapac, Barcelona, Sor.</td>
<td>9/9/92</td>
<td>7,899</td>
<td>945</td>
<td>59</td>
<td>11.13</td>
<td>26.0</td>
<td>277</td>
<td>0.41</td>
<td>78</td>
</tr>
<tr>
<td>SW 0065</td>
<td>Dancalan, Bulusan, Sor.</td>
<td>10/28/92</td>
<td>3,834</td>
<td>458</td>
<td>50</td>
<td>11.67</td>
<td>21.0</td>
<td>456</td>
<td>0.41</td>
<td>85</td>
</tr>
<tr>
<td>SW 0136</td>
<td>Layog, Barcelona, Sorsogon</td>
<td>5/19/93</td>
<td>4,000</td>
<td>750</td>
<td>66</td>
<td>10.6</td>
<td>14.1</td>
<td>857</td>
<td>0.36</td>
<td>93</td>
</tr>
<tr>
<td>SW 0154</td>
<td>Layog, Barcelona, Sorsogon</td>
<td>7/13/93</td>
<td>35,000</td>
<td>680</td>
<td>57</td>
<td>15.75</td>
<td>27.0</td>
<td>534</td>
<td>1.01</td>
<td>87</td>
</tr>
<tr>
<td>SW 0161</td>
<td>Layog, Barcelona, Sorsogon</td>
<td>8/3/93</td>
<td>2,200</td>
<td>320</td>
<td>60.5</td>
<td>18.27</td>
<td>33.0</td>
<td>839</td>
<td>0.64</td>
<td>88</td>
</tr>
<tr>
<td>SW 0183</td>
<td>Layog, Barcelona, Sorsogon</td>
<td>9/3/93</td>
<td>6,500</td>
<td>1,035</td>
<td>57</td>
<td>14.25</td>
<td>26.14</td>
<td>527</td>
<td>0.69</td>
<td>86</td>
</tr>
<tr>
<td>SW 0217</td>
<td>Layog, Barcelona, Sorsogon</td>
<td>10/10/93</td>
<td>2,500</td>
<td>373</td>
<td>49.5</td>
<td>16</td>
<td>29</td>
<td>908</td>
<td>0.64</td>
<td>87</td>
</tr>
<tr>
<td>SW 0229</td>
<td>Layog, Barcelona, Sorsogon</td>
<td>12/12/93</td>
<td>10,500</td>
<td>1,285</td>
<td>56</td>
<td>12.78</td>
<td>30.1</td>
<td>630</td>
<td>0.82</td>
<td>91</td>
</tr>
<tr>
<td>Sample code</td>
<td>Place of Collection</td>
<td>Date of Collection</td>
<td>Wet weight (g)</td>
<td>Dry Weight (g)</td>
<td>CAW (%)</td>
<td>Moisture (%)</td>
<td>Agar yield (%)</td>
<td>Gel strength (g/cm²)</td>
<td>Total sulphate (%)</td>
<td>Melting Temp. (°C)</td>
</tr>
<tr>
<td>-------------</td>
<td>--------------------------</td>
<td>--------------------</td>
<td>----------------</td>
<td>----------------</td>
<td>---------</td>
<td>--------------</td>
<td>------------------</td>
<td>----------------------</td>
<td>-------------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>SW 0003</td>
<td>Tughan, Juban, Sorsogon</td>
<td>3/16/93</td>
<td>2,306.4</td>
<td>166.9</td>
<td>52.5</td>
<td>12.50</td>
<td>23.24</td>
<td>68</td>
<td>0.20</td>
<td>88</td>
</tr>
<tr>
<td>SW0058</td>
<td>Bagacay, Gubat, Sorsogon</td>
<td>4/25/93</td>
<td>21,000</td>
<td>1,828.4</td>
<td>55</td>
<td>19.35</td>
<td>20.43</td>
<td>1051</td>
<td>0.58</td>
<td>93</td>
</tr>
<tr>
<td>SW 0112</td>
<td>Buhong, Juban, Sorsogon</td>
<td>5/18/93</td>
<td>15,620</td>
<td>1,945.3</td>
<td>55</td>
<td>18.55</td>
<td>14.91</td>
<td>982</td>
<td>0.58</td>
<td>93</td>
</tr>
<tr>
<td>SW 0 122</td>
<td>Catanagan, Juban, Sorsogon</td>
<td>6/14/93</td>
<td>6,750</td>
<td>780</td>
<td>78</td>
<td>17.45</td>
<td>8.43</td>
<td>780</td>
<td>0.52</td>
<td>91</td>
</tr>
<tr>
<td>SW 0133</td>
<td>Tughan, Juban, Sorsogon</td>
<td>6/21/93</td>
<td>11,000</td>
<td>1,100</td>
<td>51</td>
<td>9.72</td>
<td>13.92</td>
<td>750</td>
<td>0.17</td>
<td>96</td>
</tr>
<tr>
<td>SW 0002</td>
<td>Bagacay, Gubat, Sorsogon</td>
<td>5/8/92</td>
<td>5,000</td>
<td>500</td>
<td>38</td>
<td>20.5</td>
<td>14.46</td>
<td>1002</td>
<td>0.29</td>
<td>92</td>
</tr>
<tr>
<td>SW0010</td>
<td>Bagacay, Gubat, Sorsogon</td>
<td>6/18/92</td>
<td>42,320</td>
<td>4,122</td>
<td>54</td>
<td>15.86</td>
<td>15.25</td>
<td>583</td>
<td>0.31</td>
<td>88</td>
</tr>
<tr>
<td>SW 0025</td>
<td>Bagacay, Gubat, Sorsogon</td>
<td>7/14/92</td>
<td>12,000</td>
<td>1,445</td>
<td>47.5</td>
<td>16</td>
<td>23</td>
<td>820</td>
<td>0.17</td>
<td>93</td>
</tr>
<tr>
<td>SW 0054</td>
<td>Bagacay, Gubat, Sorsogon</td>
<td>9/17/93</td>
<td>27,500</td>
<td>3,825</td>
<td>61</td>
<td>15.35</td>
<td>18.36</td>
<td>324</td>
<td>0.32</td>
<td>87</td>
</tr>
<tr>
<td>SW 0179</td>
<td>Bagacay, Gubat, Sorsogon</td>
<td>9/2/93</td>
<td>3,000</td>
<td>420</td>
<td>64.5</td>
<td>16.04</td>
<td>28</td>
<td>878</td>
<td>0.16</td>
<td>89</td>
</tr>
<tr>
<td>SW 0193</td>
<td>Baruy-baruy, Samar</td>
<td>9/12/93</td>
<td>7,000</td>
<td>480</td>
<td>80</td>
<td>15.60</td>
<td>7.62</td>
<td>219</td>
<td>0.26</td>
<td>85</td>
</tr>
<tr>
<td>SW 0207</td>
<td>Calampong, Samar</td>
<td>10/26/93</td>
<td>1,000</td>
<td>48</td>
<td>16.72</td>
<td>18.29</td>
<td>239</td>
<td>0.18</td>
<td>86</td>
<td></td>
</tr>
<tr>
<td>SW 0228</td>
<td>Bongansaran, Gubat, Sor.</td>
<td>12/12/92</td>
<td>3,710</td>
<td>356</td>
<td>39</td>
<td>12.75</td>
<td>15.90</td>
<td>778</td>
<td>0.29</td>
<td>91</td>
</tr>
<tr>
<td>G. tenuistipitata</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G. firma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SW 0021</td>
<td>Tughan, Juban, Sorsogon</td>
<td>6/19/92</td>
<td>5,000</td>
<td>120</td>
<td>40</td>
<td>15.74</td>
<td>15.85</td>
<td>450</td>
<td>3.63</td>
<td>89</td>
</tr>
<tr>
<td>SW 0140</td>
<td>Binakayan, Cavite</td>
<td>5/25/93</td>
<td>31,100</td>
<td>2,522</td>
<td>46</td>
<td>17.36</td>
<td>15.21</td>
<td>808</td>
<td>0.76</td>
<td>94</td>
</tr>
<tr>
<td>SW 0156</td>
<td>Tughan, Juban, Sorsogon</td>
<td>7/13/93</td>
<td>4,000</td>
<td>479</td>
<td>51</td>
<td>17.43</td>
<td>22.6</td>
<td>1487</td>
<td>0.48</td>
<td>92</td>
</tr>
<tr>
<td>SW 0163</td>
<td>Caditaan, Magallanes, Sor.</td>
<td>8/8/93</td>
<td>9,500</td>
<td>916</td>
<td>37.5</td>
<td>19.13</td>
<td>24</td>
<td>1016</td>
<td>0.66</td>
<td>96</td>
</tr>
<tr>
<td>SW 0216</td>
<td>Tughan, Juban, Sorsogon</td>
<td>10/27/93</td>
<td>3,500</td>
<td>295</td>
<td>68</td>
<td>17.29</td>
<td>22.6</td>
<td>700</td>
<td>0.92</td>
<td>91</td>
</tr>
<tr>
<td>G. heteroclada</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
REFERENCES


Thailand

Vithya Srimanobhas,
Faculty of Environment and Resources Studies,
Mahidol University, Nakhon Pathom, Thailand.

and

Attaya Kungsuan,
Food Technologist, Fishery Technological Development Division, Bangkok, Thailand.

ABSTRACT

Seven species of *Gracilaria* were studied for their taxonomy and ecology: *G. changii*, *G. edulis*, *G. firma*, *G. fisheri*, *G. irregularis*, *G. salicornia* and *G. tenuistipitata*. In terms of agar quality, *G. fisheri* and *G. tenuistipitata* had the highest gel strength of 768 and 758 g/cm², respectively. This finding is in accord with the results reported by Dr Suwalee Chandrkrachang of BRU that *G. edulis* and *G. tenuistipitata* from Pattani have the highest potential as a source of agar in Thailand. It was recommended that further studies should be conducted on these two species with the aim of developing them as a main source of agar for the processing industry.

1. INTRODUCTION

Thailand has a coastline of 2,614 kilometers bordering the Gulf of Thailand in the east and the Andaman Sea in the west. Along the coastal shelf are shallow mud flats and rocky or sandy beaches, some parts are mangrove forests, seagrass beds or coral reefs. Coastal inhabitants have utilised several species of red seaweeds, such as *Gracilaria* spp., as supplementary foods for many years. Recently, the agar requirement of the food industries and microbiological laboratories has increased. Data from foreign trade statistics reveal that Thailand increasingly imports agar annually while small amounts are exported (Table 1). Studies on agar containing seaweeds has been made since 1986 by various institutes including the Faculty of Fisheries, Kasetsart University, concerning seaweed production, and Srinakarinvirot University, concerning agar processing. Abbott (1988) and Lewmanomont (1994) have provided excellent works on the taxonomy of *Gracilaria* spp. in Thailand.

Table 1: Agar imported and exported to Thailand from 1984-1993.

<table>
<thead>
<tr>
<th>Years</th>
<th>Imported Quantity (tonnes)</th>
<th>Value (million Baht)</th>
<th>Exported Quantity (tonnes)</th>
<th>Value (million Baht)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1984</td>
<td>259.92</td>
<td>87.98</td>
<td>1.22</td>
<td>0.08</td>
</tr>
<tr>
<td>1985</td>
<td>234.13</td>
<td>95.33</td>
<td>11.80</td>
<td>2.06</td>
</tr>
<tr>
<td>1986</td>
<td>251.87</td>
<td>108.24</td>
<td>11.66</td>
<td>2.13</td>
</tr>
<tr>
<td>1987</td>
<td>276.77</td>
<td>112.93</td>
<td>10.83</td>
<td>2.67</td>
</tr>
<tr>
<td>1988</td>
<td>262.45</td>
<td>111.10</td>
<td>5.06</td>
<td>0.97</td>
</tr>
<tr>
<td>1989</td>
<td>274.78</td>
<td>129.40</td>
<td>2.97</td>
<td>0.82</td>
</tr>
<tr>
<td>1990</td>
<td>346.56</td>
<td>199.32</td>
<td>0.76</td>
<td>0.17</td>
</tr>
<tr>
<td>1991</td>
<td>325.99</td>
<td>190.16</td>
<td>1.69</td>
<td>1.11</td>
</tr>
<tr>
<td>1992</td>
<td>435.44</td>
<td>233.17</td>
<td>5.00</td>
<td>1.67</td>
</tr>
<tr>
<td>1993</td>
<td>472.53</td>
<td>226.48</td>
<td>7.78</td>
<td>3.50</td>
</tr>
</tbody>
</table>
1.1 Material and methods

Seaweed samples were collected from the eastern and southern provinces of Thailand (Figure 1). For taxonomic study, material was placed in 5% formalin. After return to the laboratory some of the seaweeds were dried. Sections were cut by freezing microtome or free hand, stained in 1% aqueous aniline blue and 50% glucose syrup was applied as a mounting medium. For agar yield and quality studies, all materials were collected fresh and transported to the Fishery Technological Department Institute for further study.

At the Institute, the seaweeds were cleaned with water which included removing sand, stones and shells, and sun dried. Dried seaweed thus obtained was stored until extraction and analysis.

i. Extraction method.

For extraction, a 100 g sample of dried seaweed was cleaned in water 3 times and dried again to determine the final dry weight. The results obtained were calculated to find the CAW (Clean Anhydrous Weight). The dried seaweed obtained was then weighed out in samples of 40 g for agar extraction. The flow diagram of extraction method is shown in Figure 2. To extract the agar, the dried seaweed was submerged in water until soft and then the water was drained out. The wet seaweed sample was boiled with 3% sodium hydroxide (1:15 w/v) at 90 ± 4°C for one hour and washed with water until the pH was neutral. Further boiling was performed in water (1:20 w/v) at 100 °C for one hour with homogenising and addition of a filtration aid. Filtration was performed on a vacuum press and the filtrate was set and kept in a freezer at -20 °C for 48 hours. After thawing the frozen agar, the agar cake was dried in an oven overnight at 50-55 °C and further analysis of agar quality was performed.

ii. Ash content

Analysis of ash content was carried out at 550°C, until the sample turned white and had a stable weight according to a method laid out by AOAC (1980).

iii. Moisture content

Moisture content analysis was carried out at 100°C for 6 hours AOAC (1980).

iv. Gel strength.

A 1.5% aqueous solution of agar was prepared and poured into a beaker which gave an agar thickness of 3 cm and a diameter of 6 cm. The agar in these beakers was set in a water bath at room temperature for 12 hours and the gel strength of the agar cakes obtained was measured using a Nikanui gel tester.

v. Sulphate content

0.1 g of the sample was weighed out, 2 ml of saturated Mg (NO₃)₂ in HNO₃ was added and the mixture evaporated to dryness. After heating in a muffle furnace at 400 °C for 4-5 hours, the sample was cooled to 80 °C and 10 ml of 1M HCl was added. The mixture was then filtered and the volume made up to 50 ml. All of the solution was transferred to a 150 ml beaker and 5 ml of NaCl-HCl (67g of NaCl with 200 ml of water and 8 ml of HCl) was added together with 10 ml of glycerol-ether (1:2) and 0.2g of BaCl₂. The mixture was stirred for one minute and left to stand for 4 minutes, before being stirred again for 5 seconds.
Figure 1: Map of Thailand, showing the collecting places.
Figure 2: Flow diagram of agar extraction from seaweed.

Dried seaweed 40 g
- soak in water until soft
- drain the water out
- add 3% sodium hydroxide (1:16 w/v)
- heat at 90 °C for 1 h
- drain alkaline out
- wash with water until pH 7

Treated seaweed
- add water (1:20 w/v)
- boil at 95-100 °C for 1 h (with homogenizing)
- add filter aid (80 g)
- filter

Filtrate
- gel setting
- freezing at -20 °C for 2 days

Frozen agar
- thaw and decant water
- wash with water
- oven drying at 50-55 °C (overnight)

Dried agar flake
The absorbance of the final solution was measured at 425 nm. The sulphate content was then calculated from the standard curve previously prepared (ASTM, 1985; ACE, 1961).

vi. Melting temperature.
A 1.5% agar solution was prepared and poured into a test tube (2.5 x 20 cm) to a height of 10 cm and a thermometer was dipped halfway into the agar solution. After the agar had congealed completely, the test tube was heated in a water bath of 100°C. A bead was placed on the surface of the gel and the temperature was observed as the bead dropped through the solution.

2. RESULTS

2.1 Taxonomy and ecology

1. **Gracilaria changii** (Xia et Abbott) Abbott, Zhang et Xia (Figure 3).

Reference

Basionym
*Polycavernosa changii* Xia et Abbott, Phycologia 26:407, Figure 3, 1987.

Description
Plants bushy, 5-7 cm high, branches cylindrical, main branches 1.5-2.5 mm in diameter; branching alternate or irregular to four orders, most of the branches are abruptly constricted at the base and taper towards apices

Collections
Ao Len, Changwat Trat, 12° 04’ N, 102° 33’ E, NACA 002. 28 April 1993; NACA 007 8 June 1993

Ecology
*G. changii* was found growing on rocks, gravel and shells in sand-mud areas which were exposed to air at low tide. Water highly turbid, salinity 32-33 ppt.

2. **Gracilaria edulis** (Gmelin) Silva (Figure 4).

Reference


Description
Plants form a loose clump 15-25 cm high, branches cylindrical, main branches 0.7-1.0 mm in diameter, branching dichotomous or trichotomous to six orders, branches apices frequently hook-like in appearance

Collections
Ao Len, Changwat Trat, 12° 04’ N, 102° 33’ E, NACA 004. 28 April 1993

Ecology: *G. edulis* was found growing on rocks in sandy-muddy areas which are exposed to air during extreme low tide. Water was highly turbid and salinity was around 33 ppt.

3. **Gracilaria firma** Chang et Xia (Figure 5).

Reference
Stud. Mar. Sinica. I 1: 143-145, fig. 38, 1-8; fig. 39,1-4, pl 2, fig.4. 1976

Description
Plants succulent, erect 8-12 cm high; branches cylindrical, main branches 1.5-2.0 mm in diameter, branching alternate or irregular to five orders, lower branches frequently constricted at the base

Collections
Ban Learn Tian, Changwat Trat, 12O 05’ N, 102O 35’ E, NACA 001. 28 April 1993; NACA 008. 4 April 1994

Ecology: *Gracilaria firma* was found growing on gravel and rock fragments in sandy muddy areas. Not exposed to air during low tide, water turbid, salinity 31-33 ppt.
Figure 3: Habit of *Gracilaria changii*.

Figure 4: Habit of *Gracilaria edulis*. 
4. **Gracilaria fisheri** (Xia et Abbott) Abbott, Zhang et Xia (Figure 6).


Description: Plants bushy, 16-44 cm high, branches cylindrical, main branches 0.7-1.5 mm in diameter; branching alternate to four orders, constricted at the base and tapering towards the apices.

Collections: Ko Yo, Changwat Songkhla, 07° 10'N, 100° 33'E, NACA 009.21 August 1994.

Ecology: *G. fisheri* was found growing on nets of fish cages in muddy, sandy areas. Not exposed to air during low tide, water turbid, salinity 11 ppt

5. **Gracilaria irregularis** Abbott (Figure 7).


Description: Plant erect, 4-8 cm high; branches cylindrical, main branches 2.5-3.0 mm in diameter; branching mostly secund.

Collections: Ao Len, Changwat Trat, 12° 04' N, 102° 33' E, NACA 003. 28 April 1993.

Ecology: *G. irregularis* was found growing on rock fragments, gravel and shells in sandy mud areas exposed to air during low tide, water highly turbid, salinity about 33 ppt.

6. **Gracilaria salicornia** (C. Agardh) Dawson (Figure 8).


Description: Plants clump, thalli rigid, prostrate to semi-erect; branches cylindrical, frequently constricted into segments, 2.5-4 mm in diameter, branching irregular.

Collections: Leam Tian, Trat Province 12° 05' N, 102° 35'E, NACA 005. 28 April 1993; NACA 006, 8 June 1993.

Ecology: *G. salicornia* was found growing on rocks or gravel in muddy/ sandy mud areas not exposed to air during low tide. Water turbid, salinity about 31 ppt.

7. **Gracilaria tenuistipitata** var. liui Zhang et Xia (Figure 9).


Description: Plants bushy, 22-32 cm high; branches slender, cylindrical, main axes 0.5 mm in diameter, branching alternate to irregular to three orders, numerous delicate branchlets.


Ecology: *G. tenuistipitata* var. *liui* was found growing on gravels and shell in sandy mud areas, exposed to air during extreme low tide, water turbid, salinity around 31 ppt.
Figure 5: Habit of Gracilaria firma.

Figure 6: Habit of Gracilaria fisfieri
Figure 7: Habit of *Gracilaria irregularis*.

Figure 8: Habit of *Gracilaria salicornia*. 
Figure 9: Habit of *Gracilaria tenuistipitata* var. *liui*. 
2.2 Agar yield and quality

The results of agar quality are summarised in Table 2.

Table 2: Results of agar quality analysis of all samples.

<table>
<thead>
<tr>
<th>Species</th>
<th>Yield (%)</th>
<th>Ash (%)</th>
<th>Moisture Content (%)</th>
<th>Melting Temp. (°C)</th>
<th>Sulphate content (%)</th>
<th>Gel strength (g/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G. salicornia</td>
<td>4.75</td>
<td>5.60</td>
<td>16.80</td>
<td>88</td>
<td>-</td>
<td>180</td>
</tr>
<tr>
<td>G. changii</td>
<td>9.50</td>
<td>5.90</td>
<td>15.00</td>
<td>90</td>
<td>-</td>
<td>190</td>
</tr>
<tr>
<td>G. firma</td>
<td>19.96</td>
<td>2.46</td>
<td>6.90</td>
<td>93</td>
<td>2.04</td>
<td>692</td>
</tr>
<tr>
<td>G. fisheri</td>
<td>12.84</td>
<td>2.13</td>
<td>6.67</td>
<td>94</td>
<td>2.01</td>
<td>758</td>
</tr>
<tr>
<td>G. tenuistipitata var. liui</td>
<td>12.70</td>
<td>1.85</td>
<td>6.24</td>
<td>98</td>
<td>1.81</td>
<td>768</td>
</tr>
</tbody>
</table>

It was found that sample number NACA 009 (Gracilaria fisheri) and NACA 010 (G. tenuistipitata) had the highest gel strengths, with values of 768 and 758 g/cm² respectively. It was apparent that samples number NACA 006 and NACA 007, which had a very high ash content also had a very low gel strength. These results are in agreement with those reported by Chandrkrachang and Chinadit 1988, where G. fisheri from Songkhla and G. tenuistipitata from Pattani were identified as having greatest potential as raw materials for agar processing in Thailand. Further study on production of these two species should be made to eliminate the problem of raw material shortages for agar processing in Thailand.

REFERENCES


VIETNAM
Do Van Khuong,  
And  
Nguyen Van Thuc  
Research Institute of Marine Products, Ministry of Fisheries,  

ABSTRACT
Samples of *Gracilaria* spp. were collected from northern Vietnam and 8 of the 13 species reported by Nguyen Huu Dinh and Nguyen Van Thien (1993) were identified, namely: *G. asiatica* Zhang et Xia (syn. *G. verrucosa*); *G. tenuistipitata* Zhang et Xia; *G. blodgettii* Harvey; *G. arcuata* Zan.; *G. hainemensis* Zhang et Zia (similar to *G. edulis*); *G. chorda* Holm (syn. *G. lemaneiformis*), *G. gigas* Harv. and *G. bursa-pastoris* Silva.

The taxonomic identification of *Gracilaria* species usually meets with the following difficulties: variability among species is not clearly defined; morphological features of the species are influenced by environmental conditions which tend to change at different locations; and some of the specimens collected lack reproductive organs. The taxonomy of *Gracilaria* spp. is quite complicated and synonyms are commonly used. To correctly identify the taxonomic status, it is necessary to collect *Gracilaria* spp. specimens continuously when it is mature and has reproductive organs (tetrasporangium, spermatangial conceptacle and cystocarp).

Among the 8 species identified during this study, 4 were found to be of high economic value. Of these 4 species, 3 were studied and cultivated: *G. asiatica*; *G. blodgettii* and *G. tenuistipitata*. *G. chorda* still has to be studied in trial cultivation.

Analysis of results from 6 *Gradlaria* species collected from Hai Phong and Quang Ninh showed that the dried/fresh ratio, agar yield and gel strength were highest in *G. asiatica*. Its also has the lowest ash content, is widely distributed in Vietnam and could be the main species cultured in brackish water swamps in northern Vietnam. Other species of high economic value for culture and agar processing in Vietnam are *G. blodgettii* and *G. tenuistipitata*.

1. INTRODUCTION
Along the coast of Vietnam, economic red algae are widely distributed. Since ancient times, they have been naturally harvested and utilised as food and medicines in the form of raw or processed products. Only in recent decades has attention been paid to the development of seaweed culture to meet the demands of domestic consumption and the export market. In Vietnam, the cultivation and processing of economic seaweeds is quite new and has only really developed since 1987, when seaweed markets began expanding in other countries in the region.

One of the main genus of seaweeds which has been studied and cultivated is *Gradlaria*. At present, the cultivation of three species of *Gracilaria* has developed in Vietnam, namely: *Gracilaria asiatica* (syn. *G. verrucosa*); *G. blodgettii*; and *G. tenuistipitata*. In 1987, Vietnam produced about 1,500 tonnes of dried *Gracilaria* spp., but by 1993 that quantity had reached to 2,800-3,000 tonnes. Most of the dried *Gracilaria* spp. is used for agar processing for domestic consumption and the remainder is exported to China. In addition, trial cultivation of *Eucheuma gelatinae* has begun in Vietnam.
The agar extraction industry from *Gracilaria* spp. has clearly expanded. In 1987, Vietnam produced only 20 tonnes of agar, but by 1993 this had increased to 250 tonnes. In addition to industrial scale agar processing factories, many small scale and family scale ones have appeared with high economic efficiency. However, this industry only meets local demands and the agar is used for the candy and foodstuff industry. The export of agar from Vietnam is facing difficulties due to its poor quality, especially its low gel-strength and high melting point.

In Vietnam, as with other countries in the region, the identification of species of economic seaweeds belonging to the genera *Gracilaria, Eucheuma* and *Porphyra*, is difficult. Some species may be an important source of raw material for the agar extraction industry, but knowledge of the correct taxonomy and the ecological requirements for cultivation is still limited.

### 2. ECOLOGY AND TAXONOMY OF *GRACILARIA*

Morphological features traditionally used to distinguish species of *Gracilaria* are the hold fast, form of thalli, main branches and branching patterns. In recent years, attention has been given to the structure of reproductive organs which varied little in one species. Zhang, and Xia (1976) suggested using the structure of the pericarp, Yamamoto (1978) suggested using the arrangement of spermangia as an important feature to identify the species. For *Gracilaria*, taxonomists combine comparative methods of morphology, function structure, reproductive organs structure and cell rearing for taxonomic identification.

According to Nguyen Van Tien (1988), 13 species of *Gracilaria* were reported in Northern Vietnam, and *Gracilaria asiatica* Zhang et Xia with its high natural productivity, has been widely cultivated for many years. Seasonal changes in salinity in the northern coastal waters is an important factor influencing the appearance and biomass of *Gracilaria* spp. in these areas. In the littoral zone in the rainy season (end of June to early October) when the salinity reduces to 5-6 ppt, and sometimes reaches 3-4 ppt, *Gracilaria* spp. stops growing and begins to fade. In Cat Hai island, which is far from river mouths, salinities reach 14-28 ppt in the rainy season, *Gracilaria* spp. grows and develops well from April until December and fades only in January, February and March when salinity is over 30 ppt.

During 1992-1993, as part of this research programme, the following studies were carried out:

- Selected *Gracilaria* spp. resource surveys in some locations (wild and cultivated plants) in Northern Vietnam.
- Collection of specimens of *Gracilaria* spp. growing naturally and cultured in brackish water ponds.
- Determination of the ecological and environmental characteristics of the collectionsite.
- Taxonomic identification of specimens under the modern concepts and up-to-date documents on the taxonomy of *Gracilaria* spp.

### 2.1. Materials and methods

Materials were collected from various brackishwater swamps of Quang Ninh, Hai Phong, Nam Ha provinces of Northern Vietnam including all field-collected and cultivated *Gracilaria* species. Specimens of *Gracilaria* species collected were preserved in 5%...
buffered formalin/ sea-water. One part of the specimens was sliced in longitudinal and transverse sections of thallus by Microtome or razor and were stained with acetocarmin and aniline blue. Taxonomic identification of the specimens was done by Nguyen Van Tien from the Oceanographic Institute. Herbaria was comparatively studied with the Herbaria set of *Gracilaria* spp. of northern Vietnam housed at the Oceanographic Institute of Vietnam Science Institute. In addition to traditional documents, new ones were used to identify *Gracilaria* species, particularly the volumes of "Taxonomy of Economic Seaweeds" edited by Abbott and Nonis (1985; 1988).

### 2.2. Results

1. **Gracilaria asiatica** Zhang et Xia (Figures 1 and 2).

Alga solitary or large caespitose; brown or dark-brown in colour. Holdfast discoid or unattached. Thallus cylindrical or terete, about 1-1.5 mm in diameter, branching alternate, main branches elongate with shorter branchlets, acute apices. In transverse section, the central part consisted of large roundish cells, the diameter of central cells was 240-336 um. Cortex consisted of 1-3 layers of small cells.

Tetrasporangium, divided cruciate or tetrapartite in cortex scattered over the whole thallus. Cystocarps hemispherical or conical, slightly constricted at the base, without rostrum, 580-700 um in diameter. Pericarp consisted of 8-11 layers of cells, with few absorbing filaments (Code No. 92001). This species is distributed at the medium littoral zone and in brackishwater swamps along the coast of northern Vietnam from October until the following July.

2. **Gracilaria tenuistipitata** Zhang et Xia (Figure 3).

Alga solitary or big caespitose. Thallus cylindrical, delicate as thin as thread at young period 18-30 cm long or more. Main axes with maximum diameter 1-2 mm, average 0.5-0.6 mm. Dark-brown or green-brown.

Simple branching, generally of one to three orders, branching alternate with one or two orders of branching. In mature alga it is difficult to separate main axes and main branches. In transverse section, the central cell is 260-275 um in diameter, next are 2-3 layers of pericentral cells, the layer of cells reduce their size from medulla to cortex. Outermost layer is 1-2 layers of small pigmented cells. Cystocarp hemispherical with constriction at the base, with rostrum, their diameter normally same as one of branch, in transverse section of cystocarp, pericarp consists of 8-11 layers. This species is distributed in most central and northern provinces of Vietnam and is mainly a species cultivated in the brackishwater swamps of the central provinces.
Figure 1: Gracilaria asiatica

A. External features

B. Section of cylindrical thallus

C. Section of tetrasporic thallus

D. Section of pericarp
Figure 2: *Gracilaria asiatica* of Yen hung, Quang Ninh.

A. and B. Morphological cystocarp

C. Section of cystocarp

D. Section of cylindrical thallus
Figure 3: *Gracilaria tenuistipitata* of Lienvi, Quang Ninh.

A. Section of cylindrical thallus

B. Tetrasporic thallus

C. Section of tetrasporic thallus

D. External morphology of cystocarp

E. Section of cystocarp

F. Section of pericarp
3. **Gracilaria arcuata** Zan (Figure 4).

Alga caespitose, red-brown, 10-15 cm tall. Holdfast discoid. Thallus cylindrical, 1-1.2 mm in diameter, branching alternate or dichotomous, slightly constricted at the base, some branch apex curved. In transverse section, the central part is a big medullary cell and layers of pericentral cells, cortex consisted of 3-4 layers of small roundish or oval, the size of cell becomes smaller from medulla to cortex. Tetrasporangium divided tetrapartite in the cortex layer. Cystocarp hemispherical, pericarp consisted of 2-15 layers of rectangular or squared, with clearly longitudinal rows. Plants attached to rocks, abundant in spring and summer (from March - July) at lower littoral zone of Hai Phong coastal area.

4. **Gracilaria hainanensis** (Figure 5).

Alga caespitose. Holdfast discoid. Thallus terete, dark purple or dark brown, 15-20 cm tall or more, 1-1.4 mm in diameter, constriction at the base, branching sparse, no main thalli, main branch dichotomous narrowly to the base, apex almost at the same height. In transverse section, medulla cell large, surrounded by big pericentral cells. Cortex consists of 1-2 layers of small pigmented cells. Tetrasporangium divided cruciate or tetrapartite, cystocarp hemispherical, pericarp consisted of 9-11 layers of ellipse cells. Central cells of pericarp are triangular or star-shape. Plant attached on rocks at lower littoral zone, appear in winter, spring and early summer with high biomass from March - June. It is necessary to collect more samples with reproductive organs.

5. **Gracilaria chorda** (Figure 6).

Alga caespitose, reddish or yellow-red. Holdfast discoid. Thallus cylindrical usually higher than 30 cm, 1-1.3 cm in diameter, main axes, branching alternate, slightly constricted at the base. In transverse section, it consists of a large medulla surrounded by a layer of pericentral cells. The cortex consists of 3 layers of small cells.

Tetrasporangium, divided cruciate or tetrapartite. Cystocarp hemispherical, scattered on surface of thallus. Pericarp consisted of 8-11 layers of cells arranged vertically, 1-3 inner layers with roundish cells. Remainder are ellipse or oval. Plants attached to rocks at medium littoral and lower littoral zones. Abundant in Spring and Summer (from March to July ). It is necessary to collect more samples with reproductive organs.

6. **Gracilaria gigas** Harv. (Figure 7).

Alga caespitose, 30-40 cm tall, reddish or red-brown. Holdfast discoid. Thallus terete, 2-5 mm in diameter, tapering at the base, sparse branching, alternate, no or slight constriction, acute apices. In transverse section, medulla consisted of 2 layers of large multiangular cells, colourless. When the plant is old, these cells fade making hollows in the thallus. Cortex consisted of 3-4 layers of small roundish or ellipse cells.

Tetrasporangium, divided cruciate or tetrapartite, cystocarp hemispherical, seated in cortex of whole thallus, pericarp consisted of 7-9 layers of cells longitudinally arranged, inner cell star-shape or multiangular central cells oblong, outer roundish or oblong. Plants attached on rocks, shell, alluvial soil (sand) at medium littoral zone and lower littoral zone in bay, occurring abundantly in Spring and early Summer at Hai Phong, Quang Ninh. It is necessary to collect samples with reproductive organs.
Figure 4: *Gracilaria arcuata.*

A. External morphology

B. Section of tetrasporic thallus

C. Cross-section of pericarp
Figure 5: *Gracilaria hainanensis.*

A. External morphology

B. Cross-section of tetrasporic thallus
Figure 6: Gracillaria chorda

A. External morphology

Figure 7: Gracilaria gigas.

C. Section of tetrasporic thallus

B. Section of cylindrical thallus

D. Cross-section of pericarp
A and B External morphology

C. Section of cylindrical D. thallus

Section of tetrasporic E. thallus.

Cross-section of pericarp
7. **Gracilaria bursa-pastoris** Silva (Figure 8).

Alga large caespitose, 20-35 cm tall or more, reddish or red. Holdfast conical. Thallus cylindrical, brittle, coarse, 1-2 mm in diameter with main axis. Branching abundant, botryoid. On main branch, coarse branchlet large with acute apex. In transverse section, medulla consisted of 2-3 layers of quite big cells, multiangular, colourless. Cortex consisted of 1-2 layers of small roundish or oblong cells. Tetrasporangium, divided cruciate or tetrapartite, occurring sparsely among cortex cells of whole thallus. Cystocarp hemispherical or conical, tapering at the base. Pericarp consisted of 7-8 layers of small cells, irregularly. Inner cells horizon-oblong, outer vertical-oblong, medulla star-shape or multiangular with not clearly walls. Plants attached to rocks, shells at medium and lower littoral zones. Abundant from March to July. It is necessary to collect samples with reproductive organs.

8. **Gracilaria blodgettii** Harv. (Figure 9).

Alga caespitose, lightly red or dark-brown, 15-40 cm tall or more. Holdfast discoid. Thallus terete, 1. 5-2 mm cm in diameter. Branching abundant, alternate unilateral, botryoid or annular. Main branch with short-small branchlets. Branches prolonged, base hardly constricted. Apices of branches somewhat constricted.

In transverse section, medulla consisted of a central cell surrounded by a pericentral cell. Cortex consisted of 3-4 layers of roundish or elongate cells, smaller from medulla to cortex.

Tetrasporangium divided cruciate or tetrapartite. Spermatangial conceptacle oblong or oval. Cystocarp hemispherical or conical. Pericarp consisted of 9-13 layers of horizontal - oblong cells, longitudinal arranged rows.

Plants attached on rocks, shells and other plants at medium and lower littoral zones. Found abundantly from October to the next July. This species is commonly cultivated in brackish water swamps along the coastal area of Hai Phong and Cat Hai island.

2.3 Discussion

During this study, specimens of *Gradlaria* spp. were collected in northern Vietnam and 8 species of the 13 reported by Nguyen Huu Dinh and Nguyen Van Tien, (1992) were identified. The 8 species of *Gradlaria* spp. identified in this study were:

1. *Gracilaria asiatica* Zhang et Xia (Syn. *G. verrucosa*)
2. *G. tenuistipitata* Zhang et Xia
3. *G. arcuata* Zan
4. *G. hainanensis* Zhang et Xia (Similar to *G. edulis*)
5. *G. chorda* Holm (Syn. *G. lemaneiformis*)
7. *G. bursa-pastoris* Silva
8. *G. blodgettii* Harv.
Figure 8: *Gracilaria bursa-pastoris.*

A. External morphology

B. Section of cylindrical thallus

C. Cross-section of pericarp
Figure 9: *Gracilaria blodgettii*

A. External morphology

B. Section of tetrasporic thallus

C. Cross-section of pericarp

D. Section of male thallus
The determination of *Gracilaria* species usually meets with the following difficulties:

- variability among species is not clearly defined.
- morphological features of species are influenced by environmental conditions leading to changes at different locations. The specimens collected normally lack reproductive organs, for this reason, *Gracilaria verrucosa* has previously been named as follows:

  - *Fucus verrucosa* Hudson 1762
  - *F. confervoides* Linnaeus 1786
  - *Gracilaria confervoides* Greville 1830
  - *Sphaerocoscus confervoides* Martens 1886
  - *Gracilaria asiatica* Zhang et Xia 1985

The *Gracilaria* variety has also been separated into many varieties such as *Hydropuntia*, *Carollopisis*, *Gracilariopsis* and *Polycavenosa*. Recently, some researchers agreed that *Gracilariopsis rhodotricha* and 16 species of the genus *Polycavemosa* belonged to the variety *Gracilaria*.

In 1978, a well-known Japanese taxonomist (Yamamoto) used spermatangial conceptacles position as a feature to determine *Gracilaria* species. In 1985, Zhang and Xia suggested using cystocarp form and pericarp structure as important characteristics for identifying *Gracilaria* species. This seems acceptable, as the structure of these reproductive organs change little.

In general, the taxonomy of *Gracilaria* species is quite complicated and synonyms are commonly used. In order to gain the correct taxonomic status it is necessary to collect specimens continuously when the seaweed is mature and has reproductive organs (tetrasporangium, spermatangial conceptacle and cystocarp).

### 3. PROCESSING OF GRACILARIA SPECIES

The technology of agar processing from *Gracilaria* spp. in Vietnam has been studied since 1963, however, although the procedure is simple, the yield and quality of agar is low.

From 1973-1980, Vietnam and Germany co-operated in researching industrial scale procedures and installing a pilot production line with a capacity of 50 tonnes agar/year at Ha Long canning factory in Hai Phong city. In 1981, this pilot plant was put on trial operation and only had a yield of 20 tonnes/year. With the improvement of equipment and some steps in the production line, the Ha Long canning factory currently produces around 120 tonnes agar/year.

From 1985 to 1990, the Research Institute of Marine Products (RIMP) concentrated on developing a stable procedure of agar processing on a small scale (10-15 tonnes/year) and family scale (3-10 kg/day). Now the Research Institute of Marine Products continuously studies techniques to upgrade agar quality and technology transferring for other enterprises.

In implementing the research programme of NACA for agar extraction for different *Gracilaria* species, it is hoped that new species will be founded to supply more raw materials for agar processing industry in Vietnam.
3.1 Materials and methods

All specimens of *Gracilaria* species collected during 1992 and 1993 were used. However, for two of the species collected, *Gracilaria hainanensis* and *G. gigas*, there was not enough of the specimens for agar analysis. Agar extraction was carried out under the procedure set up by Research Institute of Marine Products which was modified from Chandkrachang (1992). A diagram of the agar extraction procedure of RIMP is shown in Figure 10.

The moisture content of seaweed, agar yield, gel strength and ash content were determined according to NACA, 1992.

3.2 Results

A sample of *Gradlaria* spp. was extracted and its agar yield analysed in duplicate or triplicate to gain an average figure. Analytical results were obtained from 6 *Gracilaria* species collected from Hai Phong and Quang Ninh provinces (See Table 1 and Figure 2) and showed that the dried/fresh ratio, agar yield and gel strength were highest in *G. asiatica*, and its ash content was also the lowest. It is a widely distributed species of *Gracilaria* in Vietnam and may become the main culture species in brackish water swamps in northern Vietnam.

Table 1: Results of the analysis of *Gracilaria* spp.

<table>
<thead>
<tr>
<th>Species</th>
<th>Place of collection</th>
<th>Date of collection</th>
<th>Dry/wet weight</th>
<th>Moisture Content (%)</th>
<th>Agar Yield (%)</th>
<th>Gel Strength (g/cm²)</th>
<th>Ash of Agar (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>G. verrucosa</em></td>
<td>Dinh Vu, Hai Phong</td>
<td>20.06.92</td>
<td>11.25</td>
<td>10.2</td>
<td>29.5</td>
<td>516</td>
<td>3.2</td>
</tr>
<tr>
<td><em>G. blodgettii</em></td>
<td>Phu Long, Hai Phong.</td>
<td>27.05.93</td>
<td>10.64</td>
<td>10.0</td>
<td>26.5</td>
<td>278</td>
<td>3.6</td>
</tr>
<tr>
<td><em>G. tenuistipitata</em></td>
<td>Lien Vi, Quang Ninh</td>
<td>21.06.92</td>
<td>9.60</td>
<td>11.3</td>
<td>19.7</td>
<td>338</td>
<td>3.4</td>
</tr>
<tr>
<td><em>G. chorda</em></td>
<td>Hai Ninh, Quang Ninh</td>
<td>28.05.93</td>
<td>10.40</td>
<td>12.5</td>
<td>21.5</td>
<td>290</td>
<td>4.0</td>
</tr>
<tr>
<td><em>G. arcuata</em></td>
<td>Do Son, Hai Phong</td>
<td>07.05.93</td>
<td>8.06</td>
<td>16.0</td>
<td>17.6</td>
<td>258</td>
<td>4.2</td>
</tr>
<tr>
<td><em>G. bursa-pastoris</em></td>
<td>QuanLand,Quang Ninh</td>
<td>29.05.93</td>
<td>9.65</td>
<td>15.0</td>
<td>15.2</td>
<td>128</td>
<td>4.5</td>
</tr>
</tbody>
</table>

*G. blodgettii* has an agar yield higher than that of *G. tenuistipitata*, but its gel strength is lower. *G. boldgettii*, having a high productivity, has been cultured a lot in Hai Phong and Cat Hai island, whereas *G. tenuistipitata* is cultured in the central provinces of Vietnam.

*G. chorda* has a agar yield higher than that of *G. tenuistipitata* and its gel strength is higher than that of *G. blodgettii*. The latter has not been studied for the purpose of cultivation but it is a species which should be paid more attention to in Vietnam.

*G. arcuata* and *G. bursa-pastoris* have little natural biomass and the yield and quality of agar are lower than those of other 4 species above-mentioned. These species are not principal objects for study in Vietnam.
Figure 10: Agar extraction procedure at RIMP.

Dried Seaweed
↓
Washing
↓
Alkaline treatment (2.4-3% NaOH, 90-98°C, 1-2 hours)
↓
Cleaning
↓
De colouring (0.7-1g NaOCl/1,30°, cleaning)
↓
Citric acid treatment (0.2-0.3%, 30-60°, cleaning)
↓
Cooking (add CH₃COOH and Na₂B₄O₇·10H₂O)
↓
Filter (at 85-95 °C depending on species)
↓
Cooling
↓
Cutting
↓
Semi-dewater press
↓
Freezing
↓
Defrosting
↓
Drying
↓
Powder Grinding
↓
Packaging
The quality of *Gracilaria* spp. and its agar in one species differed in various areas depending on ecological conditions. For cultivated *Gracilaria* spp., the yield and quality of agar still depends on culturing methods (seeding density, water-changing ability, fertilising, harvesting). In this study, *Gracilaria asiatica* samples were collected from various areas of 4 the coastal provinces of Northern Vietnam and analysed for the quality of seaweed and its agar (See Table 2). The analytical results showed that *Gracilaria asiatica* growing in Dinh Vu, Hai Phong and Yen Hung, Quang Ninh had the best quality. These areas are determined as having rich nutritional waters and other ecological conditions which are suitable for the growth and development of *Gracilaria* spp.

Table 2: Results of the analysis of *Gracilaria* spp. from different locations.

<table>
<thead>
<tr>
<th>Place of collection</th>
<th>Date of collection</th>
<th>Dry/wet weight</th>
<th>Agar Yield (%)</th>
<th>Gel Strength (g/cm²)</th>
<th>Ash of Agar (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dinh vu, Hai Phong</td>
<td>20.06.92</td>
<td>11.25</td>
<td>29.5</td>
<td>516</td>
<td>3.2</td>
</tr>
<tr>
<td>Bang La, Hai Phong</td>
<td>20.06.92</td>
<td>11.4</td>
<td>27.0</td>
<td>456</td>
<td>3.4</td>
</tr>
<tr>
<td>Cat Hai, Hai Phong</td>
<td>20.06.92</td>
<td>10.5</td>
<td>24.5</td>
<td>319</td>
<td>3.6</td>
</tr>
<tr>
<td>Yen hung, Quang ninh</td>
<td>20.06.92</td>
<td>10.86</td>
<td>31.0</td>
<td>474</td>
<td>3.25</td>
</tr>
<tr>
<td>Thai thuy, Thia binh</td>
<td>20.06.92</td>
<td>11.0</td>
<td>28.0</td>
<td>396</td>
<td>3.2</td>
</tr>
<tr>
<td>Hai hau, NamHa</td>
<td>20.06.92</td>
<td>10.5</td>
<td>26.9</td>
<td>387</td>
<td>3.4</td>
</tr>
</tbody>
</table>

### 3.3. Discussion

The identification of 3 *Gracilaria* species: *G. asiatica*, *G. blodgettii* and *G. tenuispitata* with the highest quality among 13 species existing in Northern Vietnam corresponds with previous research results by Do and Nguyen (1990) and Nguyen Van Tien (1992). The above 3 species have also been researched and cultured for raw material for agar extraction in Vietnam. In addition, to the above, *Gracilaria* species and *G. chorda* with high economic value are also being studied.

The cultivation of *Gracilaria* species for high quality agar depends on many factors including site selection, the structure of ponds (in case culturing in brackish water area), culture methods, suitable harvest time and semi-processing. Studies on the biological characteristics of *Gracilaria* spp. are necessary in order to produce high-grade, quality products. During previous years, 3 species of cultivated *Gracilaria* spp. have been thoroughly studied in Vietnam. The introduction of new culture species is needed as are relevant studies to gain products of high economic value.

### 4. CONCLUSIONS

- During this study, only 8 of the 13 species in northern Vietnam, listed by NguyenHuu Dinh and Nguyen Van Tien (1990), were found.

- Among the 8 species found, 4 had a high economic value. Of these 4 species, 3species were studied and cultivated, namely: *Gracilaria asiatica*, *G. blodgettii* and *G.tenuispitata*. *G. chorda* needs further study and trial cultivation.

- The quality of raw materials, quality and yield of agar depend on the ecologicalconditions of each region and harvesting time. Specimens should be collected at the peak of development during the mature period for correct analytical results.
• The collection of specimens in season (the period that *Gracilaria* spp. has reproductive organs), will make identification of the species easier. It is necessary to supply enough funds for field-collecting continuously at the mature period in order to gain specimens with reproductive organs.

5. PROPOSALS

• It is necessary to invest in the systematic study of the components and ecosystems of the *Gracilaria* spp. flora of Vietnam. On the basis of this an accurate estimate of the natural resources, preservation measures as well as a development plan can be made for cultivating and agar processing in whole country.

• Further study is needed and the application of new-techniques to culture seaweeds with higher economic value in Vietnamese coastal areas. More attention should be paid to species that can be cultured in gulfs and bays.

• New technologies in agar processing should be promptly applied to produce high-quality products which can meet the requirements of both domestic and international markets.

• Vietnam must co-operate with other countries in the region for training and study of *Gracilaria* spp. and agar processing. Taxonomic methods for *Gracilaria* spp. should be standardised and manuals for culture methods and analysis of agar quality produced.

• Regional Centres for research into *Gracilaria* spp. and agar should be established under NACA so that researchers can exchange information on ecology, taxonomy, biophysiology, biochemistry, technique of cultivation of *Gracilaria* spp. and agar processing technology.

REFERENCES


Studies on Intertidal Algae in Southern Coasts of Iran  
(Oman Sea and Persian Gulf)  

H. Shoghi*  
Iranian Offshore Fisheries Research Centre  
Port of Chabahar, Iran  

A number of studies have been carried out to identify and map the distribution of Iranian coastal algae. The first attempt to identify algae in the Persian Gulf was made by Diesing and Eadlicher in 1845. This study was followed by Boergesan and Koie (1939) and Newton (1955). It is worth mentioning that all these studies were carried out in the Persian gulf coasts and this is the first time that an article has been presented describing Iranian algae in Oman sea.

During four study trips fielded in 1993, 23 species of brown algae, 16 species of green algae and 29 species of red algae were identified in coasts of Persian Gulf and Oman Sea.

It was observed that Sargassum spp., Ulva lactuca, and Chondrus crispus species were common in the Oman Sea and Padina spp., Enteromorpha intestinalis, Chodrus crispus species were quite well distributed in the Persian Gulf.

The Iranian Fishing Company (Shilat) has a programme to develop the culture of economic and commercial algal species in Iranian waters and to establish new processing industries to obtain agar, alginate and carrageenan from algae. Any co-operation with relevant companies and organisations would be accepted and appreciated. Iran looks forward to joining the regional co-operation efforts in seaweed development organised under NACA.

* Iran did not participate in the regional study, but expressed interest in participation in the Final Workshop, and presented a country-status paper, only an abstract of which is available
SRI LANKA
V. Pahalawattaarchchi*,
National Aquatic Resources Agency,
Crow Island, Colombo 15, Sri Lanka.

ABSTRACT

Of the 260 seaweed species found growing along the coast of Sri Lanka, *Gracilaria* species are the most commonly used for food. Two species, *G. verrucosa* and *G. edulis*, occur in commercially valuable quantities in Kalpitiya, Trincomalee and Mannar.

*G. edulis* was recorded in the 1950's in the Kalpitiya area. Large quantities of *G. verrucosa* (*G. confervoides*) have been recorded in Koddiyar Bay near Trincomalee. *G. edulis* has the highest yield of good quality agar when extracted at pH 5. The ideal heating time was 4 hours. Comparison of cultured and wild seaweeds showed that the latter can give a satisfactory amount of gel content and strength. Comparative studies of three of the most common species found in the northern part of Sri Lanka, namely *Hypnea* spp., *Gracilaria lichenoides* and *Gelidium* spp. have shown a seasonal variation in agar content in these species, the plants showing the highest agar content in January. During May to November, wild *Gracilaria* spp. grows well in the Puttalam lagoon. This coincides with the south-west monsoon period and also the season for prawn fishing in the lagoon.

Sri Lanka exported an average of 100 tonnes/year of dried raw *Gracilaria* spp. since early in the last century. Exports have, however, dwindled over the years for various reasons. There is an increasing domestic demand for agar especially for medical research purposes and the confectionery industry. During 1985, Sri Lanka imported a wide range of seaweed products processed from red and brown seaweeds.

The National Aquatic Resources Agency launched a project in 1988 at Kalpitiya (north-west coast) to find out optimum growth conditions and culture sites for *G. edulis* in Puttalam lagoon. Two methods were followed for cultivation, namely vegetative and spore setting techniques. The culture of *Gracilaria* on a commercial scale and improvement of processing methods are considered of immense importance to national development from seaweed products and cut down the import expenditure on refined agar. Polyculture of *Gracilaria* spp. with brackish water fish species is recommended for future development.

* Sri Lanka did not participate in the study. The status paper presented by the Sri Lankan participant at the Final Workshop is presented here.

1. INTRODUCTION

There are nearly 260 species of seaweeds growing along the Sri Lankan coastline (which is approximately 1700 km long), of which only 2 species are of commercial interest (Durairtnam, 1961). *Gracilaria* varieties, known as "Ceylon moss" are the varieties most commonly used as food. *G. verrucosa* and *G. edulis* are the two main species of commercial importance at present and occur in commercially valuable quantities in three main areas of Sri Lanka (Figure 1), namely Kalpitiya, Trincomalee and Mannar (Anon., 1952).
Gracilaria edulis was reported in the 1950's in the Kalpitiya area. Large quantities of Gracilaria verrucosa (Gracilaria confervoides) have been recorded in Koddiyar Bay near Trincomalee (Duiriratnam 1965). According to the author G. verrucosa gives a 20-25 % yield of agar. Recent studies regarding the agar yield of G. edulis revealed that it has the highest constant (48.7%) of best quality agar when extract at pH 5. The ideal heating time was 4 hours, on the basis of yield and quality.(Jayasinghe & Jayasinghe 1994). According to the comparison of cultured and wild seaweeds, the latter has given satisfactorily amounts of gel content, working strength, moisture content, ash content and insoluble ash contents (Jayasinghe & Jayasinghe ,1994, unpublished data). Studies in the northern part of Sri Lanka, have shown a seasonal variation in agar content in G. lichenoides, the plant showing the highest agar content in January.(Table 1).
Table 1: Agar content (dry weight) of different red algae from northern Sri Lanka (Mandativu).

<table>
<thead>
<tr>
<th></th>
<th>1977</th>
<th>1978</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>October</td>
<td>December</td>
</tr>
<tr>
<td>Gracilaria</td>
<td>.37%</td>
<td>-</td>
</tr>
</tbody>
</table>

Source: Arumugam et al. (1981)

During May to November wild Gracilaria spp. grows well in the Puttalam lagoon (Subasinghe & Jayasuriya 1989a). This coincides with the south west monsoon period and also the season for prawn fishing in the lagoon. Figure 2 shows the areas of Puttalam Lagoon where natural beds of seaweeds are found. People who collect seaweeds are mainly engaged in prawn fishing and only when the local buying agents request seaweeds do they collect them (Subasinghe, & Jayasooriya, 1989b).

Figure 2: Locations in the Puttalam Lagoon where natural beds of seaweeds are found.
2. PRESENT STATUS OF THE GRACILARIA INDUSTRY

2.1 Wild stocks of Gracilaria

Sri Lanka exports about 100 tonnes/yr of dried raw Gracilaria spp., which has been actively collected, dried and exported since early in the last century, though there has been a recent decline in wild stocks (Subasinghe & Jayasuriya, 1989a). Figure 3 shows the distribution of seaweed beds of commercial importance. The quantity exported in 1972 was around 50 tonnes with an export value of Rs.76,000 (FOB). In 1986, these exports increased to 150 tonnes. Of these, 70 tonnes were produced from the Kalpitiya area, the balance came from the Trincomalee area (Subasinghe & Jayasooriya 1989a,b).

Over the last 7 years, no harvesting of Gracilaria spp. has been done in Trincomalee due to the unsettled conditions prevailing in the northern part of the country. According to export data, only 10 tonnes of dried Gracilaria spp. were exported in 1987. In 1988, it was further reduced to 5 tonnes. At present, according to the information provided by the export companies there is a very high renewed demand for this resource. Hence, the export companies find it difficult to cope with the present demand due to limited natural stocks.

2.2 Status of the seaweed processing industry

Most countries exporting seaweeds have realised the benefits of exporting processed seaweed products rather than the dried seaweeds. Understanding the distinct advantages of exporting unprocessed seaweed products, the government launched several pilot projects to examine the feasibility of extracting alginic acid and agar from local seaweeds, during the period of 1960-1970. Even at that time those products were found to be of international standard.

The government decided to expand this industry by setting up a network of collecting centres and processing plants under the management of District development councils. To accelerate the programme, the government introduced an import quota system for manufactures. Nevertheless, a change in government policy in the mid 1970's cancelled the import quota system and the local market once again started to import seaweed products. Since then Sri Lanka has remained as an exporter of dried seaweed.

2.3 Demand for the seaweed products in Sri Lanka

In Sri Lanka, with the development of new industries, there is a demand for agar especially for medical research purposes and the confectionery industry. During 1985, Sri Lanka imported a wide range of seaweed products processed from red and brown seaweeds at a value of Rs 14 million. (Customs Department Report).

2.4 Present status of culture practices of Gracilaria spp.

An alternative way to prevent the exploitation of natural seaweed stocks is cultivation. Taking into account the importance of establishing a steady source of Gracilaria spp. for that purpose, the National Aquatic Resources Agency launched a project in 1988 at Kalpitiya (north west coast) to establish optimum growth conditions and culture sites for G. edulis in Puttalam lagoon (Jayasooriya 1993).

Two culture methods were used, namely vegetative and spore setting techniques. From the vegetative propagation technique the plant growth was 0.42 cm/day and from the spore setting technique it was 0.25 cm/day. Gracilaria edulis showed high relative growth rates in Puttalam lagoon during the south west and the north east monsoon.
Figure 3: Distribution of seaweed of commercial importance in Sri Lanka.
The most effective substratum found for the vegetative propagation was plastic nets in an optimum depth of 20 cm with optimum density of 100 fragments/m² (Jayasooriya 1993). The author also recommended the spore settlement technique for the development of small scale culture practices oilier than commercial culture due to the lack of spore bearing plants in the lagoon during parts of the year. The author also revealed that fluctuations in environmental factors such as salinity and turbidity of water adversely affected the growth of *Gracilaria* spp.. Hence, more work is needed on the aspect of turbidity in relation to light attenuation for finding suitable sites in the lagoon for the culture of *Gracilaria edulis*.

The culture of *Gracilaria* spp. on a commercial scale and improvement of processing methods are considered to be of immense importance to increase the GNP from seaweed products and cut down the import expenditure on refined agar.

Polyculture of *Gracilaria* spp. with edible brackish water fish species can be recommended for future development of seaweed culture.

REFERENCES


SUMMARY OF THE RESULTS OF THE REGIONAL STUDY

1 POSITION OF THE COUNTRY REPORTS

1.1 Taxonomy and ecology

Bangladesh
No report of *Gracilaria* spp., but it is possible that the survey done may have been limited to riverine areas. More detailed surveys towards the outer part of the bay will probably reveal some *Gracilaria* spp.

China
Scientific names appear to be correctly applied for three species - *G. lemaneiformis*, *G. tenuistipitata* and *G. asiatica*. Taxonomic description complete for three species. *Gracilaria* sp. needs verification, more materials and samples are needed.

Ecological data: Complete except for depth and habitat (exposed/not exposed during low tides).

India
Scientific names appear to be correctly applied, except for *G. crassa*. Taxonomic descriptions lack information on male reproductive structures.

Ecological data: Complete except for depth and habitat (intertidal/subtidal or exposed/not exposed during low tides).

Indonesia
Scientific names are correctly applied for *G. eucheumoides* and *G. salicornia*, but *G. edulis*, *G. lemaneiformis* and *Gracilaria* spp. need verification. More materials are needed.

Ecological data: Lacks information on habitat, type of substrate, whether intertidal or subtidal and exposed or not during low tides.

Malaysia
Taxonomic description very incomplete. Needs taxonomic description of each species. The scientific names applied have to be verified, especially *G. changii* and *Gracilaria* sp.

Ecological data: Notes are incomplete and should be summarised in a table.

Myanmar
Taxonomic description complete but the format should conform to the regular format used in taxonomic studies. The identity of *G. verrucosa* should be re-evaluated as this species is not found in Asia. *G. crassa* should be changed to *G. salicornia*.

Ecological data: Should be condensed in the table "Summary……".

Philippines
No taxonomic description of the species which should be a major part of the report. At least the species analysed for agar should be included. The species in the data sheets are all *Gracilaria* sp.

Thailand
Taxonomic description present but incomplete. Only gross morphology of the genus is described, a more detailed description is needed. The scientific names applied are probably correct but the lack of important taxonomic characters in the description is a source of doubt.

Ecological data Lacking some ecological notes on habitat, abundance and reproductive status.
Vietnam

Taxonomic notes available but the binomids should be placed at the start of the description. The species need to be verified especially in the absence of note of the male structures.

Ecological data: Lack of data on habitat, type of substrate and depth.

1.2 Processing technology of *Gracilaria*

China

Complete analysis of agar quality in almost all of the essential parameters. In addition, different techniques of agar extraction are also reviewed and compared. Good quality agar is mostly from *Gracilaria tenuistipitata* collected from June to September. Although it should be noted that the values of agar content are very high and need to be verified. Due to its long experience in seaweed processing, China has a leading role in the seaweed industry. With this expertise, they are able to help other NACA member countries to develop seaweed resources, as well as the analysis of seaweed products.

**Culture of Gracilaria:**

i. **Candidates for culture:**
   - *G. asiatica*
   - *G. lemaneiformis*
   - *G. hainanensis*
   - *G. tenuistipitata var. liui*

   All of these species, especially *G. asiatica* and *G. tenuistipitata var. liui* have been studied in detail with respect to their reproduction, ecological characteristics and culture methods, as well as their agar contents and qualities.

ii. **Culture methods**
   - Intertidal culture - Suitable for all candidate species.
   - Floating culture - Suitable for *G. asiatica*, *G. lemaneiformis* and *G. hainanensis*.
   - Pond culture - Suitable for *G. tenuistipitata var. liui* because it is hard to find its sexual organs and tetrasporangia over whole seasons. It is not like *G.asiatica*, therefore the thalli would decay after releasing the spores and gametes.

iii. **Other aspects**
   - Genetic studies. Use the experience gained on seaweed genetic studies of *Laminaria* and *Porphyra* to introduce and select new varieties with fast growth rates and higher agar quality.
   - Tissue culture Develop new techniques to improve methods of spore collection

India

Most of the agarophyte seaweed resources in India are *G. edulis* with low gel strength. As *G. edulis* is the major seaweed resource attempts should be made to improve agar extraction techniques.

i. **Candidates for culture:**
• *G. edulis*
• *G. tenuistipitata*

The natural resource of *G. edulis* is abundant and the species is adapted to the local climate and other natural conditions, it is therefore much easier to promote local seaweed farming industries. The species, *G. tenuistipitata var. liui* is not mentioned in the country report.

ii  **Culture methods**

Intertidal culture and pond culture are good for Indian natural conditions. Particularly using polyculture techniques to farm seaweed and shrimp or finfish together.

iii  **Suggested projects**

Intensify studies on taxonomy and ecology of *Gracilaria* spp. and conduct suitable extension methods for culture.

**Indonesia**

From the results of analysis of agar quality, there are at least two species of *Gracilaria* which give good agar quality in terms of gel strength, *G. edulis* and *G. lemaneiformis*. Moreover, most of the species have low gelling temperatures, which is an important characteristic of agar quality preferred for applications in microbiological mediums. There is an opportunity to develop high-value agars. Further study should be carried out on process development as well as development of the high-value agar product. Improvement of the agar extraction process should be studied to increase agar yields and clarity.

**Malaysia**

The method of agar extraction in the study was done without alkali pre-treatment and with simple processing equipment which was provided by NACA after the first workshop. However, the quality of selected agar samples showed relatively high gel strength. There is a high possibility of the process development for agar extraction to obtain an agar product with high gel strength. The support of equipment as well as training of the personnel is recommended for the development of seaweed processing technology in Malaysia since local seaweed resources are readily available and there is potential for good quality agar.

**Myanmar**

The local available species which give a similar gel quality are *G. verrucosa*, *G. edulis* and *G. crassa*. More development in both processing techniques and product development is recommended. More research work is needed. Support for equipment and well trained personnel should be provided.

Myanmar gave descriptions of 7 species: *G. crassa*; *G. edulis*; *G. eucheumoides*; *G. foliifera*; *G. millardetii*; *G. textorii*; and *G. verrucosa*. They also reported agar analysis from species mentioned above, except *G. eucheumoides*. However, ecological data was obtained from only 2 species, *G. edulis* and *G. foliifera*. Culture experiments showed that *G. edulis* can be grown eight months of the year with monthly harvests from April to September, suggesting commercial potential. It also grows well in the natural conditions and is suitable for culture both in inland ponds and in the open seas
Philippines  
From the results of agar extraction and analysis, there are several species of *Gracilaria* which give good agar quality, such as *G. heteroclada*, *G. tenuistipitata* and *G. firma*. With high agar yields of 16-20 %, these *Gracilaria* species will be important raw material sources for agar production. Systematic and well-developed experience in analysis of agar is the most important resource to facilitate the development of the agar industry in the Philippines. Funding support would aid the progress of the development of the seaweed industry in the Philippines.

**General comments on report.**

1. Supply taxonomic description of species
2. Supply data on: ecological parameters; gelling temperature; seasonally in reproductive states; and abundance
3. Species recommended for farming include only three species  
   *G. heteroclada*, *G. tenuistipitata* and *G. firma*

Thailand  
The *Gracilaria* which gave the best quality agar were *G. fisheri* and *G. tenuistipitata* which grows naturally in the southern coastal area of the country. However, the availability of the seaweed raw material is quite limited in Thailand. Incentives to farm seaweed are needed for the local coastal people to increase production. The seaweed processing industry is promoted by the Royal Thai government, for instance by the reduction of the import tax for seaweed raw materials and research funding for seaweed production and processing. The initiative of the seaweed processing industry needs to be promoted in terms of its economic, social and environmental benefits.

Thailand reported 7 species collected from eastern and southern Thailand: Trat, Songkhla and Pattani. They are *G. changii*, *G. edulis*, *G. firma*, *G. fisheri*, *G. irregularis*, *G. salicornia*, and *G. tenuistipitata*. Only 5 species, one sample each were used for agar analysis. The results show that *G. fisheri* and *G. tenuistipitata* were the most valuable followed closely by *G. firma*. In contrast, *G. salicornia* and *G. changii* gave lower yields and low quality agar. It was recommended that *G. fisheri* and *G. tenuistipitata* be further studied as useful resources for agar production.

Vietnam  
*Gracilaria verrucosa* gave high yields and high gel strength of agar. Improvement of the agar extraction method should be further studied with international support. Co-operation among local institutions to strengthen personnel and resource ability are recommended for the development of the seaweed processing industry in Vietnam.

The resource persons questioned why this study was limited to a small region in the north of the country and the exact method of extraction. There was also no data about ecological parameters of the 3 strains of *G. verrucosa*.
1.3 General comments on the country reports

Vietnam and Indonesia

- Ecological parameters are missing, for example, for Vietnam we do not know if samples come from culture or the wild.
- What are the annual relationships between sexual maturation, growth rate and agar yield and quality.
- It is essential to standardise the taxonomy, methods of measurement and extraction of agar (date of samples, water temperature, drying methods, gelling temperature etc.). It is necessary to make a Centre of Taxonomy and Methodology supervised by NACA as point of reference.

Bangladesh

- Bangladesh reported an absence of *Gracilaria* but has recommended introduction of *Gracilaria* for culture. Also, they recommended studies of *Hypnea* and *Sargassum*.

1.4 Recommendations (Thailand, Myanmar and other countries)

- Studies on taxonomy and ecological parameters which are important information for cultivation should be followed-up.
- Collection must be done more frequently, at least once a month during the growing season.
- For species determination, the male and female plants should be obtained.
- Participants should give descriptions from the specimens they collected.
2. AGAR QUALITY CHARACTERISTICS OF THE DIFFERENT SPECIES IN THE COUNTRIES IN THE REGION

Quality of agar according to Japanese (Chandrkrachang and Chinadit, 1988)

<table>
<thead>
<tr>
<th>Grade</th>
<th>Mean gel strength (g/cm²)</th>
<th>Species name</th>
<th>Mean Yield of Agar (%)</th>
<th>No of countries observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Special grade agar</td>
<td>608</td>
<td>G. asiatica*</td>
<td>48.2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>570</td>
<td>G. lemaneiformis*</td>
<td>28.48</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>892</td>
<td>G. heteroclada</td>
<td>20.05</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>649</td>
<td>G. firma</td>
<td>18</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>850</td>
<td>G. eucheumoides</td>
<td>17</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>758</td>
<td>G. fisheri</td>
<td>12.8</td>
<td>1</td>
</tr>
<tr>
<td>Grade I</td>
<td>578</td>
<td>Gracilaria sp. *</td>
<td>51.3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>584</td>
<td>G. tenuistipitata *</td>
<td>22.2</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>356</td>
<td>G. salicornia</td>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>565</td>
<td>Gracilaria sp.</td>
<td>10.1</td>
<td>1</td>
</tr>
<tr>
<td>Grade II</td>
<td>278</td>
<td>G. blodgettii</td>
<td>26.5</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>290</td>
<td>G. chorda</td>
<td>21.5</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>257</td>
<td>G. changii</td>
<td>20.0</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>292</td>
<td>G. verrucosa</td>
<td>20</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>G. fastigiata</td>
<td>19.3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>280</td>
<td>G. edulis o</td>
<td>15.3</td>
<td>3</td>
</tr>
<tr>
<td>Grade III</td>
<td>34.4</td>
<td>G. corticata var.</td>
<td>22.4</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>corticata o</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Gracilaria sp.</td>
<td>22.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>47.2</td>
<td>G. corticata var</td>
<td>19.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>cylindrica o</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>128</td>
<td>G. bursa-pastoris</td>
<td>15.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>85</td>
<td>G. textorii</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>76</td>
<td>G. millardetii</td>
<td>14.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>64.5</td>
<td>G. foliifera</td>
<td>13.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>62.4</td>
<td>G. crassa o</td>
<td>12.1</td>
<td>2</td>
</tr>
</tbody>
</table>

* These results take into account the data from China that give % yield of agar about 4 times higher than the other countries.

° These results take into account the data from India that give gel strengths ten times higher than the other countries.

* The countries involved are Bangladesh, China, India, Indonesia, Malaysia, Myanmar, Philippines, Thailand and Vietnam.

3. SUMMARY OF RESULTS ON GRACILARIA SPECIES

Table 3.1: China
Table 3.2: India
Table 3.3: Indonesia
Table 3.4: Malaysia
Table 3.5: Myanmar
Table 3.6: Philippines
Table 3.7: Thailand
Table 3.8: Vietnam
### 3.1 Summary of Results on *Gracilaria* Species in China (1 of 2)

<table>
<thead>
<tr>
<th>Species</th>
<th>Date</th>
<th>Abundance</th>
<th>Salinity (ppt)</th>
<th>Temp (°C)</th>
<th>Substrate</th>
<th>Depth (cm)</th>
<th>Rep. Stage</th>
<th>Yield (%)</th>
<th>Gel strength (g/cm²)</th>
<th>Gelling Temp. (°C)</th>
<th>Melting Temp. (°C)</th>
<th>Method of extraction</th>
<th>Code (Table IV, Country Report)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G. lemaneiformis</td>
<td>1-6-94</td>
<td>+</td>
<td>28.6-31.0</td>
<td>23-26</td>
<td>Rocky</td>
<td></td>
<td>Y</td>
<td>46.4</td>
<td>848</td>
<td>93.5</td>
<td>91.0</td>
<td>5% NaOH</td>
<td>1A Quingdao</td>
</tr>
<tr>
<td>G. lemaneiformis</td>
<td>27-8-94</td>
<td>+</td>
<td>28.6-31.0</td>
<td>23-26</td>
<td>Rocky</td>
<td></td>
<td>Y</td>
<td>47.6</td>
<td>872</td>
<td></td>
<td></td>
<td></td>
<td>1B Quingdao</td>
</tr>
<tr>
<td>G. asiatica</td>
<td>1-6-94</td>
<td>+</td>
<td>28.6-31.0</td>
<td>23-26</td>
<td>Rocky-sand</td>
<td></td>
<td>Y</td>
<td>44.1</td>
<td>682</td>
<td>80.0</td>
<td>92.0</td>
<td></td>
<td>2A Quingdao</td>
</tr>
<tr>
<td>G. asiatica</td>
<td>24-7-94</td>
<td>+</td>
<td>32.3</td>
<td>28-29</td>
<td>Muddy-sand</td>
<td></td>
<td>Y</td>
<td>38.2</td>
<td>482</td>
<td>92.5</td>
<td>95.0</td>
<td>3A Shanyao</td>
<td>3A Shanyao</td>
</tr>
<tr>
<td>G. tenuispitata</td>
<td>31-7-94</td>
<td>+</td>
<td>32.3</td>
<td>31.0</td>
<td>Muddy-sand</td>
<td></td>
<td>V.P.</td>
<td>44.7</td>
<td>634</td>
<td>95.0</td>
<td>93.5</td>
<td></td>
<td>3B Shanyao</td>
</tr>
<tr>
<td>G. tenuispitata</td>
<td>3-9-94</td>
<td>+</td>
<td>23.0</td>
<td>29.0</td>
<td>Muddy-sand</td>
<td></td>
<td>V.P.</td>
<td>35.0</td>
<td>1001</td>
<td>103.2</td>
<td>96.8</td>
<td>4B Dongshan Island</td>
<td>3C Shanyao</td>
</tr>
<tr>
<td><em>Gracilaria</em> sp.</td>
<td>17-7-94</td>
<td>+++</td>
<td>28.3</td>
<td>37-39</td>
<td>Muddy-sand</td>
<td></td>
<td>V.P.</td>
<td>48.7</td>
<td>609</td>
<td>96.8</td>
<td>93.0</td>
<td></td>
<td>4C Dongshan Island</td>
</tr>
<tr>
<td><em>Gracilaria</em> sp.</td>
<td>17-7-94</td>
<td>+++</td>
<td>24.2</td>
<td>37-39</td>
<td>Muddy-sand</td>
<td></td>
<td>V.P.</td>
<td>57.5</td>
<td>578</td>
<td>93.0</td>
<td>94.0</td>
<td></td>
<td>4D Dongshan Island</td>
</tr>
<tr>
<td><em>Gracilaria</em> sp.</td>
<td>20-8-94</td>
<td>+++</td>
<td>23.1</td>
<td>28-29</td>
<td>Muddy-sand</td>
<td></td>
<td>V.P.</td>
<td>47.6</td>
<td>549</td>
<td>94.0</td>
<td>94.0</td>
<td></td>
<td>4D Dongshan Island</td>
</tr>
<tr>
<td>G. <em>tenuispitata</em> v. <em>liui</em></td>
<td>26-6-94</td>
<td>+++</td>
<td>11.7</td>
<td>34.7</td>
<td>Muddy-sand</td>
<td></td>
<td>V.P.</td>
<td>49.7</td>
<td>363</td>
<td>93.5</td>
<td>97.2</td>
<td></td>
<td>5A Quiantou</td>
</tr>
<tr>
<td>G. <em>tenuispitata</em></td>
<td>15-6-94</td>
<td>+++</td>
<td>20.4</td>
<td>29</td>
<td>Muddy-sand</td>
<td></td>
<td>V.P.</td>
<td>38.0</td>
<td>720</td>
<td>99.2</td>
<td>97.2</td>
<td></td>
<td>5B Diancheng</td>
</tr>
<tr>
<td>G. <em>tenuispitata</em></td>
<td>18-8-94</td>
<td>0</td>
<td>27.2</td>
<td></td>
<td>Muddy-sand</td>
<td></td>
<td>V.P.</td>
<td>33.3</td>
<td>446</td>
<td>97.2</td>
<td>97.2</td>
<td></td>
<td>5C Diancheng</td>
</tr>
</tbody>
</table>
### 3.1 Summary of Results on Gracilaria Species in China (2 of 2)

<table>
<thead>
<tr>
<th>Species</th>
<th>Date</th>
<th>Abundance</th>
<th>Salinity (ppt)</th>
<th>Temp (°C)</th>
<th>Substrate</th>
<th>Depth (cm)</th>
<th>Rep. Stage</th>
<th>Yield (%)</th>
<th>Gelstrength (g/cm²)</th>
<th>Gelling Temp. (°C)</th>
<th>Melting Temp. (°C)</th>
<th>Method of extraction</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>G. tenuistipitata var. liui</td>
<td>16.-8-94</td>
<td>+++</td>
<td>7.33-8.62</td>
<td>29.2-31.0</td>
<td>Sandy-mud</td>
<td>V.P.</td>
<td>44.9</td>
<td>373</td>
<td>92.8</td>
<td>&quot;</td>
<td>&quot;</td>
<td>7A Rongshan</td>
<td></td>
</tr>
<tr>
<td>G. tenuistipitata var. liui</td>
<td>12.-6-94</td>
<td>+</td>
<td>22.1</td>
<td>33-36</td>
<td>Muddy</td>
<td>V.P.</td>
<td>42.8</td>
<td>360</td>
<td>94.5</td>
<td>&quot;</td>
<td>&quot;</td>
<td>8A Beihai</td>
<td></td>
</tr>
</tbody>
</table>

Y = Reproductive structures present  
V.P = Vegetative propagation.
### Summary of Results on *Gracilaria* Species in India (1 of 2)

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>Date</th>
<th>Abundance</th>
<th>ECOLOGICAL PARAMETERS</th>
<th>AGAR YIELD AND QUALITY</th>
<th>REMARKS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Salinity (ppt)</td>
<td>Temp (°C)</td>
<td>Substrate</td>
</tr>
<tr>
<td><strong>G. edulis</strong></td>
<td>4-6-92</td>
<td>+++</td>
<td>31.5</td>
<td>30</td>
<td>Sand/coral</td>
</tr>
<tr>
<td></td>
<td>14-8-92</td>
<td>+</td>
<td>35.84</td>
<td>28.4</td>
<td>Sand/coral</td>
</tr>
<tr>
<td></td>
<td>29-9-92</td>
<td>+</td>
<td>34.62</td>
<td>29.2</td>
<td>Sand/coral</td>
</tr>
<tr>
<td></td>
<td>24-12-92</td>
<td>+</td>
<td>32.46</td>
<td>27.4</td>
<td>Sand/coral</td>
</tr>
<tr>
<td><strong>G. edulis</strong></td>
<td>6-2-93</td>
<td>+++</td>
<td>35.86</td>
<td>32.4</td>
<td>Sand/coral</td>
</tr>
<tr>
<td><strong>G. edulis</strong></td>
<td>28-5-94</td>
<td>+++</td>
<td>30.8</td>
<td>29.6</td>
<td>Sand/coral</td>
</tr>
<tr>
<td><strong>G. edulis</strong></td>
<td>24-6-94</td>
<td>+++</td>
<td>35.2</td>
<td>27.6</td>
<td>Sand/coral</td>
</tr>
<tr>
<td><strong>G. edulis</strong></td>
<td>12-7-94</td>
<td>+++</td>
<td>34.6</td>
<td>27.4</td>
<td>Sand/coral</td>
</tr>
<tr>
<td><strong>G. edulis</strong></td>
<td>10-8-94</td>
<td>+++</td>
<td>35.6</td>
<td>28.6</td>
<td>Sand/coral</td>
</tr>
<tr>
<td><strong>G. corticata var. corticata</strong></td>
<td>6-9-94</td>
<td>+++</td>
<td>34.6</td>
<td>29.2</td>
<td>Sand/coral</td>
</tr>
<tr>
<td><strong>G. corticata var. cylindrica</strong></td>
<td>27-5-94</td>
<td>+++</td>
<td>32.4</td>
<td>29.4</td>
<td>Rocky</td>
</tr>
<tr>
<td><strong>G. corticata var. cylindrica</strong></td>
<td>23-6-94</td>
<td>+++</td>
<td>35.2</td>
<td>27.6</td>
<td>Rocky</td>
</tr>
</tbody>
</table>
### 3.2 Summary of Results on *Gracilaria* Species in India (2 of 2)

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>Date</th>
<th>Abundance</th>
<th>Salinity (ppt)</th>
<th>Temp (°C)</th>
<th>Substrate</th>
<th>Depth</th>
<th>Rep</th>
<th>Stage</th>
<th>Yield (%)</th>
<th>Gel strength (g/cm²)</th>
<th>Gelling Temp. (°C)</th>
<th>Melting Temp. (°C)</th>
<th>Method of extraction</th>
<th>REMARKS</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>G. crassa</em></td>
<td>23-7-94</td>
<td>+++</td>
<td>34.6</td>
<td>27.6</td>
<td>Rocky</td>
<td>Y</td>
<td>17</td>
<td></td>
<td>17</td>
<td>34.62</td>
<td>98</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>G. corticata var. corticata</em></td>
<td>24-8-94</td>
<td>+++</td>
<td>34.8</td>
<td>28.4</td>
<td>Rocky</td>
<td>Y</td>
<td>25</td>
<td></td>
<td>25.0</td>
<td>38.69</td>
<td>98</td>
<td></td>
<td>5%NaOH</td>
<td></td>
</tr>
<tr>
<td><em>G. corticata var. corticata</em></td>
<td>15-9-94</td>
<td>+++</td>
<td>34.26</td>
<td>29.6</td>
<td>Rocky</td>
<td>Y</td>
<td>15.8</td>
<td></td>
<td>15.8</td>
<td>31.69</td>
<td>95</td>
<td></td>
<td>&quot;</td>
<td></td>
</tr>
<tr>
<td><em>G. corticata var. cylindrica</em></td>
<td>28-5-94</td>
<td>+++</td>
<td>30.8</td>
<td>29.6</td>
<td>Sand/rock</td>
<td>Y</td>
<td>18.4</td>
<td></td>
<td>18.4</td>
<td>36.69</td>
<td>98</td>
<td></td>
<td>&quot;</td>
<td></td>
</tr>
<tr>
<td><em>G. corticata var. cylindrica</em></td>
<td>24-6-94</td>
<td>+++</td>
<td>31.2</td>
<td>27.6</td>
<td>Sand/rock</td>
<td>Y</td>
<td>24.6</td>
<td></td>
<td>24.6</td>
<td>65.69</td>
<td>98</td>
<td></td>
<td>&quot;</td>
<td></td>
</tr>
<tr>
<td><em>G. corticata var. cylindrica</em></td>
<td>12-7-94</td>
<td>+++</td>
<td>34.6</td>
<td>27.4</td>
<td>Sand/rock</td>
<td>Y</td>
<td>25.2</td>
<td></td>
<td>25.2</td>
<td>58.69</td>
<td>98</td>
<td></td>
<td>&quot;</td>
<td></td>
</tr>
<tr>
<td><em>G. corticata var. cylindrica</em></td>
<td>10-8-94</td>
<td>+++</td>
<td>35.6</td>
<td>28.4</td>
<td>Sand/rock</td>
<td>Y</td>
<td>14.8</td>
<td></td>
<td>14.8</td>
<td>36.69</td>
<td>98</td>
<td></td>
<td>&quot;</td>
<td></td>
</tr>
<tr>
<td><em>G. corticata var. cylindrica</em></td>
<td>8-9-94</td>
<td>+++</td>
<td>34.62</td>
<td>28.6</td>
<td>Sand/rock</td>
<td>Y</td>
<td>20.8</td>
<td></td>
<td>20.8</td>
<td>40.60</td>
<td>98</td>
<td></td>
<td>&quot;</td>
<td></td>
</tr>
<tr>
<td><em>G. crassa</em></td>
<td>27-5-94</td>
<td>+++</td>
<td>32.4</td>
<td>29.4</td>
<td>Stone pieces</td>
<td>Y</td>
<td>5.6</td>
<td></td>
<td>5.6</td>
<td>28.69</td>
<td>98</td>
<td></td>
<td>5%NaOH</td>
<td></td>
</tr>
<tr>
<td><em>G. crassa</em></td>
<td>29-6-94</td>
<td>+++</td>
<td>33.6</td>
<td>27.8</td>
<td>Stone pieces</td>
<td>Y</td>
<td>5.4</td>
<td></td>
<td>5.4</td>
<td>27.69</td>
<td>98</td>
<td></td>
<td>&quot;</td>
<td></td>
</tr>
<tr>
<td><em>G. crassa</em></td>
<td>12-7-94</td>
<td>+++</td>
<td>33.8</td>
<td>28.2</td>
<td>Stone pieces</td>
<td>Y</td>
<td>7.8</td>
<td></td>
<td>7.8</td>
<td>28.69</td>
<td>98</td>
<td></td>
<td>44</td>
<td></td>
</tr>
<tr>
<td><em>G. crassa</em></td>
<td>10-8-94</td>
<td>+++</td>
<td>35.4</td>
<td>28.4</td>
<td>Stone pieces</td>
<td>Y</td>
<td>5.1</td>
<td></td>
<td>5.1</td>
<td>29.69</td>
<td>98</td>
<td></td>
<td>44</td>
<td></td>
</tr>
<tr>
<td><em>G. crassa</em></td>
<td>7-9-94</td>
<td>+++</td>
<td>34.42</td>
<td>28.8</td>
<td>Stone pieces</td>
<td>Y</td>
<td>6.2</td>
<td></td>
<td>6.2</td>
<td>29.69</td>
<td>98</td>
<td></td>
<td>&quot;</td>
<td></td>
</tr>
</tbody>
</table>
### 3.3 Summary of Results on *Gracilaria* Species in Indonesia

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>Date</th>
<th>Abundance</th>
<th>Salinity (ppt)</th>
<th>Temp (°C)</th>
<th>Substrate</th>
<th>Rep Stage</th>
<th>Yield (%)</th>
<th>Gel strength (g/cm²)</th>
<th>Gelling Temp. (°C)</th>
<th>Melting Temp. (°C)</th>
<th>Method of extraction</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>G. edulis</em></td>
<td>20-9-92</td>
<td>++</td>
<td>28</td>
<td>29</td>
<td></td>
<td></td>
<td>8.13</td>
<td>610</td>
<td>36</td>
<td>68</td>
<td>SW 01</td>
</tr>
<tr>
<td><em>G. edulis</em></td>
<td>8-10-92</td>
<td>+++</td>
<td>29</td>
<td>29</td>
<td></td>
<td></td>
<td>13.61</td>
<td>840</td>
<td>39</td>
<td>90</td>
<td>SW 03</td>
</tr>
<tr>
<td><em>G. edulis</em></td>
<td>15-6-93</td>
<td>+++</td>
<td>29</td>
<td>28</td>
<td></td>
<td></td>
<td>15.77</td>
<td>575</td>
<td>32</td>
<td>79</td>
<td>SW 10</td>
</tr>
<tr>
<td><em>G. lemaneiformis</em></td>
<td>20-9-94</td>
<td>+++</td>
<td>28</td>
<td>29</td>
<td></td>
<td></td>
<td>9.96</td>
<td>880</td>
<td>34</td>
<td>85</td>
<td>SW 02</td>
</tr>
<tr>
<td><em>Gracilaria sp.</em></td>
<td>9-10-92</td>
<td>++</td>
<td>29</td>
<td>29</td>
<td></td>
<td></td>
<td>8.18</td>
<td>780</td>
<td>28</td>
<td>86</td>
<td>SW 04</td>
</tr>
<tr>
<td><em>Gracilaria sp.</em></td>
<td>4-6-93</td>
<td>+</td>
<td>33</td>
<td>28</td>
<td></td>
<td></td>
<td>12.12</td>
<td>550</td>
<td>28</td>
<td>89</td>
<td>SW 09</td>
</tr>
<tr>
<td><em>G. saliconia</em></td>
<td>21-5-93</td>
<td>++</td>
<td>31</td>
<td>28</td>
<td></td>
<td></td>
<td>5.47</td>
<td>580</td>
<td>32</td>
<td>75</td>
<td>SW 05</td>
</tr>
<tr>
<td><em>G. saliconia</em></td>
<td>4-6-93</td>
<td>+</td>
<td>33</td>
<td>28</td>
<td></td>
<td></td>
<td>11.07</td>
<td>625</td>
<td>31</td>
<td>72</td>
<td>SW 08</td>
</tr>
<tr>
<td><em>G. eucheumoides</em></td>
<td>21-5-93</td>
<td>+</td>
<td>31</td>
<td>28</td>
<td></td>
<td></td>
<td>16.99</td>
<td>850</td>
<td>29</td>
<td>32</td>
<td>SW 06</td>
</tr>
</tbody>
</table>
### 3.4 Summary of Results on *Gracilaria* Species in Malaysia

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>Date</th>
<th>Abundance</th>
<th>Salinity (ppt)</th>
<th>Temp (°C)</th>
<th>Substrate</th>
<th>Depth (m)</th>
<th>Rep Stage</th>
<th>Yield (%)</th>
<th>Gel strength (g/cm²)</th>
<th>Gelling Temp. (°C)</th>
<th>Melting Temp. (°C)</th>
<th>Method of extraction</th>
<th>REMARKS</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>G. changii</em></td>
<td>13-5-92</td>
<td>001</td>
<td>28</td>
<td>28</td>
<td></td>
<td>4m</td>
<td></td>
<td>41.7</td>
<td>39</td>
<td></td>
<td></td>
<td>Non alc treat</td>
<td>T = 90 cm</td>
</tr>
<tr>
<td><em>G. changii</em></td>
<td>28-5-92</td>
<td>002</td>
<td>28</td>
<td>28</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>G. changii</em></td>
<td>9-7-92</td>
<td>003</td>
<td>32</td>
<td>28</td>
<td></td>
<td>4m</td>
<td></td>
<td>35.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>T = 85 cm</td>
</tr>
<tr>
<td><em>G. changii</em></td>
<td>9-9-92</td>
<td>004</td>
<td>32</td>
<td>30</td>
<td>4m</td>
<td>1.5m</td>
<td></td>
<td>30.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>T = 115 cm</td>
</tr>
<tr>
<td><em>Hypnea sp.</em></td>
<td>9-9-92</td>
<td>005</td>
<td>28</td>
<td>29</td>
<td>1.5m</td>
<td></td>
<td></td>
<td>13.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>T = 115 cm</td>
</tr>
<tr>
<td><em>G. changii</em></td>
<td>24-4-93</td>
<td>006</td>
<td>28-32</td>
<td>28-30</td>
<td>sandy/muddy</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Sheltered, not exp.</td>
</tr>
<tr>
<td><em>G. fastigiata</em></td>
<td>24-4-93</td>
<td>007</td>
<td>30</td>
<td>29</td>
<td></td>
<td>2.5m</td>
<td></td>
<td>15.8</td>
<td>89</td>
<td></td>
<td></td>
<td></td>
<td>Sheltered, exp. (T=28 cm)</td>
</tr>
<tr>
<td><em>G. changii</em></td>
<td>14-5-93</td>
<td>008</td>
<td>28-32</td>
<td>28-30</td>
<td></td>
<td>3.8m</td>
<td></td>
<td>25.4</td>
<td>174</td>
<td></td>
<td></td>
<td></td>
<td>Sheltered, not exp. (T=80 cm)</td>
</tr>
<tr>
<td><em>Gracilaria sp.</em></td>
<td>14-4-94</td>
<td>009</td>
<td></td>
<td></td>
<td>muddy</td>
<td></td>
<td></td>
<td>20.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Sheltered, exp.</td>
</tr>
<tr>
<td><em>Gracilaria sp.</em></td>
<td>27-7-94</td>
<td>010</td>
<td></td>
<td></td>
<td>muddy</td>
<td></td>
<td></td>
<td>24.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Sheltered, exp.</td>
</tr>
<tr>
<td><em>G. changii</em></td>
<td>17-9-94</td>
<td>011</td>
<td>28-30</td>
<td></td>
<td>shrimp pond</td>
<td></td>
<td></td>
<td>25.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Sheltered, not exp.</td>
</tr>
<tr>
<td><em>G. changii</em></td>
<td>8-10-94</td>
<td>012</td>
<td>28-30</td>
<td></td>
<td>shrimp pond</td>
<td></td>
<td></td>
<td>22.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Sheltered, not exp.</td>
</tr>
</tbody>
</table>

Key: exp. = exposed at low tide. not exp.: = not exposed at low tide. T = turbidity
### 3.5 Summary of Results on *Gracilaria* Species in Myanmar

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>Date</th>
<th>ECOLOGICAL PARAMETERS</th>
<th>AGAR YIELD AND QUALITY</th>
<th>Method of extraction</th>
<th>Code: NACA-</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Abundance</td>
<td>Salinity (ppt)</td>
<td>Temp (°C)</td>
<td>Substrate</td>
</tr>
<tr>
<td>G. verrucosa</td>
<td>9-22</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G. edulis</td>
<td>10-22</td>
<td>60-120</td>
<td>27-39</td>
<td>52-73</td>
<td>&quot;</td>
</tr>
<tr>
<td>G. crassa</td>
<td>12-21</td>
<td>70-120</td>
<td>28-40</td>
<td>55-73</td>
<td>&quot;</td>
</tr>
<tr>
<td>G. foliifera</td>
<td>11-19</td>
<td>60-98</td>
<td>18-39</td>
<td>50-82</td>
<td>&quot;</td>
</tr>
<tr>
<td>G. millardetii.</td>
<td>10-19</td>
<td>60-92</td>
<td>29-39</td>
<td>50-80</td>
<td>&quot;</td>
</tr>
<tr>
<td>G. textorii</td>
<td>11-19</td>
<td>60-110</td>
<td>27-39</td>
<td>52-73</td>
<td>&quot;</td>
</tr>
<tr>
<td>G. edulis</td>
<td>23-8-92</td>
<td>18.22</td>
<td>92.51</td>
<td>64-69</td>
<td>wild</td>
</tr>
<tr>
<td>G. edulis</td>
<td>24-11-92</td>
<td>18.87</td>
<td>116.5</td>
<td>45-62</td>
<td>cultured 3/92. Mushwe-key, Rakhine</td>
</tr>
<tr>
<td>G. foliifera</td>
<td>18-2-92</td>
<td>12.85</td>
<td>50.07</td>
<td>73-74</td>
<td>wild</td>
</tr>
<tr>
<td>G. edulis</td>
<td>18-2-92</td>
<td>22.18</td>
<td>116.91</td>
<td>68-71</td>
<td>wild</td>
</tr>
</tbody>
</table>
### 3.6 Summary of results on *Gracilaria* species in the Philippines

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>Date</th>
<th>Abundance</th>
<th>Salinity (ppt)</th>
<th>Temp (°C)</th>
<th>Habitat</th>
<th>Depth (cm)</th>
<th>Rep</th>
<th>Stage</th>
<th>Yield (%)</th>
<th>Gel strength (g/cm²)</th>
<th>Gelling Temp (°C)</th>
<th>Melting Temp. (°C)</th>
<th>Method of extraction</th>
<th>% SO₂⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>G. fastigiata</em></td>
<td></td>
<td>++</td>
<td>25-35</td>
<td>25-35</td>
<td>open reef flat Sandy/rocky</td>
<td>not</td>
<td></td>
<td>19.24</td>
<td>250</td>
<td>-</td>
<td>79</td>
<td>5%NaOH</td>
<td>3.28</td>
<td></td>
</tr>
<tr>
<td><em>G. saliconia</em></td>
<td></td>
<td>+</td>
<td>25-35</td>
<td>25-35</td>
<td>open reef flat Sandy/rocky</td>
<td>c.c.</td>
<td></td>
<td>20.07</td>
<td>287</td>
<td>-</td>
<td>80</td>
<td>5%NaOH</td>
<td>1.86</td>
<td></td>
</tr>
<tr>
<td><em>G. changii</em></td>
<td></td>
<td>++</td>
<td>25</td>
<td>34</td>
<td>open reef flat Coral</td>
<td>C.C.</td>
<td></td>
<td>20.67</td>
<td>583</td>
<td>86</td>
<td>5%NaOH</td>
<td>0.56</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>G. tenuistipitata</em></td>
<td></td>
<td>++</td>
<td>10-25</td>
<td>25-33</td>
<td>Brackish Sandy-mud</td>
<td>c.c</td>
<td></td>
<td>16.18</td>
<td>726</td>
<td>88</td>
<td>5%NaOH</td>
<td>1.26</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>G. firma</em></td>
<td></td>
<td>+++</td>
<td>25-35</td>
<td>25-35</td>
<td>Mangrove Sandy-mud</td>
<td>c.c (nov)</td>
<td></td>
<td>17.61</td>
<td>606</td>
<td>89</td>
<td>5%NaOH</td>
<td>0.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>G. heteroclada</em></td>
<td></td>
<td>++</td>
<td>10-25</td>
<td>25-33</td>
<td>Brackish pond -mud.</td>
<td>n.o.</td>
<td></td>
<td>20.05</td>
<td>892</td>
<td>92</td>
<td>5%NaOH</td>
<td>0.45</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Key: n.o = not observed; c.c = cystocarpa
## 3.7 Summary of results on *Gracilaria* species in Thailand

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>Date</th>
<th>ECOLOGICAL PARAMETERS</th>
<th>AGAR YIELD AND QUALITY</th>
<th>REMARKS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Abundance</td>
<td>Salinity</td>
<td>Temp (°C)</td>
</tr>
<tr>
<td><em>G. firma</em></td>
<td>28-4-93</td>
<td>31 34</td>
<td>Sandy-mud</td>
<td>-</td>
</tr>
<tr>
<td><em>G. changii</em></td>
<td>28-4-93</td>
<td>33 37</td>
<td>Sandy-mud exposed</td>
<td>-</td>
</tr>
<tr>
<td><em>G. irregularis</em></td>
<td>28-4-93</td>
<td>33 37</td>
<td>Sandy-mud exposed</td>
<td>-</td>
</tr>
<tr>
<td><em>G. edulis</em></td>
<td>28-4-93</td>
<td>31 34</td>
<td>Sandy-mud</td>
<td>10-20</td>
</tr>
<tr>
<td><em>G. salicornia</em></td>
<td>28-4-93</td>
<td>- -</td>
<td>Sandy-mud</td>
<td>30-50</td>
</tr>
<tr>
<td><em>G. salicornia</em></td>
<td>8-6-93</td>
<td>- -</td>
<td>Sandy-mud</td>
<td>30-50</td>
</tr>
<tr>
<td><em>G. changii</em></td>
<td>8-6-93</td>
<td>32 -</td>
<td>Sandy-mud</td>
<td>- F</td>
</tr>
<tr>
<td><em>G. firma</em></td>
<td>4-4-94</td>
<td>33.5 36</td>
<td>Sandy-mud</td>
<td>30 F</td>
</tr>
<tr>
<td><em>G. fisheri</em></td>
<td>21-8-94</td>
<td>11 -</td>
<td>Muddy sand</td>
<td>100 F</td>
</tr>
<tr>
<td><em>G. tenuistipitata</em></td>
<td>20-8-94</td>
<td>31 -</td>
<td>Muddy sand</td>
<td>30 F</td>
</tr>
</tbody>
</table>

F = Female
### 3.8 Summary of results on *Gracilaria* species in Vietnam

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>Date</th>
<th>Abundance</th>
<th>SALINITY (ppt)</th>
<th>TEMP (°C)</th>
<th>SUBSTRATE</th>
<th>DEPTH (cm)</th>
<th>REP STAGE</th>
<th>YIELD (%)</th>
<th>GEL STRENGTH (g/cm²)</th>
<th>GELLING TEMP. (°C)</th>
<th>MELTING TEMP. &lt;°C</th>
<th>METHOD OF EXTRACTION</th>
<th>REMARKS</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>G. verrucosa</em></td>
<td>20-6-92</td>
<td>+++</td>
<td>15-16</td>
<td>23</td>
<td>Rocky</td>
<td>50</td>
<td>Nov-Jul</td>
<td>29.5</td>
<td>516</td>
<td>-</td>
<td>85-87</td>
<td>-</td>
<td>Dinh vu, Haiphong</td>
</tr>
<tr>
<td><em>G. blodgettii</em></td>
<td>27-5-93</td>
<td>+++</td>
<td>23</td>
<td>24</td>
<td>Muddy-sand</td>
<td>70</td>
<td>Mar-Jul</td>
<td>26.5</td>
<td>278</td>
<td>85-87</td>
<td>-</td>
<td>2-4%NaOH</td>
<td>Phulong, Haiphong</td>
</tr>
<tr>
<td><em>G. tenuistipitata</em></td>
<td>21-6-92</td>
<td>+++</td>
<td>16</td>
<td>22-24</td>
<td>Rocky</td>
<td>50</td>
<td>Jan-Jun</td>
<td>19.7</td>
<td>338</td>
<td>89-92</td>
<td>-</td>
<td></td>
<td>Lienvi, Quangninh</td>
</tr>
<tr>
<td><em>G. chorda</em></td>
<td>28-5-93</td>
<td>++</td>
<td>23</td>
<td>22</td>
<td>Rocky</td>
<td>85</td>
<td>Jan-Jul</td>
<td>21.5</td>
<td>290</td>
<td>85-87</td>
<td>-</td>
<td></td>
<td>Haininh, Quangninh</td>
</tr>
<tr>
<td><em>G. arcuata</em></td>
<td>7-5-93</td>
<td>++</td>
<td>22</td>
<td>23</td>
<td>Rocky</td>
<td>85</td>
<td>Mar-Jul</td>
<td>17.6</td>
<td>258</td>
<td>92-93</td>
<td>-</td>
<td></td>
<td>Dovon, Haiphong</td>
</tr>
<tr>
<td><em>G. bursa-pastoris</em></td>
<td>7-5-93</td>
<td>++</td>
<td>26</td>
<td>23</td>
<td>Rocky</td>
<td>85</td>
<td>Jan-Jul</td>
<td>15.7</td>
<td>128</td>
<td>92-93</td>
<td>-</td>
<td></td>
<td>Quanlan, Quangninh</td>
</tr>
<tr>
<td><em>G. verrucosa</em> (1)</td>
<td>20-6-92</td>
<td>++</td>
<td>16</td>
<td>23</td>
<td>Rocky</td>
<td>50</td>
<td>Nov-Jun</td>
<td>29.5</td>
<td>516</td>
<td>82-85</td>
<td>-</td>
<td></td>
<td>Dinh vu, Hai Phong</td>
</tr>
<tr>
<td>(2)</td>
<td>20-6-92</td>
<td>++</td>
<td>16</td>
<td>23-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>27.0</td>
<td>456</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Bang la, Haiphong</td>
</tr>
<tr>
<td>(3)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>24.5</td>
<td>319</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Cathai, Haiphong</td>
</tr>
<tr>
<td>(4)</td>
<td>20-6-92</td>
<td>++</td>
<td>15</td>
<td>23</td>
<td>Rocky</td>
<td>45</td>
<td>Jan-Jul</td>
<td>31.0</td>
<td>474</td>
<td>82-85</td>
<td>-</td>
<td>-</td>
<td>Yin hung, Quang Ninh</td>
</tr>
<tr>
<td>(5)</td>
<td>20-6-92</td>
<td>-</td>
<td>15</td>
<td>23-</td>
<td>Rocky</td>
<td>50</td>
<td>-</td>
<td>28.0</td>
<td>396</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Thai thuy, Thai binh</td>
</tr>
<tr>
<td>(6)</td>
<td>20-6-92</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Rocky</td>
<td>80</td>
<td>Jan-July</td>
<td>26.9</td>
<td>387</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Hai hau, Nam Ha.</td>
</tr>
<tr>
<td><em>G. gigas</em></td>
<td>28-5-93</td>
<td>++</td>
<td>25</td>
<td>23</td>
<td>Rock/sand</td>
<td>80</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td>Campha, Quangninh</td>
</tr>
</tbody>
</table>
4. SEAWEED PROCESSING TECHNOLOGY IN PARTICIPATING COUNTRIES

4.1 China

Methods used for Agar Extraction

*G. tenuistipitata*

First Method of Extraction

1. Seaweed
2. 5% NaOH at 95±3°C, 1 hour
3. Wash out alkaline
   - neutralize by HCl
4. Cutting seaweed
   - boil with 1% Sodium Hexametaphosphate solution at pressure 1 ± 0.3 kg/cm², 90 min.
5. Filter by nylon gauze

- Filtrate
- Residue

- Filtrate
- Filtrate

- Freeze and thaw
- Agar

Second Method of Extraction

25% NaOH treatment, the rest same as 1st method
Third Method of Extraction

Seaweed

5% NaOH heat at 105 ± 3°C, 20 min.

Wash out alkali, neutralize with HCl (dilute)

+ 0.08% BaClO, 10 min.

Wash out with water

+ sodium acetic buffer, 20 min.

Filter and chopped

+ 0.1 sodium hexametaphosphate

Extraction as method 1
4.2 India

*Gracilaria* species¹
- *G. edulis* (11)
- *G. crassa* (6)
- *G. corticata var. cylindrica* (9)

**Method of Extraction**

1. Seaweed
   - wash with freshwater, sun dry (bleaching)

2. Clean seaweed
   - 5% NaOH, 90°C for 1 hr.

3. Wash-out alkali
   - neutralize with HCl

4. Extraction with water

5. Filter through cloth bag with pressing tool

6. Setting the gel at room temperature
   - dry at 50-55°C

7. Freeze and thaw

8. Agar

¹Numbers within brackets indicate the number of samples
4.3 Indonesia

Gracilaria species
- G. edulis (3)
- G. lemaneiformis (1)
- G. spp. (2)
- G. salicornia (2)
- G. eucheumoides (1)

Method of Extraction
Adopted from Chandrkrachang, 1992
Water extraction as the first step, then alkali treatment to the agar afterwards.

4.4 Malaysia

Gracilaria species
- G. changii (8)
- G. fastigiata (1)
- G. spp. (2)
- Hypnea sp. (1)

Method of Extraction

1. Clean sun dry seaweed
2. Freshwater extraction, 1 hour
3. Pressing filter through nylon cloth (2-layered)
4. Agar solution cooling
5. Cutting
6. Freezing and Thawing
7. Sundry
8. Agar for analysis
4.5 Myanmar

*Gracilaria* species

- G. verrucosa
- G. edulis
- G. crassa
- G. foliifera
- G. millardetii
- G. textorii

Method of Extraction

1. Sun-bleached *Gracilaria*
   - 2-6% NaOH solution at 90°C for 1½ hours
2. Wash alkali out
3. Neutralize with HCl
4. Extraction with water
   - at 100°C for 2 hours
5. Filtration
6. Gel setting at room temperature
7. Cutting
8. Freezing and Thawing
9. Sun dry
10. Agar
4.6 Philippines

**Gracilaria species**
- *G. fastigiata* (8)
- *G. salicornia* (2)
- *G. spp.* (3)
- *G. changii* (9)

**Method of Extraction**

1. Dried *Gracilaria*
   - 5% NaOH at 90°C for 3 hours
2. Wash out alkaline
   - Adjust by acetic acid to pH 5.6-6.0
3. Extraction with water
   - 2 hours at 85-90°C
4. Add filter aid
5. Filter under pressure at 60-80 psi
6. Agar gel setting at room temp.
7. Freeze and thaw
8. Drying facilitate by IPA
9. Agar
4.7 Thailand

**Gracilaria species**
- *G. salicornia* (1)
- *G. changii* (1)
- *G. firma* (1)
- *G. fisheri* (1)
- *G. tenuistipitata* (1)

**Method of Extraction**

1. **Dried seaweed**
   - soak in freshwater; drain water out; add 3% NaOH, heat at 90°C for 1 hour

2. **Wash alkali out with water (pH7)**

3. **Extraction with water at 95-100°C for 1 hour**

4. add filter aid

5. **Pressure Filter at 60-80 psi**

6. **Agar gel setting**

7. **Freezing and Thawing**
   - dry at 50-55°C

**Agar**
4.8 Vietnam

**Gracilaria species**
- G. verrucosa (7)
- G. blodgetti (1)
- G. tenuistipitata (1)
- G. chorda ()
- G. arcuata (1)
- G. bursa-pastoris (1)

**Method of Extraction**

1. Dried seaweed
2. Washing
   - 2-3-4% NaOH, 90-98°C, 1-2 hours
3. Wash out alkali
4. Decolouring (0.7-1 gm NaOCl/liter)
5. Wash, cleaning
6. Citric acid treatment (0.2-0.3% at 30-60°C)
7. Wash, cleaning
8. Extraction (add acetic acid and Na₂B₄O₇·10H₂O)
9. Filter at 85-95°C
10. Agar gel cool
11. Freeze and Thawing
12. Agar
A REVIEW PAPER ON THE TAXONOMY OF \textit{GRACILARIA} IN ASIAN COUNTRIES

Khanjanapaj Lewmanomont,
Professor, Department of Fishery Biology,
Faculty of Fisheries, Kasetsart University, Bangkok, Thailand

ABSTRACT

From 9 countries in the region only Bangladesh reported the absence of \textit{Gracilaria} spp. The reports from the other eight countries, namely: China; India; Indonesia; Malaysia; Myanmar; Philippines; Thailand; and Vietnam, included 25 identified species with 3 varieties and 4 unidentified species. Among these, 10 species appear to be correctly identified, the rest have to be confirmed. The most common species was \textit{G. edulis}, followed by \textit{G. tenuistipitata}, \textit{G. salicornia} and \textit{G. changii}. The species recommended for culture were \textit{G. edulis}, \textit{G. changii} and \textit{G. tenuistipitata}.

The main problem in identifying the species of \textit{Gracilaria} was the lack of sexual reproductive organs. Only a few species could be identified by their morphological characteristics such as \textit{G. eucheumoides} and \textit{G. salicornia}.

1. INTRODUCTION

Marine red algae, or red seaweeds, comprise the largest and most diverse assemblage of the marine plants. They are important to the marine environment and used as raw materials for the extraction of valuable products. \textit{Gracilaria} is one of the red seaweeds used as an important source of agar extracts and related products. This seaweed grows naturally in many Asian countries, but the taxonomic status and ecological requirements for culture of the various species are not properly known.

2. STUDIES ON \textit{GRACILARIA}

The genus \textit{Gracilaria} was established by Greville in 1830 and consisted of four species: \textit{G. confervoides}; \textit{G. compressa}, \textit{G. purpurascens} and \textit{G. erecta}. In 1852, J. Agardh revised and redefined the generic circumscription for the genus, and designated \textit{G. confervoides} as its species. Early concepts of the species of \textit{Gracilaria} were mainly based on external structure. A detailed anatomical study was first reported by Sjostedt in 1926 with \textit{G. confervoides}, \textit{G. compressa} and \textit{G. robusta}.

In 1949, Dawson regarded nutritive filaments as a diagnostic characteristic at the generic level and distinguished the genus \textit{Gracilariopsis} from \textit{Gracilaria} on the basis of the absence of nutritive filaments and the small size of the gonimoblast cells. In 1966, Papenfuss reported that the presence of nutritive filaments could not always be confirmed in British material of \textit{Gracilaria verrucosa}. He concluded that the presence of nutritive filaments could not be used as a basis for separating \textit{Gracilaria} and \textit{Gracilariopsis} and, for that reason, he reduced \textit{Gracilariopsis} to synonymy with the original genus \textit{Gracilaria}.

In 1963, Chang and Xia described the genus \textit{Polycavernosa} with \textit{P. fastigiata} as the type species. This genus consists of rhizome-like creeping parts from which arise erect, free branches; a creeping portion fastened at frequent intervals by a disc-like attachment; spermatangial conceptacles in clusters and basal absorbing filaments with many long branches. Many authors supported the recognition of \textit{Polycavernosa} and some species of \textit{Gracilaria} were transferred into \textit{Polycavernosa} and new species have been described. Wynne (1989), re-instated \textit{Hydropuntia} Montagne (1842)
which is shown to be the earliest validly published name with *H. urvillei* as the type species. He transferred 14 species of *Polycavernosa* and two species of *Gracilaria* to *Hydropuntia*.

In 1991, Abbott, Zhang and Xia described a new species, *Gracilaria mixta*, according to the mixture of two kinds of spermatangial conceptacles, *Verrucosa* type and *Polycavernosa* type, in the same branchlets. Re-examination of male plants of the western Pacific taxa placed in *Polycavernosa (= Hydropuntia)* also shows both types of configurations in the same thalli. Therefore, 16 species of *Hydropuntia* were transferred to *Gracilaria*.

The genus *Gracilaria* is cosmopolitan in its distribution. It has been reported from most parts of the world, the Arctic, temperate, tropical and even Antarctic region. According to Bird *et al.* (1982), the apparent centre of distribution lies in the tropics with the largest number of species, and a rapid decline in species numbers occurs with increasing latitude in both directions. More than 150 species have been reported. The identification of the species of *Gracilaria* is difficult owing to the great variability of the plants and poorly understood species limits. Anatomical characteristics seem to be more stable than morphological features.

### 2.1 Life cycle of *Gracilaria* (Figure 1)

*Gracilaria* shows an alternation of isomorphic generations between haploid gametophyte and diploid tetrasporophyte. The gametophytes are dioecious. The fertile male thallus produces spermatangia and the female thallus produces carpogonia. After fertilisation a structure called a "cystocarp" is formed. The cystocarps are prominent, protruding, globose or hemispherical, with or without rostrum, scattered on the surface of the female thallus. Each cystocarp consists of pericarp, gonimoblast filaments and carposporangia, with or without absorbing filaments. Carpospores are liberated through a small hole or ostiole at the top of the cystocarp and germinate into tetrasporic thalli or tetrasporophytes.

*Figure 1: Life cycle of Gracilaria*
The mature tetrasporophyte produces tetrasporangia occurring generally in the cortex of the thallus. The tetrasporangium is cruciately divided and forms four spores or tetraspores which germinate into four gametophytic thalli or gametophytes, of which two are male and two are female thalli.

3. **CHARACTERISTICS OF *GRACILARIA***

The genus *Gracilaria* is characterised by an erect, fleshy and succulent thallus which consists of a small discoid holdfast with lateral branches. The thalli of most species are cylindrical, some are compressed (*G. eucheumoides*) and some are foliose (*G. textorii*). Important characteristics used for identifying species of *Gracilaria* are:

A. **Morphological features (external features)***

1. From of thalli  
   - cylindrical or terete  
   - compressed or flattened  
   - foliose  
2. Branching  
   - alternate  
   - di or trichotomous  
   - secund  
   - irregular  
3. Constriction of branches - frequently *Gracilaria* shows a constriction at the point where a lateral branch joins a main axis.
4. Branch apex  
   - attenuate  
   - rostrate  
   - tapering  
   - apiculate  
   - obtuse  
   - blunt

B. **Anatomical characteristics (internal features)***

1. Size and number of medullary cells  
2. Number of cortical cells  
3. Change of cells in medullary layers

C. **Characteristics of reproductive organs***

1. Types of spermatangial conceptacles:
   i. *Chorda* type - superficial  
   ii. *Textorii* type - shallow cavity  
   iii. *Verrucosa* type - deep pot-like cavity  
   iv. *Polycavernosa* type - compound *Verrucosa* type

2. Characteristics of cystocarps:
   i. Form  
   ii. Basal constriction  
   iii. With or without rostrum
3. Anatomy of cystocarp:
   i. Pericarp
   ii. Gonimoblast cells
   iii. Carposporangia
   iv. Absorbing filament

The most important characteristics used in identifying the species are the male and female reproductive organs. Without these, it is almost impossible to determine the specific names.

3.1 Studies on *Gracilaria* in Asian waters

Taxonomic studies of *Gracilaria* in Asian waters were reported in "Taxonomy of Economic Seaweeds", publications of California Sea Grant College, Volume 1-4. A review of work carried out in seven Asian countries is presented below.

Studies on *Gracilaria* have been actively carried out in China by Zhang and Xia. They described many new species and revised the name of some similar species previously identified. In total, 25 species were reported from China.

Japan has produced many taxonomic works on *Gracilaria* especially those by Yamamoto. Seventeen species were reported with some replaced names for the previous identifications. Phang (1994), reported 5 species of *Gracilaria* from Malaysia and Singapore, two of which are new records. These five species were added to the three previously known in Malaysia.

Taxonomic studies of *Gracilaria* in the Philippines have been conducted since 1963. Twenty four species are listed, about half are mis-identified or have had their names changed recently. Abbott (1994), examined specimens that had been collected in the Philippines and reported 9 species. Three of the nine were added as new records and two new species were described by Yamamoto and Trono (1994).

In Taiwan, Chiang (1985) reported only 8 species with key, list, description and distribution of the species.

Studies of *Gracilaria* in Thailand have been conducted recently. Abbott (1988), described 4 species of *Gracilaria* and 4 species of *Polycavernosa*, two of these are new species. Lewmanomont (1994) described 13 species of *Gracilaria* from Thailand, four are new records and one new species.

In Vietnam, Nguyen (1992) re-examined the specimens of *Gracilaria* and *Gracilariopsis* and reported 15 species.

In conclusion, 53 species of *Gracilaria* are listed in this region, almost half are mis-identified or have had their names changed recently. In some cases, the old names are repeated, but the new one has also been added.

3.2 Species determination of *Gracilaria* from Asian countries

Examination of herbarium specimens from member countries in the region found that most of them lacked male reproductive organs and there were some without both male and female ones. This made it difficult to determine the species. Only a few species could be identified by their morphological characteristics, such as G. *eucheumoides* by its compressed, prostrate, thick and succulent thallus with dentate margins, and G. *salicornia* by its prostrate to semi-erect thallus with constricted segments.
From the reports of the countries, 25 species with 3 varieties were identified and 4 species were unidentified (Table 1). Among these, some names are not currently used. They are:

1. **G. crassa** - current name *G. salicornia*. Xia (1986), examined specimens from various countries and found all *Gracilaria* with constricted segments either at the main axes or lateral branches, namely *G. cacalia*, *G. crassa*, *G. minor*, *G. canaliculata* and *G. salicornia* were the same species. Since *G. salicornia* is the oldest available name for this group, the other names were placed in synonymy.

2. **G. fastigiata** - this name is supposed to be *Polycavernosa fastigiata* which was transferred to its former name *G. edulis* (Abbott et al. 1991).

3. **G. verrucosa** - this is the most confused species and many countries reported its occurrence. It is not a regular inhabitant of Asian waters, it belongs to European algal flora.

According to Abbott et al. (1985) "G. verrucosa", was recorded from "everywhere" with the widest kinds of ecological conditions. Some of the descriptions or specimens in general match those from the English coast (type locality), but critical comparisons show differences in details of the gonimoblast, pericarp, or spermatangia. The Japanese and Chinese specimens referred to *G. verrucosa* were found to be a similar taxon, but taxonomically not the same as the English *G. verrucosa*. Further, it is different from the Taiwan specimens under that name (Yang and Chiang, 1982) especially in the gonimoblast structure. Also different were specimens from California called "G. verrucosa" (Abbott and Hollenberg, 1976). Clearly, it is necessary to understand the features and their variation by which the British topotype material of *G. verrucosa* is to be recognised.

The Taiwanese *G. verrucosa* reported by Chiang (1985, p.81) was re-examined and identified as *G. tenuistipitata* var. *liui* by Zhang and Xia (1988). The Chinese *G. verrucosa* reported by Zhang and Xia (1985) was named as *G. asiatica*.

### 3.3 Problems with identification of *Gracilaria*

The taxonomy of this economically important agar-producing genus is confused by the high degree of morphological and anatomical variation exhibited within species. Although *Gracilaria* is a very polymorphic genus vegetatively, it has remarkably constant reproductive structures.

The main problems in identification of *Gracilaria* are the lack of sexual reproductive organs in most of the specimens collected and also lack sufficient numbers of specimens for an evaluation of the boundaries of species. The comparison of the type specimens is not easy because the type specimens are widely distributed and many specimens are difficult to find.
Table 1: Distribution of *Gracilaria* in some Asian countries (From: Taxonomy of Economic Seaweeds. Vol. 1-4).

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>CPR</th>
<th>JAP</th>
<th>MAL</th>
<th>PHI</th>
<th>TAI</th>
<th>THA</th>
<th>VIE</th>
<th>Male</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. <em>G. arcuata</em></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>V</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. <em>G. articulata</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. <em>G. asiatica</em></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>V</td>
<td></td>
<td></td>
<td>Replaced name for <em>G. verrucosa</em></td>
</tr>
<tr>
<td>4. <em>G. bangmeiana</em></td>
<td>X</td>
<td></td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td>p</td>
<td></td>
<td>Replaced name for <em>Polycavernosa ramulosa</em></td>
</tr>
<tr>
<td>5. <em>G. blodgettii</em></td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>T</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. <em>G. bursa-pastoris</em></td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>T</td>
<td></td>
<td>Replaced name <em>G. compressa</em> for</td>
</tr>
<tr>
<td>7. <em>G. cacalia</em></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>V</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. <em>G. canaliculata</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>V</td>
<td></td>
<td></td>
<td>Current name is <em>G. salicornia</em></td>
</tr>
<tr>
<td>9. <em>G. changii</em></td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td>V</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10. <em>G. chorda</em></td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11. <em>G. choae</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>T</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12. <em>G. compressa</em></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>T</td>
<td></td>
<td></td>
<td>Synonym for <em>G. bursa-pastoris</em></td>
</tr>
<tr>
<td>13. <em>G. confervoides</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>V</td>
<td></td>
<td></td>
<td>Synonym for <em>G. verrucosa</em></td>
</tr>
<tr>
<td>14. <em>G. coronopifolia</em></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>V</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15. <em>G. crassa</em></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td>V</td>
<td></td>
<td></td>
<td>Current name is <em>G. salicornia</em></td>
</tr>
<tr>
<td>16. <em>G. cuneifolia</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>T</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17. <em>G. dactyloides</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18. <em>G. denticulata</em></td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>T</td>
<td></td>
<td></td>
<td>Current name is <em>G. vieillardii</em></td>
</tr>
<tr>
<td>19. <em>G. disticha</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20. <em>G. edulis</em></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>P</td>
<td></td>
<td>Replaced name for <em>P. fastigiata</em></td>
</tr>
<tr>
<td>21. <em>G. eucheumoides</em></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22. <em>G. firma</em></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>V</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23. <em>G. fisheri</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>V</td>
<td></td>
</tr>
<tr>
<td>24. <em>G. gigas</em></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
<td>T</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25. <em>G. glomerata</em></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>T</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26. <em>G. hainanensis</em></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>V</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>27. <em>G. heteroclada</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td>C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>28. <em>G. incurvata</em></td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>29. <em>G. irregularis</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>30. <em>G. lacinulata</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>31. <em>G. lemaneiformis</em></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td>C</td>
<td></td>
<td>Synonym of <em>G. edulis</em></td>
</tr>
<tr>
<td>32. <em>G. lichenoides</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>33. <em>G. manilaensis</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>V</td>
<td></td>
<td>Replaced name <em>G. verrucosa</em> for</td>
</tr>
<tr>
<td>34. <em>G. megaspora</em></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>35. <em>G. minor</em></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>V</td>
<td></td>
<td></td>
<td>Current name is <em>G. salicornia</em></td>
</tr>
<tr>
<td>36. <em>G. minuta</em></td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>V</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No.</td>
<td>Species</td>
<td>CPR</td>
<td>JAP</td>
<td>MAL</td>
<td>PHI</td>
<td>TAI</td>
<td>THA</td>
<td>VIE</td>
<td></td>
</tr>
<tr>
<td>-----</td>
<td>-------------------------</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>G. percurrens</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>38</td>
<td>G. punctata</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>39</td>
<td>G. purpurascens</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>G. rubra</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>41</td>
<td>G. salicornia</td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>V</td>
<td></td>
<td></td>
</tr>
<tr>
<td>42</td>
<td>G. spinulosa</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>43</td>
<td>G. sublittoralis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>44</td>
<td>G. subtilis</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>V</td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>G. sullivani</td>
<td></td>
<td></td>
<td>uk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>46</td>
<td>G. tenuistipitata</td>
<td>X</td>
<td></td>
<td>X</td>
<td>X</td>
<td>T</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>47</td>
<td>G. textorii</td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>T</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>G. turgida</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>49</td>
<td>G. urvillei</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>G. verrucosa</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>V</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>51</td>
<td>G. vermiculophylla</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>V</td>
<td></td>
</tr>
<tr>
<td>52</td>
<td>G. vieillardii</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>T</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**TOTAL** 30 17 8 24 8 13 15

Key:
- **V** = Verrucosa type
- **T** = Textorii type
- **C** = Chorda type
- **P** = Polycavernosa type

Countries:
- **CPR** = China
- **JAP** = Japan
- **MAL** = Malaysia
- **PHI** = Philippines
- **TAI** = Taiwan
- **THA** = Thailand
- **VIE** = Vietnam

Replaced name for **G. verrucosa**
Replaced name for **G. denticulata**
Table 2: List of *Gracilaria* reported by the eight participating countries.

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>CPR</th>
<th>IND</th>
<th>INS</th>
<th>MAL</th>
<th>MYA</th>
<th>PHI</th>
<th>THA</th>
<th>VIE</th>
</tr>
</thead>
<tbody>
<tr>
<td>G. arcuata</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>G. asiatica</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G. blodgettii</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>G. bursa-pastoris</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>G. changii</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>G. chorda</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>G. corticata var. corticata</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G. crassa&lt;sup&gt;1&lt;/sup&gt;</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G. eucheumoides</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G. fastigiata&lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>G. firma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G. fisheri</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>G. foliifera</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>G. gigas</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>G. hainanensis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>G. heteroclada</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>G. irregularis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>G. lemaneiformis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G. manilaensis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>G. millardetii</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>G. salicornia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>G. tenuistipitata</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>G. textorii</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>G. vemicosa&lt;sup&gt;3&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
</tbody>
</table>

<sup>*</sup> Name changed to *G. asiatica*

1 Current name is *G. salicornia*
2 Current name is *G. edulis*
3 Does not exist in Asian waters

**REFERENCES**


Chiang, Y.M. 1985. *Gracilaria* from Taiwan; Key, list and distribution of the species. Taxonomy of Economic Seaweeds. 1: 137-150


CULTIVATION AND USES OF GRACILARIA

by

R. Perez and O. Barbaroux,
IFREMER Centre de Nantes, Rue de l'Île d'Yue,
Nantes, France.

ABSTRACT

There are four main uses for cultured Gracilaria: fodder for fish and mollusc aquaculture; human consumption; fertiliser; and high quality agar. Fish farmers in China and the Philippines were probably the first to cultivate this algae by cutting it into little pieces and throwing it into ponds where herbivorous fish (milkfish) are grown. In this symbiotic system, algal growth is stimulated by nitrogen excretion by the fish, which also feed on the seaweeds. Gracilaria spp., was not considered an agarophyte before 1949 when there was a shortage of Japanese agar and alkali treatment techniques were developed to improve agar quality. Production has increased steadily since that time.

Despite the simplicity of culture, two major problems remain to achieve production of high quality agar: (i) taxonomic problems due to difficulties in reliably determining species; and (ii) increasing culture yields lowers agar quality and vice versa.

1 INTRODUCTION

1.1 Geographical distribution

Gracilaria spp. has a world-wide distribution (Figure 1) because of its ability to withstand great ecological variations. It is found in all the seas of the world, from 60° north to 40° south latitude (Kling, 1978) and from cool temperate to tropical waters. Chapman (1977), supposed that it could exist up to 47° north latitude, and Mayer (1986) down to 45° south latitude. Development is more limited in cool temperate areas, but the quality of the agar is similar to that of bacteriological agar. The grey distribution zone is valid for all oceans and not just for the Atlantic.

Figure 1: Worldwide distribution of Gracilaria spp. between 47°N and 45°S.

1.2 Bathymetric distribution

*Gracilaria* spp. is found on tidal mudflats, in sandy or muddy basins with scattered rocks where it is rarely out of the water (Lefebvre, 1986). In the sea, it occurs to depths of up to 25 m, although 98% of the population is found between 0.5 m and 10 m, with an optimum depth of 3 to 4 m (Kim, 1970). It cannot remain out of the water for more than an hour. By means of its basal disk, it attaches to all sorts of substrates (sand, shell debris, rocks), even to mussel byssus. It can continue to develop even when partly buried in the sand.

2. BIOLOGY

2.1 Regeneration

Like most Gigartinales, *Gracilaria* possesses the remarkable ability of regeneration at the level of breaks or damaged areas in order to reconstitute missing tissues and ensure growth. This property concerns attached plants as well as detached fragments and the basal disk. The thallus, once cut, can thus be expected to grow again, and a piece can be used as a cutting to produce an entire frond. The cutting buried 10 cm in the sand, which prevents any photosynthesis, can subsist on its own reserves for more than 90 days without any increase in size or weight (Santelices *et al.*, 1984). Re-growth only occurs once movements of the sea bottom displace the sand and uncover the fragment. These fragments as well as basal disks are as important as sexual reproduction in maintaining and expanding populations.

Lefebvre (1986), noted that low temperatures inhibit the emergence of fronds but favour the development of the disk, which becomes a means of resistance and survival. Thus, during severe winters in which fronds are destroyed, the base survives and produces re-growth the following year.

2.2 Growth

Growth is ensured by the apex of the main stem and those of ramifications. Growth rate and development differ depending on whether the plants live in warm areas without great seasonal variations, in marshes in which the water is replenished or in brackish waters. In Israel, Friedlander and Lipkin (1982) found a maximal growth of 6.6 g.m$^{-2}$.day$^{-1}$ (wet weight). Doty (1977) noted 5.6 g.m$^{-2}$.day$^{-1}$ and Edelstein (1977) 8.9 g.m$^{-2}$.day$^{-1}$. Optimal theoretical production can attain 125 tons.hectare$^{-1}$.year$^{-1}$ (dry weight), i.e., one of the highest productions in the plant world.

2.3 Variables affecting production

The fact that *Gracilaria* spp. is a cosmopolite is indicative of its capacity to withstand considerable environmental variations.

**Salinity:** *Gracilaria* spp. is euryhaline, adapting to salinities of 15 to 50 ppt. The best growth is obtained between 20 and 35 ppt.

**Temperature:** Optimal development occurs between 20 and 28°C (Causey *et al.* 1946).

**Lighting:** Light intensity does not appear to be a basic factor since the species can survive with very reduced lighting.
**Epiphytism:** Beginning in June, certain *Gracilaria* spp. plants bear epiphytes which become increasingly abundant, ultimately causing cessation of growth and degeneration of thalli.

**Turbidity:** As *Gracilaria* spp. lives preferentially in sandy areas, it needs to withstand great variations in turbidity which limit photosynthesis by reducing light intensity. Some fields implanted near the estuaries of streams carrying considerable silt are more fragile and paler than those growing in clearer waters.

**Immersion:** When the climate is quite humid, *Gracilaria* spp. can survive an entire day out of the water, but in sunlight it resists no more than an hour.

**Nutrition:** As *Gracilaria* spp. develops in quite varied sites, it has been impossible by natural observation to demonstrate the influence of nutrient levels on the metabolism of the species. Jones (1959) reported that, paradoxically, the greatest growth takes place when the nitrogen level is lowest.

3. **NATURAL POPULATIONS AND COLLECTION METHODS**

The largest resources in *Gracilaria* spp. are found in the following locations:

- in Asia, along the Pacific coasts of Japan and the Philippines; along the shores of Java, Sri Lanka and south India; in the Bay of Bengal; around Taiwan and Hong Kong; in Thailand, Vietnam, Cambodia and Malaysia;
- in Africa, along the coasts of Mozambique and South Africa;
- in South Europe (southern and Adriatic Italy);
- in South America, along most of the coast of Peru and Chile; along the southern shores of Argentina and Brazil.

Exploitation is particularly active in Taiwan, the Philippines and Chile. In Taiwan and the Philippines, harvesting is done manually in the marshes and along the coast where plant populations show high densities (4.2 kg. m$^{-2}$) and growth rates of 4.70 g. m$^{-2}$ day$^{-1}$. Peak growth rates are reached between November and February when the sea is calm, salinity high and rains infrequent. The minimal period for harvesting corresponds to July and August when the south-west monsoon brings big waves, violent rains and a drop in salinity.

In Chile, the dominant *Gracilaria* have been identified as *G. lemaneiformis* (Bory) Weber Van Bosse by Santelices et al. (1979), *G. chilensis* by Bird et al. (1987), *Gracilaria verrucosa* (Hudson) Papenfuss by Tawaga, Kojima and Kono (1963) and *G. confervo'ides* by the Department of Hunting and Fishing (Cable, 1974). Harvesting is performed manually at mid-thigh level in the intertidal zone and by boat using a tool in the subtidal zone. Three types of tools are used (Figure 2): a long-handled rake; the "trebol" a hook attached perpendicularly to a pole; and the "arana" or "spider", which looks like an umbrella turned inside out. The two traditional systems used (trebol and araña) are in fact being abandoned in favour of the rake and diving which provide higher yields and cause less plant deterioration.
4. CULTIVATION OF GRACILARIA

The first efforts at cultivating *Gracilaria* spp. were undertaken in Taiwan about 25 years ago. They were initially empirical, but the technique was improved as scientific knowledge increased. Chile began this type of activity around 1983 and now produces 1,200 tonnes of cultivated *Gracilaria* spp. The government plans to increase production to 4,000 tonnes, having granted 420 concessions for a total of 2,700 hectares. Other countries will probably follow this example since many experiments are now in progress.

4.1 Sowing from cuttings

*Cultivation in Taiwan, Vietnam and China*

Cultivation is performed by scattering fragments of *Gracilaria* spp. in marshes communicating with the sea by a system of sluices or in basins dug directly in the intertidal zone.

*Type of marshes:* Each marsh covers a mean area of one hectare and is connected to the sea as well as to a fresh water source, allowing salinity to be maintained at around the optimal value (25 ppt). Water depth is 50 to 150 cm, and the bottom should be about 10 cm below sea level at low tide. The substrate is theoretically sandy. *Gracilaria* spp. prefers fine sand (3.9 mm in diameter) to very fine sand (less than 2 mm in diameter) and mud which are more easily resuspended by turbulence. If the marsh area is greater than a hectare, it is necessary to erect a bamboo fence around it to keep out the wind which would otherwise cause currents and waves and displace the alga, accumulating it in piles. A net with 15-cm mesh is placed downstream from the marsh in order to capture alga which would otherwise be carried away. The pH must be kept slightly alkaline (8 to 9) by replenishing the water.
Sowing: The farmer selects a stock of *Gracilaria* spp. whose good condition is attested by its elasticity, dark colour and ramification. Cuttings (20 to 30 cm) are scattered uniformly at a rate of 4,000 to 5,000 kg per hectare (rarely more), corresponding to a density of 0.4 to 0.5 kg m\(^{-2}\).

Growth management: Replenishment of the water allows the marsh to be maintained at near-optimal conditions for algal growth. In general, water replenishment 2 to 3 times per day represents 50 to 70% of the total volume. The water layer above the algae must not be less than 30 cm between March and June, nor less than 80 cm from July to October.

Epiphytes and commensal algae: It is not unusual for commensal algae and epiphytes to invade the marshes and cuttings, reducing light and rapidly absorbing nutrient salts. The invaders include *Ulva* spp., *Enteromorpha* spp., *Chaetomorpha* spp., *Cladophora* spp., *Ectocarpus* spp., *Elachista* spp., *Polysiphonia* spp., *Ceramium* spp. and diatoms. The fight against these "weeds" is particularly difficult. The farmer must necessarily remove them with a rake or by hand while they are still floating. The second means of reducing epiphytes consists in working with a high density of thalli per m\(^2\) (2 to 3 kg). Thirdly, browsing animals can be used, which prefer the tender fronds of the epiphytes to the tougher ones of *Gracilaria* sp.. Among these animals, the fishes *Chanos chanos*, *Fundulus heteroclitus* and *Tilapia* sp. are most often employed to destroy Chlorophyceae and Cyanobacteria. These fish must of course be removed once the epiphytes are eliminated since they are then likely to attack the young ramifications of *Gracilaria* spp.

Harvesting: The first harvest can be made 2 to 3 months after sowing, with subsequent ones every 20 to 40 days from June to November depending on algal growth. As the tide allows only 1 or 2 daily replenishments of water, the yield is between 11 and 27 tonnes heactare\(^{-1}\)year\(^{-1}\) (dry weight). Total annual production for Taiwan has been around 13,000 tonnes (dry weight) since 1981 (Trono, 1988).

Product conditioning: The harvest is carefully washed in marsh water to remove mud, sand, fish or mollusc larvae and shellfish. Epiphytes are removed by hand. The plants are then spread out on the ground or on a layer of bamboo or on a horizontal trellis until water content is reduced to 18 to 20%.

*Cultivation methods in other parts of the world*

Figure 3 shows common methods of *Gracilaria* culture in Chile: cuttings are pushed down into the sand with a pitchfork (top) and a spade is used to partially bury the cuttings (middle). The bottom of Figure 3, shows the system used in deep areas: cuttings are attached to a sausage-shaped plastic bag filled with sifted sand; many fields are created in this way. The advantage of the method concerns the rapidity with which the cuttings can be planted. The disadvantage is that the plastic bags are not biodegradable and thus a source of pollution. The mean yield from such plantings is 20 tons hectare\(^{-1}\) year\(^{-1}\) (dry weight).

The stretched rope method is used in Chile and the Caribbean: (Figure 4) This method is similar to the "mono-line" approach used in the Philippines for *Eucheuma*. It is used when the bottom is too muddy or, on the contrary, too stony. The algae benefit from more growing space, and water circulates both above and below the plants. Although better growth is obtained and losses are relatively low with this system, it is rather time-consuming to set up. Yields of 50 to 60 tonnes hectare\(^{-1}\) year\(^{-1}\) can be obtained in winter (the warmest season in Chile).

Other methods of cultivation include raft culture (Figure 5) where the rafts either float (cuttings being placed every 10 cm along ropes hanging in the water) or are anchored so
as to be near the surface at low tide and under 5 to 6 m of water at high tide (in which case the ropes with the cuttings ar horizontal).

![Figure 3 Cultivation procedures in Chile.](image)

![Figure 4: Stretched lime method tested in the Caribbean.](image)

Culture trials in raceways (ponds with water current) have also been undertaken. Cultivation by spraying: *Gracilaria* thalli are placed on a plastic-coated grid and continually sprayed with enriched seawater at a rate of 50 l.m\(^{-2}\) min\(^{-1}\). The water recovered under the grid is returned to the sprayer by a pump.
Different systems for cultivating *Gracilaria* in the Caribbean are shown in Figure 6. Cuttings are placed between strands on rafts (A) submerged 2 to 3 m below the surface. The crop is cut, and the part remaining under the strand generates a new tuft. Figure 7 shows cultivation management during development of the cuttings, those with low growth (A) are replaced with fragments obtained from plants with high growth (B). The farmer thus "homogenizes" the development of the plants.

According to Lopehandia (1986), 10% of the production in Chile is obtained by the profitable method using sausage-shaped plastic bags of sand. This is why 420 new concessions were requested and granted in 1987. Nearly 2,700 hectares are currently devoted to the cultivation of *Gracilaria*, which may soon make Chile the world's leading producer. The crop, once carefully dried, provides food agar of good quality, with a gel strength of 900 g.cm$^2$ after alkaline extraction. In some cases, the production can be used as bacteriologic agar.

Figure 5: Cultivation on rafts in the Caribbean and the Republic of China.
Figure 6: Different systems for cultivating *Gracilaria* in the Caribbean.
In all cases, harvesting takes place when the tufts are between 80 and 100 cm in length. It is done on foot in the intertidal zone and by diving in deep waters. The alga is cut with a knife or sickle, leaving in place a piece 15 to 20 cm long which will generate a new plant. If sowing is performed in January, February or March, the first crop can be obtained in May and subsequent ones in September-October, December-January, April-May, etc.

4.2 Sowing from spores or carpospores

The use of reproductive elements is more difficult than that of fragments, in as much as the factors causing sudden, massive emission must be controlled. Besides, the interval between germination of the reproductive element and the first crop is much longer than that between attachment of the cutting and the first harvest. As Gracilaria is a small alga, the creation of populations by means of cuttings requires considerable work and manpower, implying higher costs. The use of spores would allow rapid sowing on a large number of supports, rocks, nets or ropes. It would also be possible to produce hybridizations to improve either growth capacities or gel quality.

At Santa Lucia in the Caribbean, ropes were seeded using spores placed within a dense population of Gracilaria. However, an element of chance enters into such operations since the ropes must be placed in the water precisely at the peak of the emission (which is difficult to predict) in order to prevent too many commensal algae and animals from colonising them, which in fact is what generally happens.
Good seeding by means of spores or carpospores can only be obtained in the laboratory where a massive release can be produced at the desired time by acting on certain factors. The most efficient process would appear to be that used in Thailand.

5. **USES OF GRACILARIA**

*Gracilaria* spp. is used for agriculture, human food, animal feed and agar extraction. In agriculture, it provides a means of enrichment either in the form of fermented algae mixed with seeds sown in the spring or of liquid or powder spread over roots or leaves. People in the Philippines, Indonesia, Korea and China consume *Gracilaria* spp. either fresh (known as "gonori"), in salads, dried or in a jelly made from powdered fronds.

5.1 **Agar production**

When extracted according to the process applied to Gelidiales, agar from *Gracilaria* spp. has no market value since its gel strength (upon gelation) is very low. Thus, it is preferable first to apply an alkaline treatment with soda for 50 to 70 min at 95°C, in the proportion of 5 tonnes of algae (dry weight) to 100,000 litres of 3 or 5% soda. This operation eliminates sulphuric esters and converts α-L-galactopyranose units into 3,6-anhydro-α-L-galactopyranose, thereby increasing gel strength (Wang and Yang, 1980) but not to the level of that of the polymer extracted from Gelidiales. These are generally food and not pharmaceutical agars.

**Content:** Agar content differs appreciably depending on the variety of *Gracilaria* spp. considered and, for the same variety, according to the collection site, season, physiological state, development stage, conditions of cultivation, degree of cleaning, extraction mode, etc.

**Quality:** Quality is directly related to the composition of the molecule which, even more than the content, is subject to variations.

6. **THE MARKET FOR GRACILARIA**

The annual average global production of *Gracilaria* amounts to about 370,000 tonnes (wet weight). At present, cultivation accounts for a little more than a third of this output (130,000 tonnes). Taiwan remains the leading cultivator, followed by Chile. Contrary to what happens for carrageenans and alginates, agar is extracted by many small units located near the producing areas.

Seaweed production is not exclusively used for agar extraction. Its used for human food in the Far East (notably Southeast Asia) during Islamic holidays, and for feeding farmed fish and molluscs (mainly abalone). These uses absorb a good part of the total production, to the extent that fluctuations in the agarophyte market have only a limited impact on prices (except in South America where *Gracilaria* is used only for its agar content). The prices charged depend more on local negotiations than on international rules, except when it is a question of exportation, in which case the price of a tonne of dried, clean algae ranges between $500 and $700. The production cost per year and per hectare has been determined (Table 1).

The operation, whether gathering, extensive farming or intensive farming, takes the form of a family business involving a hectare (rarely two), with the use of additional labour only during sowing and harvesting. It generally depends on mixed farming since *Gracilaria*, shrimp, crabs and fish are developed harmoniously in the same marshes, thereby increasing income appreciably.
Table 1: Production cost and income for a hectare of *Gracilaria* cultivated at Taiwan in 1989.

<table>
<thead>
<tr>
<th>Costs/Income</th>
<th>Cost (US $)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Variable costs</strong></td>
<td></td>
</tr>
<tr>
<td>Labour</td>
<td>$1,800</td>
</tr>
<tr>
<td>Investment</td>
<td>$1,450</td>
</tr>
<tr>
<td>(cuttings, fertilisers, maintenance)</td>
<td></td>
</tr>
<tr>
<td>Taxes and losses</td>
<td>$ 350</td>
</tr>
<tr>
<td><strong>TOTAL COSTS</strong></td>
<td>$3,600</td>
</tr>
<tr>
<td><strong>SALE PRICE</strong></td>
<td>$7,350</td>
</tr>
<tr>
<td><strong>PROFIT</strong></td>
<td>$3,750</td>
</tr>
</tbody>
</table>

The selection of efficient individuals or the creation of laboratory strains through genetic engineering might change this situation. In fact, the uncertain nomenclature for the genus does not facilitate studies but probably reflects the existence of various genomes whose combinations could be enriching. There is also a possibility for the future use of the protoplasts that Christiaen and Stadler (personal communication, 1986) have been able to obtain. The cultivation of *Gracilaria* is in fact in abeyance. If research currently in progress leads to cost-efficient cultivation of species of the *Gelidium* genus, which provides high quality agar, that of *Gracilaria* could be limited to the quantity intended for human and animal food. On the other hand, *Gracilaria* might well remain the agarophyte of recourse.

The degradation of the economic situation in Eastern European countries, which are high consumers of food agars but currently not very creditworthy, led to a decrease in 1991 in the world demand for agar and thus for agarophytes of the *Gracilaria* type. At the same time, production prices dropped. Even Chile, despite the production of good quality *Gracilaria*, is in difficulty. It is not easy to know how this situation will evolve. Moreover, "gelanes" (competing colloids of bacterial origin) have made their appearance, showing comparable properties to those of Floridean agar.

7. AGARS

Algae used for agar production belong to three orders:

- **Gelidiales**, including the genera
- **Gelidium**, **Gelidiella** and **Pterocladia**.
- **Gigartinales**, with the genus
- **Gracilaria**. **Ceramiales**, with the genus **Ceramium**.

*Gelidium* is harvested mainly in France, Spain, Portugal, Morocco, South Africa, Japan, Korea, China, the United States and Chile, involving notably *G. corneum*, *G. cartilagineum*, *G. pacificum*, *G. pristoides*, *G. latifolium*, *G. sesquipedale* and *G. amansii*. Mexico now has an annual production of 1,200 tonnes of *G. robustum*. *Gelidiella* comes mainly from Egypt, India and Madagascar. *Pterocladia* is abundant in the Azores (*P. capillacea*) and New Zealand (*P. lucidd*).

The genus *Gracilaria* is found world-wide, and its many species yield agars of quite variable quality. Noteworthy ones are *Gracilaria* sp., *G. crassa*, *G. picheno'ides*, *G. canaliculata*, *G. lemaeiformis*, *G. foliifera*, *G. corticata* and *G. multipartita*. These species are harvested in Chile, Argentina, Peru, Brazil, the Caribbean, Japan, China, Taiwan, Indonesia, Vietnam, India, Sri Lanka and South Africa. Other algae, such as *Anhfeltia*...
plicata (Japan, Sakhalin), Acanthopeltis japonica and Ceramium sp., are blended with these species. The difference in quality among the agars is such that it is often necessary to indicate not only the genus and species but also the country of origin and the site at which the alga was harvested.

Table 2: World agar production in 1990.

<table>
<thead>
<tr>
<th>COUNTRY</th>
<th>Tonnes</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Japan</td>
<td>2,260</td>
<td>31.3</td>
</tr>
<tr>
<td>Spain and Portugal</td>
<td>1,910</td>
<td>26.4</td>
</tr>
<tr>
<td>Korea</td>
<td>1,440</td>
<td>19.9</td>
</tr>
<tr>
<td>Chile</td>
<td>900</td>
<td>12.5</td>
</tr>
<tr>
<td>Morocco</td>
<td>250</td>
<td>3.5</td>
</tr>
<tr>
<td>USA and Mexico</td>
<td>150</td>
<td>2.1</td>
</tr>
<tr>
<td>Canada</td>
<td>150</td>
<td>2.1</td>
</tr>
<tr>
<td>France</td>
<td>80</td>
<td>1.1</td>
</tr>
<tr>
<td>India</td>
<td>10</td>
<td>0.1</td>
</tr>
<tr>
<td>Argentina</td>
<td>10</td>
<td>0.1</td>
</tr>
<tr>
<td>Others</td>
<td>70</td>
<td>-</td>
</tr>
<tr>
<td>TOTAL</td>
<td>7,230</td>
<td>-</td>
</tr>
</tbody>
</table>

The total harvest of agarophytes amounted to 540,000 tonnes (wet weight), comprising 120,000 tonnes for Gelidium, 50,000 tonnes for Pterocladia and Gelidiella and 370,000 tonnes for Gracilaria. Agar contents relative to dry weight differed greatly (19 to 29%) from one alga to another and for a given place and time. The two great areas of cultivation are Taiwan and Chile (Figure 8).
Figure 8: World agarophyte sources.
7.1 Extraction

The general process is based on the fact that agar is insoluble in cold water and soluble in warm water. The principle consists in obtaining a solution containing about 1% of agar at the end of extraction. Beyond this value, the separation between agar and water becomes practically impossible if a suitable product is to be obtained.

AGAR EXTRACTION

Preparation of the raw material
- Water bath + acid (0.05%); 2 to 3 h
- Rinsing
- Alkaline bath - Na₂CO₃ (0.5%); 30 min

Isolation of the agar solution
- Algal bath + water + pH 4 to 8; 3 to 4 h
- Filtration: rotative or plate filter + infusorial earth

1% agar juice

either

Obtaining agar

- Gelation

or

- Gelation

- Pressure syneresis (agar 25% water)

- Thawing (agar 92% water)

- Air or heat drying

- Evaporation

Grinding

Except for these two points, it is impossible to describe an extraction method that is valid for all agarophytes. It is essential each time to adapt the technique to the physicochemical characteristics of the alga to be processed.

Freezing: Freezing is based on the fact that agar below 0°C becomes insoluble in water. The process consists in freezing the filtrate F containing 1% agar at a temperature between -2 and -10°C. Until 1960, the prevailing method consisted in placing the filtrate F with 1% agar in 45 x 30 x 5 cm wooden boxes until gelation occurred. The gel was then cut into thin slices. The slices were treated differently according to season. In winter they
were exposed to bad weather so that they could undergo a series of freezings and thawings. In summer they were soaked in a saline solution at -5°C. In both cases, the effect was the same. The water separated partly from the agar, taking pigments and other impurities with it.

![Cooling circuit allowing isolation of the agar.](image)

Since 1967, the agar solution is cooled progressively (Figure 9), and the agar, having become insoluble, floats on the surface. A comb channels the flocculent masses toward a grid where they are drained. This type of rather costly separation is only used for high quality agars (Gelidium or Pterocladia agar).

**Figure 9:** Cooling circuit allowing isolation of the agar.

Syneresis: This method is applied more particularly to the Gracilaria genus. Syneresis is the gel property which tends to compress and exude the water which it contains. This evacuation of water can be accelerated by applying pressure, in which case energy consumption is relatively low since dehydration requires only 8,360 to 9,196 kJ per kg of agar. However, implementation of the industrial technology is not easy.

### 7.2 Special case of Gracilaria and Porphyra in East Asia

Species of the Gracilaria and Porphyra genera were previously considered to provide soft gels or nongelling viscous solutions. Now they can be made to produce an acceptable gel by subjecting the alga to a highly alkaline heat treatment using an O.025 to 0.5 N soda solution, depending on the species and place of origin. This operation, known as "alkaline hydrolysis", converts the a-L-galactopyranose-6-sulfate of the second monomer of neoagarobiose into 3,6-anhydro-a-L-galactopyranose.

### 7.3 Characteristics of the extraction factory

The operation of an agar-producing factory should take four basic elements into account: availability of water, the need for a control laboratory, maintenance of extreme cleanliness and the existence of well-designed storage areas.

### 7.4 Agar properties

Although agar possesses a certain number of characteristics which make it a quite special colloid, the basic property is its spontaneous ability to provide very resistant gels at a low concentration.
**Gelation**

When agar is mixed with water, the resulting solution has a gelling power. It is only necessary to heat the solution to 85-90°C and let it cool again. Other components need not be added, contrary to the situation for alginic acid (bivalent and trivalent cations and acids are required to produce gels), carrageenans (gelation occurs only in the presence of proteins or cations such as K⁺ or Ca²⁺) and pectins (sugars or acids must be added to produce gels).

**Measurement of gel strength**

Measurement of gel strength constitutes the basic control element in the agar business. Industrialists and their customers generally use the Nikan Sui process which consists in determining the weight load capable of breaking a standard 1.5% gel in 20 seconds.

Gel strengths range from 150 to 1,200 g.cm⁻². Top quality values are between 600 and 900 g.cm⁻² medium quality between 400 and 600 g.cm⁻² and poor quality less than 350 g.cm⁻². Only *Gelidia*, because of their high agarose level, produce gels reaching and sometimes exceeding *(Gelidium latifolium)* a break force of 1,000 g.cm⁻², which corresponds to a gel strength 10 times as great as that of other colloids with the same concentration measured in the same conditions.

**Gel properties**

Agar produces a translucent gel which is tasteless and odourless and can therefore be used for the gelation of food products without altering colour or flavour. It enhances the taste and acts as a long-term aromatic fixative. The refractive index can be increased by the adjunction of sugar (glucose) or glycerine, giving an attractive appearance. Agar gel has excellent reversibility.

- It can be melted and gelled repeatedly without losing its original properties.
- It can withstand heat treatment above 100°C, which allows for good sterilisation.
- It can be used with a wide range of pH (5 to 8, and sometimes above).
- A 1.5% solution gels between 34 and 38°C for *Gelidium* agar and between 40 and 52°C for *Gradlaria* agar. Fusion only occurs between 85 and 90°C.

7.5 **The special case of bacteriological agar**

One of the greatest uses of agar is in the bacteriologic field. In fact, bacteriology is indebted to agar for the progress it has made since 1881 when Robert Koch used this product for the first time to solidify culture media. Bacteriologic agar is prepared mainly from *Gelidium* and sometimes from *Pterocladia*. For these genera, a 1% solution reaches gel state between 34 and 38°C, temperatures which allow bacterial culture. However, the extract obtained from *Gracilaria* and *Gelidiella* solidifies at 41 to 43°C, temperatures too high for bacterial life.

**Agarose**

Agarose is the chemical component of agar which is the poorest in sulphuric esters and the richest in 3,6-anhydro-α-L-galactopyranose. It is very difficult to obtain agarose in pure state, i.e., free both of residues from the extraction process and of the chemical products used to isolate it from agar. Various techniques (roughly 15) have been
developed to reduce purification costs or more particularly to ensure the elimination of impurities incompatible with certain applications.

Agarose is defined by

- its physicochemical properties: colour, transparency, moistness, ash content, gelstrength, fusion temperature and gelation temperature;
- its purity: maximal reduction of electronegative groups, tending toward a neutral product (the presence of these groups would induce the binding of positively charged substances such as proteins);
- characteristics relative to its use when intended for a specific type of electrophoresis.

8. AGAR APPLICATIONS

Twenty years ago, agar was used mainly as a thickener of textile colorants. For this purpose, it has been totally replaced by alginates.

8.1 Use in the food industry

The very wide range of agar applications in the food industry is attributable to its particular gelation characteristics not found in any other vegetable or animal colloid. Accordingly, its price on the world market is higher than that of other colloids. As agar was the first phycocolloid used, some 300 years ago, it can be safely assumed that it has no toxicity for man. The countries with the most demanding regulations have approved its use. Under the code E 406, it is used mainly for gelation but also as a stabiliser for controlling viscosity. The customary doses are less than 1%. Studies have shown that human agar digestion is imperfect since less than 10% is assimilated. Given its limited calorific contribution, reduced assimilation and low percentage in foods, agar cannot be said to modify the calorific value of the foods to which it is added. Its numerous applications in the food industry include the following:

- In the bakery trade, cake toppings and icings (agar ensures the good adherence of chocolate without occurrence of cracks).
- In confectionery, for the preparation of jellies and to avoid the dehydration of preparations.
- In fruit salad desserts, in which the fruits cut in small pieces are mixed with sugared and coloured cubes of agar. The agar used in this preparation must withstand sterilisation without melting or losing its edges. Only certain Gelidiun agars can meet these requirements.
- In making jams, where agars are used instead of pectins which require large amounts of sugar for gelation. Apricot, peach, apple or even coffee jelly can be obtained in this way (2 g of agar, 4 g of instant coffee, 300 ml of water + sugar).
- Preparation of low-acid yoghurts in which agar replaces casein to maintain the consistency.
- Jellied meat: pieces of beef, chicken or ox tongue in jelly.
- In sauces, in which it serves as a stabiliser or thickener, as well as in alcoholic drinks.
• For packaged sauces, in which it allows sterilisation without any loss of viscosity or gelling power.
• For food preparations, a high concentration of agar added to glycerine and glycol (a preservative) provides a hard protective gel. Moulds and bacteria cannot penetrate this barrier nor develop within it because of low water content.
• The production of wafers (for mass) in which agar is mixed with starch.

### 8.2 Uses of agar in agriculture

Seeds are preserved from bacteria and moulds by an agar gel of the same type as that used for the protection of food preparations. The fight against insects requires the raising of insect larvae, a great number of which are then sterilised before being released. Sterile males play the role of fertile males with regard to females, so that there is no reproduction. The harmful action of the fly *Pectinopora glosipeis*, which attacks cotton plantations, has been limited in this way. Agar enters into the preparation of food for the larvae of these insects.

In orchid nurseries, agar is the substrate which receives the culture medium for meristems or cell tissues for clone formation. The agar must be absolutely free of growth inhibitor. This application has increased considerably since meristem cell cultures have become the classic technique in agriculture for producing and reproducing certain varieties.

### 8.3 Use of agar for pharmaceutical purposes

Agar was first used as a bulk laxative (Molagar) and is now used for many other purposes. It is an expedient in pharmaceutical preparations and enters into the composition of gastric plasters (phosphalugel, gelogastrin, anacidase). Agar is used to thicken and stabilise many cholesterol solutions. It is also used as an emulsifier in creams, suppositories and surgical liquids and as a dispersive agent in tablets. It is mixed with potentially inflammable alcohol concentrations to avoid this risk. Added to dietetic substances, it serves as an appetite suppressant by expanding within the stomach.

### 8.4 Use of agar for moulding and casting

Agar gel allows precision moulding to be achieved in sculpture and archaeology as well as in dentistry where it is used most. One advantage of agar is that a series of equally fine secondary moulds can be made based on the first one.

### 8.5 Use of agar in bacteriology

Applications in bacteriology constitute one of the most remarkable uses of agar. A great number of culture media use agar as basic substrate. Agar has contributed to the development of most vaccines. To prevent syneresis, an 0.75%-5% agar-gelatine mixture is prepared. As agar is not altered by bacteria and does not affect them, their biology can be studied as a function of the substances introduced into the gel. A typical medium is composed of:

- agar 15 to 30 g
- sodium chloride 5g
- water 1,000 g
- pectin 10g
- protein 50 g
The medium is different for each bacterial species, but the main ones are the following:

- glycerine-agar
- blood-agar (from the horse or sheep)
- serum-agar
- egg-agar

8.6 Use of agar in biochemistry and biotechnology

Agarose is generally used for protein separation, mainly in analytic laboratories. Industrial applications have also appeared because of advances in genetic engineering productive of substances such as interferons, interleukins and insulin which are often separated and purified on an agarose support.

Electrophoresis

Electroendosmotic gels constitute the most suitable medium for the separation of polyelectrolytes according to their charge or mass.

- Separation according to the electric charge is based on the difference in the migration velocity of substances with different electric charges.
- Separation according to the mass or the size of the molecules depends on their ability to migrate through gel pores: small molecules migrate faster than large ones.

There are numerous applications for this property, including analysis of biological fluids, separation of DNA combinations and development of the genetic map, recovery of isolated substances, preservation of electrophoretic plates, isoelectric focusing and two-dimensional gel electrophoresis.

Immunology

There are many applications of agarose in immunology for the detection and study of antigenic material, particularly that responsible for diseases.

Culturing of micro-organisms

Agar is a suitable substrate for culturing micro-organisms and cells. However, even those agars considered typically bacteriologic can contain infinitesimal proportions of unknown substances capable of disturbing the development of micro-organisms and animal or vegetable cells. For this reason, scientists prefer to use agarose which is known to have a high degree of purity and consistency.

Gel-filtration and affinity chromatography

1. Immobilisation of biological systems. Agarose in capsule or bead form is used to enclose active coal and ion-exchange resins during blood infusions to detoxify patients after an overdose.

2. Other uses. Agar is also used for many special purposes, including lubrication of certain parts involved in the processing of tungsten and tantalum; graphite preparation; protection of aluminium upon contact with caustic environments; stabilisation of nitroglycerin; manufacture of ultra-sensitive photographic films and of paint, batteries and storage batteries; and preparation of primers or glues for wallpapering.
9. WORLD MARKET

The quality standards used are generally based on those established by the Japanese merchants who have dominated the agar market for about 50 years. The four qualities differentiated by them are summarised in Table 3.

Table 3: Characteristics of the different commercial agars.

<table>
<thead>
<tr>
<th>QUALITY</th>
<th>SPECIAL</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water content (%)</td>
<td>22</td>
<td>22</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>Gel resistance (g.cm⁻²)</td>
<td>+ than 600</td>
<td>+ than 350</td>
<td>+ than 250</td>
<td>150</td>
</tr>
<tr>
<td>Protein content (%)</td>
<td>1.5</td>
<td>1.5</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Warm insoluble matter (%)</td>
<td>0.5</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Maximum ash (%) at 550°C for 4 h</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>5.5</td>
</tr>
</tbody>
</table>

The sale price ranges from $36 per kg (special) to $10 per kg (quality 3). Food agar represents the greatest part used in the world (88 to 93%), mainly in Asia. Contrary to that of carrageenans, agar production is performed in relatively small factories, of which there are 220 in the world (170 in Japan). The two largest factories, in Spain and Chile, have an annual capacity of 400 tonnes.

The market for bacteriologic agar represents only 4 to 5% of the 7,530 tonnes produced world-wide, and the kg sells for $36 to $100. The price of agarose ranges from $190 to $1,170 per kg and stands for 2% of the bacteriologic agar. Table 4 shows the quantities of agar consumed in each country in 1990:

Table 4: Quantities of agar consumed in the world in 1990.

<table>
<thead>
<tr>
<th>COUNTRY</th>
<th>Consumption in tonnes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Japan</td>
<td>3,300</td>
</tr>
<tr>
<td>United States</td>
<td>830</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>700</td>
</tr>
<tr>
<td>Germany</td>
<td>600</td>
</tr>
<tr>
<td>Korea</td>
<td>215</td>
</tr>
<tr>
<td>Denmark and Spain</td>
<td>250</td>
</tr>
<tr>
<td>France</td>
<td>100</td>
</tr>
<tr>
<td>Italy</td>
<td>150</td>
</tr>
<tr>
<td>Argentina</td>
<td>125</td>
</tr>
<tr>
<td>Brazil</td>
<td>100</td>
</tr>
<tr>
<td>Chile</td>
<td>90</td>
</tr>
<tr>
<td>Africa</td>
<td>100</td>
</tr>
<tr>
<td>South Sea Islands</td>
<td>170</td>
</tr>
<tr>
<td>Eastern Europe</td>
<td>400</td>
</tr>
<tr>
<td>Others</td>
<td>400</td>
</tr>
<tr>
<td>TOTAL</td>
<td>7,530</td>
</tr>
</tbody>
</table>

References


Doty, M. S. 1977. Seaweed resources and their culture in the South China Sea Region. FAO Publication SCS/7/WP60 (Workshop in Manila), 22p.


A REVIEW OF GRACILARIA CULTURE IN ASIA-PACIFIC AND DIRECTIONS FOR FUTURE DEVELOPMENT

Gavino C. Trono Jr.,
Marine Science Institute, College of Science, University of the Philippines,
Quezon City, Philippines.

ABSTRACT
The current status of Gracilaria culture in the region, different culture methods, production of planting materials and the future development of Gracilaria culture are discussed in this paper.

1. INTRODUCTION
At present, a large proportion of the agarophytes used in the manufacture of agar still come from natural stocks. There are no accurate data available on the contributions of agarophytes produced through cultivation but, based on the genera imported by Japan in 1984 from different countries (Armisen and Galatas, 1987), about one half of the seaweed processed into agar is harvested from natural stocks. The main genera of seaweeds utilised in the manufacture of agar are: Gracilaria; Gelidium; Pterocladia; Gelidiella; Ahnfeltia; and Ceramium. In 1984, approximately 6,683 tonnes of agar were produced by 14 countries (Armisen and Galatas, 1987). About 48% of this amount was processed from Gelidium and Pterocladia and the other 52% from "other seaweed" which undoubtedly would include other genera such as Gracilaria and Gelidiella. More recent estimates place world agar production at 7-10,000 tonnes (Globefish, 1988).

As far as is known today only the genus Gracilaria is presently produced in commercial quantities though cultivation. In the early 1980's, Chile, Brazil, Taiwan and the Philippines were the main suppliers of cultured Gracilaria to Japan. Recently, Vietnam, Indonesia, Thailand, Hawaii and others have applied culture techniques in Gracilaria production. Even now a large proportion of Gracilaria production still comes from natural stocks. Gelidium and Pterocladia are used mainly for the production of bacteriological agar and agarose and are still derived from the harvest of natural stocks. Their commercial production through farming is still a long way from realisation.

2. GRACILARIA CULTURE

2.1 Species of Gracilaria presently cultured
Seven species of Gracilaria have been reported to be cultured in the region. These are: Gracilaria changii (Xia et Abbott) Abbott, Zhang and Xia; G. flrma Chang et Xia; G. fisheri (Xia et Abbott) Zhang et Xia; G. heteroclada Zhang et Xia; G. gigas Harvey, G. lemanaeformis (Bony) Weber van Bosse; G. lichenoides (Linne) Harvey and G. tenuistipitata Chang et Xia.

2.2 Selection of culture areas
Site selection for farms is an important consideration which partly determines the success or failure of the farming venture. In general, sites which support natural stocks of the species to be cultured are considered good sites. However, if culture is to be carried out in areas where there are no natural stocks of the species, the area should have comparable ecological conditions to the sites where the natural stocks are found.
Basic ecological parameters which should be evaluated are: salinity; water temperature; turbidity; water movement; depth at low tides; and nutrients.

Natural stocks of many species found in commercial quantities grow quite well in brackish and highly fertilised waters like protected bays and lagoons. Some species with potential for culture are also found on reefs, sandy flats to sandy-rocky wave exposed habitats. The culture technology to be applied should be adapted to the ecological conditions of the sites.

2.3 Selection of highly productive seedstocks

The utilisation of highly productive species/ strains with high quality agar is always a plus factor in the successful farming of *Gracilaria*. In species where it is easy to manipulate the individuals to improve hybridisation, using the gametes may be a normal and logical way of improving the seedstocks. However, in *Gracilaria* and other species where the technique in the manipulation of the sexual process to produce hybrids is not well known, other methods may be used in selecting the species.

One of these methods is through species/strain selection. Different strains/species present in a natural populations are selected, e.g. different species, various coloured strains, morphologically different thalli. These different strains/species are then multiplied through cloning. Comparative eco-physiological studies are then conducted using these stocks. Physiological studies to determine the tolerance range of the various species/strains to the changes in ecological factors may be done employing manometric techniques using a respirometer. The photosynthetic/respiratory responses can be monitored under varying conditions of stress. Analyses of yields and agar qualities of the various species/strains are also an indispensable tool in determining the qualities of the various strains or species as a basis for the selection of seedstocks.

*In situ* studies on seasonality and production capacities of the various strains/species, relative to changes in ecological factors (such as salinity, temperature, nutrients and water movement), are important in determining the field conditions which influence the productivity of the various strains as well as the seasonal aspects of the reproductive states and regenerative capacities of the various strains/species.

2.4 Production of seedstocks

The term "seeds" refers both the vegetative propagules (cuttings) and spores which can be utilised as planting materials. The availability of local stocks as seed materials is important. *Gracilaria* exhibits triphasic alternation of generation consisting of the gametophyte, sporophyte and carposporophyte. The large somatic stages, the gametophyte and sporophyte are sources of cuttings and spores.

Vegetative propagules (cuttings)

Healthy thalli which are fleshy and elastic in texture, dark-reddish brown in colour, robust, well-branched with smooth and shiny surfaces should be selected as planting materials. These must be clean, free of dirt and epiphytes. Preparation of the "seeds" varies depending on the type of culture used, which are described later in this paper.

Spore and seeding materials

Two methods are presently used in the production of sporelings from spores: natural spore-recruitment and induced spore-shedding in hatcheries.
Natural spore recruitment
In the spore recruitment method, artificial substrates like wooden stakes, rocks and netting materials may be used. However, braided ropes and netting materials are generally preferred because of the ease in handling during the process of recruitment as well as during cultivation. The ropes are anchored or tied to wooden stakes below the low tide level among the dense populations of *Gracilaria*. These are left in the area for about two weeks to allow the naturally shed spores to settle on them. The sporelings developing from the spores become visible after 3 to 4 weeks. The seeded ropes with sporelings are then transferred to the culture sites for outgrowing.

Hatchery production of sporelings from spores
The production of sporelings from spores requires some skill to recognise fertile materials. The fertile female materials are quite easy to recognise because of the presence of cystocarps. These fertile structures appear as elevated mammillate "bumps" on the surface of the thalli with dark coloured contents. Cystocarps which have already shed spores ("empty") are pale in colour. In contrast, the fertile sporophytic thalli is quite hard to recognise because the tetrasporangia are microscopic and do not form apparent recognisable structures. The tetrasporangia appear as dark purplish microscopic spots on the surface of the thalli when examined under a stereomicroscope. Their presence, however, has to be counter checked by the examination of sections of the suspected thalli under a compound microscope. The tetrasporangia are of the cruciate type and are embedded in the cortex of the thallus.

Hatchery production of sporelings requires a land-based set-up consisting of a seeding tank with provision for control of water depth and an adjustable seeding material support structure. These structures consist of a wooden frame with monofilament netting material which can fit into the seeding tank and a mechanism to adjust the height from the bottom of the tank. Various types of substrate can be seeded, *e.g.*, pieces of gravel, shells or lines. These materials are placed at the bottom of the seeding tank. The use of lines as setting substrate requires additional structures, *i.e.*, a frame which also fits the tank when laid flat on the bottom. The line may be nylon braided ropes (ca. 3mm dia) or plastic tying raffia materials. The "raffia line" or rope is wound evenly to the seeding frame. The frame with "raffia line" is placed at the bottom of the tank.

Fertile materials of *Gracilaria* are collected from the natural stocks and brought to the seeding facility. The moist materials are placed in containers such as styrofoam boxes provided with aeration holes to avoid stress. The fertile *Gracilaria* are then placed on the seeding support structure and submerged in water in the seeding tank. The distance of the seeding materials from the seeding substrates (rocks, stones, or raffia in frames) should be adjusted to ensure the uniform distribution and density of the spores. The seeding materials are left in the tanks for 2 to 3 days to allow the shedding of spores.

The seeded materials may be left: in the tank for another 1 to 3 days to allow the spores to germinate and completely attach to the raffia or substrates. The seeded rope is transferred to a holding tank or to a nursery area while waiting for the spores to develop into sporelings.
3. CULTURE METHODS PRESENTLY USED

3.1 Pond culture

The determination of pond culture system for Gracilaria consists of the following stages:

*Site selection*

The success in pond culture of Gracilaria is highly dependent on the selection of appropriate sites. The following criteria are used in the selection of sites for pond culture:

- the site should be located near seawater and freshwater sources;
- the site should be protected from strong winds;
- the pond bottom should be at or near the zero tide level; and
- the pH of seawater should be slightly alkaline, e.g., 8.2 to 8.7.

Many species of Gracilaria are euryhaline and can grow in brackish water under a wide range of salinities. A salinity range of 15 to 24 ppt has been found to be optimal. Salinity increases during the sunny months due to evaporation, reaching values as high as 35 ppt or drops to as low as 8 ppt during the rainy season. These variations have been shown to be detrimental to the crop. The maintenance of optimal salinity in ponds requires available sources of both freshwater and seawater. The ponds should be located in areas protected from strong winds to prevent the Gracilaria accumulating on the leeward side of the pond. The formation of thick heaps of Gracilaria on one side of the pond has adverse effects on growth due to shading. Water movement is greatly influenced by tidal changes in relation to the elevation of the pond bottom. Ponds located in areas where the bottom is at, or a little above, the zero tide level can easily be managed as water exchange is easy.

*Culture ponds*

The average size of ponds for the culture of Gracilaria is about one hectare or smaller. Smaller ponds are easier to manage than larger ones. Pond management is also easier when Gracilaria is polycultured with shrimp and or crab. Provisions of entrance and exit gates facilitate proper water management.

The depth of ponds vary from 50 to 80 cm. The bottom generally, is of clayish loam, silly loams or sandy loam. It was observed that Gracilaria easily gets buried in ponds with soft sandy or muddy bottom. This problem can be prevented by increasing the depth of the water during windy periods. In large ponds, wind breakers consisting of bamboo slots are installed perpendicular to the direction of the wind to prevent the seaweed being transported to one side of the pond.

*Preparation and stocking of ponds*

The ponds are dried for several days after which water is men introduced. Healthy stocks (characterised by their elastic feel to touch, reddish brown colour, brittle texture and well branched thalli) are selected as planting materials. The seedstocks are transported from the source to the pond site early in the morning to prevent exposure to the sun. During long-distance transport, it is frequently sprinkled with seawater and perforated bamboo or plastic pipes are inserted into the bottom of the heap to provide aeration. The plants must immediately be placed in the pond upon arrival. The planting materials are cleansed, cut into pieces and broadcast uniformly on the bottom of the pond. The stocking rate is 5,000 to 6,000 kg of chopped Gracilaria per hectare.
Pond management
The water is maintained at a depth approximately 30 to 40 cm above the top of the algae. However, the depth is increased to 60 to 80 cm during the warm summer months to prevent a significant rise in water temperature. Water depth is also increased during the cold winter months to avoid temperatures dropping to below 8 °C, which is lethal to Gracilaria.

Frequent exchange of water is necessary to maintain optimum water temperatures in the ponds. About 50 to 75% of the pond water is drained and replaced with fresh seawater every two to three days.

Fertilisation with organic or inorganic fertiliser is necessary to enhance the growth of Gracilaria. Weekly application of three kilograms of urea per hectare was found to be sufficient. Fermented pig manure may be applied at a rate of 160 to 180 kg per hectare two to three days after the exchange of the water. The manure is placed in sacks and the sacks are placed at strategic areas in the pond.

Harvest and post-harvest activities
Under optimum conditions, the crop may be harvested 2 to 3 month after seeding. Cropping may be done every 10 to 40 days manually by using scoop nets. The frequency of harvest is primarily dictated by the amount of biomass, the market price and the season. Approximately 30 to 40% of the biomass is harvested during each cropping. The crop is thoroughly washed in pond water to remove silt, sand, pieces of shell and other extraneous materials, such as snails and other algae. The clean Gracilaria is spread uniformly on bamboo screens or plastic sheets for drying. An average wet to dry ratio of 7:1 is generally attained.

3.2. Field culture
There are several methods used in the field culture of Gracilaria. The "seeds" used in these various methods are cuttings or sporelings.

Fixed off-bottom long line method
In this method, three-strand polyethylene ropes 3 to 4 mm in thickness and 5 meters long (or longer) are generally used. Other type of materials, such as coir or abaca ropes, may be used as a substitute. The seeding of long lines using cuttings consists of untwisting the rope and inserting bunches of cuttings between the strands of the rope, the seedlings passing two or three times through the strands. This method ensures that the cuttings are securely tucked in place. The seeding of the ropes is done in the shade and the seedlings are placed in basin of sea water to prevent the cuttings from dehydrating.

The support system for the long line consists of wooden stakes driven into the ground. These are usually arranged in rows, the distance between stakes in the row is about one meter while the distance between rows of stakes varies between 4 to 5 meters. One end of the seeded rope is tied to a stake, stretched tightly and the other end is tied to the opposite stake in the next row.

The same technique is applied for ropes seeded with spores. Unbraided plastic raffia seeded with hatchery produced sporelings are used as long lines. Longer ropes may be used but additional stakes are utilised as support to prevent the ropes from sagging to the ground. The distance of the seeded ropes from the ground vary depending on the depth and clarity of the water.
Raft method
The raft method is popularly used in deep areas and where bamboo is available. The raft generally measures 2 x 4 meters in size. Seeded long-lines are also used. One end of the seeded rope is tied to the shorter bamboo frame, stretched tightly and the other end fixed to the opposite side of the raft. The distance between ropes vary but about 12 to 14 lines or more may be attached to one raft. In India, the use of coir ropes fabricated into nets of 7 cm mesh size was used on a 2 x 2 m raft. The same method of seeding was used. Variations of this method have been developed in the People's Republic of China. Pieces of rope about 70 cm long seeded with *Gracilaria* cuttings are hung horizontally on the low fixed raft or vertically along a long line floating raft.

Improvement of substrates
This method can only be utilised in areas where natural beds are found. The introduction of artificial substrates such as rocks, shells, bamboo or wooden stakes to the site provides additional substrates for the spores to settle or fragments of thalli to attach. Most of the commercially important species of *Gracilaria* (beds) are found in shallow bays and coves where the substrate is generally particulate (sandy-muddy). The survival and success of spores growing in this habitat is greatly enhanced by the availability of solid substrates. Results of studies on the influence of solid substrate on biomass production has shown that production more than doubles in portions of the beds where solid substrates were introduced.

33 Polyculture with shrimp and/or crab
*Gracilaria* can be polycultured with shrimp (*Penaeus monodon*) and/or crab (*Scylla serrata*). Stocking material for a hectare of farm consists of 4,000 to 5,000 kg of *Gracilaria*, 5,000 to 10,000 crab and 10,000 to 20,000 shrimp. Crushed trash fish and snails are generally used as feed for the crabs. Crabs are harvested after three months, the shrimp after four to seven months. Survival rates as high as 80% for crabs and 80 to 90% for shrimp, have been documented making this polyculture one of the most profitable aquaculture methods in Taiwan. The net income from polyculture has been proven to be three times as much as from monoculture.

3. Polyculture with fish in cages
*Gracilaria* may be polycultured with carnivorous fish species in cages. Seeded nylon twines are attached to a support structure at the opposite sides of the cages. The distance of the seeded lines below the surface of the water is determined by the light intensity requirements of the species for maximum photosynthesis.

4. FUTURE DIRECTION FOR DEVELOPMENT

4.1 Selection and development of fast growing seedstocks with high quality agar
Two of the factors which influence the income of farmers are the production capacity of the farms and the agar quality of the seedstock. Thus, comparative studies of local species of *Gracilaria* should be done to select the most productive, fast growing species or strain with high quality agar. The selected species should then be developed and multiplied and used as seedstocks for commercial culture.

4.2 Polyculture
Studies on polyculture with other species in ponds should be done to increase the productivity of the farms and make efficient use of the space and nutrients available.
4.3  **Appropriate methods and culture site**

The species respond differently to the culture methods being used, thus the method to be applied to the species should be one which will enhance the ecological conditions resulting in an increase in the productivity of the species. Culture sites to be selected should conform to the ecological requirements of the species to be cultured.

4.4  **Environmental aspects of polyculture**

Although the polyculture of *Gracilaria* with other species is definitely known to improve the quality of pond effluents, the absorption and accumulation of pesticides and other toxic substances by the seaweeds should be a primary concern of the farmers which should be resolved. Studies on the pesticides which are not harmful to humans should be carried out.

4.5  **Improvement in the post harvest methods**

The quality of the crop is by large influenced by the post harvest methods used. The removal of dirt and associated materials by thorough washing and sorting and the use of fast sun-drying methods are some of the easy but important post harvest activities which will enhance the quality of the crop.
REFERENCES


TAXONOMY AND CULTURE OF GRACILARIA IN THE ASIA - PACIFIC REGION

Chen Jiaxin,
Director, Yellow Sea Fisheries Research Institute,
19 Laiyang Road, Qingdao, China 266003.
and
Xia Bang Mei,
Institute of Oceanology, Academic Sinica,
7 Nan Hai Road, Qingdao, China 266071.

ABSTRACT

This review report combines the country papers presented by China, India, Myanmar and Vietnam and the literature dealing with genus *Gracilaria*. Detailed biological characteristics of *Gracilaria* spp., including basic features of taxonomy, life cycle and ecological conditions for cultivation are described. Sixty-six species of *Gracilaria* and their distribution in the region are listed and 25 species are introduced in detail. Culture methods, such as pond culture and floating-raft culture, are briefly discussed.

1. INTRODUCTION

Red seaweeds of the genus *Gracilaria* have been the main source of agarophytes in the world since the 1960s, due to their euryhaline and eurythermal characteristics, as well as their higher growth ratios and agar contents. For example, 450 tonnes of agar products were produced in China in 1993, of which 260 tonnes were from *Gracilaria* spp. The taxonomic status of many of the *Gracilaria* species is not properly known in many countries of the Asia-Pacific region because *Gracilaria* species vary a great deal, even within the same habitat and population. This has often created confusion when comparing the information and data provided by different countries on red seaweeds with respect to production, culture methods, ecological parameters and phycocolloid properties. In addition, several other algae occurring in the region form an important resource of the phycocolloid industry, but little is known about their correct taxonomic status and ecological conditions for cultivation. In the absence of information on the productivity, biological and bio-chemical characteristics of the different strains and species, it is difficult to develop a viable culture technology for the lesser known species that would be economically efficient and sustainable. This paper is a review report that is based on the country reports presented by China, India, Myanmar and Vietnam, as well as a number of references, including, "Taxonomy of Economic Seaweeds" edited by Isabella A. Abbott.

2. A BRIEF HISTORY OF THE TAXONOMIC STUDY OF GENUS GRACILARIA

The genus *Gracilaria* was established by Greville in 1830 and consisted of four species: *G. confervoides*, *G. compressa*, *G. purpurascens* and *G. erecta*. In 1852, J. Agardh revised and redefined the generic circumscription for the genus and designated *G. confervoides* as its species. Early concepts of the species of *Gracilaria* were mainly based on external structure. A detailed anatomical study was first reported by Sjostedt in 1926 with *G. confervoides*, *G. compressa* and *G. robusta*.

In 1949, Dawson regarded nutritive filaments as a diagnostic characteristic at the generic level and distinguished the genus *Gracilariopsis* from *Gracilaria* on the basis of the absence of nutritive filaments and the small size of the gonimoblast cells. In 1966, Papenfuss reported that the presence of nutritive filaments could not always be
confirmed in British samples of *Gracilaria vermicosa*. He concluded that the presence of nutritive filaments could not be used as a basis for separating *Gracilaria* and *Gracilariopsis* and for that reason he reduced *Gracilariopsis* to a synonymy with the original genus *Gracilaria*.

In 1963, Chang and Xia described the genus *Polycavernosa* with *P. fastigiata* as the species type. This genus consists of rhizome-like creeping parts from which arise erect, free branches. The creeping portion is fastened at frequent intervals by a disc-like attachment; spermatangial conceptacles are in clusters and basal absorbing filaments have many long branches. Many authors supported the recognition of *Polycavernosa*, some species of *Gracilaria* were transferred into *Polycavernosa* and new species were described. Wynne (1989), re-instated *Hydropuntia* Montagne (1842) which was shown to be the earliest validly published name with *H. urvillei* as the species type. He transferred 14 species of *Polycavernosa* and two species of *Gracilaria* to *Hydropuntia*.

In 1991, Abbott, Zhang and Xia described a new species, *Gracilaria mixta* according to the mixture of two kinds of spermatangial conceptacles, *Verrucosa* type and *Polycavernosa* type, in the same branches. Re-examination of male plants of the western Pacific taxa placed in *Polycavernosa* (= *Hydropuntia*) also shows both types of configurations in the same thalli. Therefore, 16 species of *Hydropuntia* were transferred to *Gracilaria*.

The genus *Gracilaria* is cosmopolitan in distribution. It has been reported in most parts of the world, the Arctic, temperate, tropical and even Antarctic regions. According to Bird *et al* (1982), the apparent centre of distribution lies in the tropics with the largest number of species and a rapid decline in species numbers occurs with increasing latitude in both directions. More than 150 species have been reported. The species of *Gracilaria* in the region were listed, of which 27 species were from China, 32 species were from India, 7 species were from Myanmar, 9 species were from Peninsular Malaysia and Singapore, 13 species were from Thailand, 25 species were from the Philippines and 13 species were from Vietnam. The identification of all the species of *Gracilaria* listed here still remains a vexing problem owing to the great variability of the plants and poorly understood species limitations. Nevertheless, the information provided by country reports gives us an opportunity to exchange information with each other.

3. FEATURES OF *GRACILARIA* AND THEIR TERMINOLOGY

3.1 Morphological features

**Thallus construction**

A group of apical meristematic cells divided into multitaxial form thallus. The main structure of thallus is composed of pseudo-parenchymatous or parenchymatous cells. Throughout the thallus, commonly isodiametric cells of outer cortex layers and inner medulla layers are observed (Figure 1.1).

**Apical cells**

A group of apical cells are situated at the meristematic region of the thallus. It is difficult to distinguish them from the neighbouring cells of the broad apical region, except when a little protrusion occurs at the sharp apex (Figure 1.2).

**Cortex**

The cortex is the outer tissue of the thallus consisting of 1 to 4 layers of cortical cells. The cells are small, ovoid, strongly pigmented, densely protoplasmic and arranged in anticlinal plane. The
Figure 1: Morphological features of *Gracilaria* spp.

1.1: Transversal and longitudinal section of a sterile plant
   a. Cortex layers
   b. Medulla layers

1.2 Longitudinal section of an apical tip.
   a. Apical cells
   b. Cortex cells
   c. Medulla layers

1.3 Transversal section of a sterile frond, showing several florideam starch grains

1.4 A holdfast and its longitudinal section.
outermost cortical cells (surface cells) are more or less elongated and are smaller than the inner cells. Sometimes more cortical layers are observed in the basal part due to the secondary growth of the outermost cells (Figures 1.1-1.2).

Medulla
The term medulla has been used for the pseudo-parenchymatous tissue of the thallus beneath the cortex and consists of non-pigmented, irregularly polygonal, large cells with thin to thick walls. The medulla may be separated into two parts, the central medulla (inner medullar) usually with empty cells and peripheral layer (outer medulla) which is generally filled with the floridean starch grains. The transition from outer medulla to cortex is either abrupt or gradual (Figures 1.1-1.2 and Figures 1.3).

Hairs
Elongated, deciduous, colourless, unicellular hairs up to 40 long, are found abundantly on the cortical layer of the thallus in most species, but are scarcely present in G. verrucosa. Hairs are attached by means of a special foot-cell or hair base-cell. Hair base-cells are distinctly large, darkly staining and different in shape from other cortical cells.

Branches
Branches are erect or decumbent, cylindrical, compressed or flattened. The cylindrical branches may be conspicuously articulated into oblong, obovate to ovoid segments in some species (e.g. G. crassa). Branching may be highly variable irregularly or regularly dichotomously, sometimes trichotomously branched in one plant (e.g. G. foliifera). The shape and breadth of the branches may be uniform throughout the thallus (e.g. G. foliifera) or slightly broad or narrow at the apex and narrow at the base (e.g. G. textorii). The apices of the branches may be acute, obtuse or rounded. The base of the branches are slightly or distinctly constricted.

Branchlets
The branchlets are short and are probably potentially indeterminate branches arising on all sides of the branches. These occur generally at the upper parts of the branches (e.g. G. edulis).

Margins
The margins may be entire, sometimes undulated or spiny with minute processes or outgrowths.

Holdfast (Figure 1.4)
The plants are attached on the substratum by means of a discoid holdfast. One to several branches may arise from a single holdfast.

Haptera
The decumbent branches may be produce multi-cellular haptera or secondary attachment organs on reaching a substratum (e.g., G. crassa).

3.2 Reproductive features
Spermatangia (Figure 2.1)
Spermatangia are found on the surface of the thallus in variously shaped cavities. The spermatangia are ovoid to pyriform, 2.5-6.0 μm in diameter, more weakly staining than the spermatangial mother cells which are arranged compactly or loosely in anticlinal position on the surface cells of spermatangial cavities.
Figure 2: Reproductive features of *Gracilaria* spp.

2.1: Different types of spermatangia

a-b. Chorda-type; c-d Textorii-type; e-f Verrucosa-type

2.2 Carpongonial branches

a. Unfertilised carpongonial branch to show clearly trychogyne.
b. Early post-fertilisation stage of the carpongonial apparatus.
2.3 A mature cystocarp.
   a. Gonimoblast
   b. Carposporangia
   c. Nutritive filaments
   d. Pericarp

2.4 Carposporangia.

2.5 Tetrasporangia showing various styles of division.

*Spermatangial conceptacles* (Figure 2.2)
The shape of spermatangial conceptacles varies with different species. Three types of spermatangial cavities: (a) chorda type with superficial or wide opening, (b) textorii type with shallow cup-like cavities and (c) verrucosa type with deep and ovoid to oblong conceptacles with narrow openings. Dawson (Searles and Hommersand, 1971) proposed a system for classifying genera and species of Gracilariaceae based on spermatangial cavities.

*Carpogonial branches* (Figure 2.2)
The carpogonial branch is two to four cells and occurs singly (unicarpogonical) and outwardly on a supporting cell which is an inner cortical cell of the thallus. The carpogonium is conical and the basal cell is usually ovoid or angular and generally large, sometimes similar or smaller in size to the carpogonium. The trichogyne is long, not constricted at the base, projecting directly outward or slightly twisted at the tip. The supporting cell is large, ovoid, darkly stained and also produces two to four deeply stained celled vegetative filaments which are similar in size and shape to carpogonial branch.

*Post-fertilisation* (Figure 2.2)
After fertilisation, the fertilised carpogonium probably fuses directly with an adjacent cell from one of the vegetative filaments.

**Auxiliary cell**
Richly protoplasmic cell from one of the vegetative filaments borne on the supporting cell of the carpogonial branch probably functions as the auxiliary cell.

**Fusion cell**
The results of the extended connection of the fertilised carpogonium with neighbouring cells is a large ramified fusion cell consisting of carpogonium and adjacent cells of the vegetative filaments bearing from the supporting cell.

**Gonimoblast** (Figures 2.3, a)
After the formation of the fusion cell, gonimoblast initial cells arise from several points of the fusion cell outwardly. These further develop into gonimoblast tissue in the same direction. Earlier formed gonimoblast cells are large, polygonal in shape, densely protoplasmic and form a parenchymatous tissue around the upper part of the fusion cell. Gonimoblast parenchyma produces short-celled filaments which are irregularly shaped, rounded toward the tips and transformed into carposporangia.

**Carposporangia** (Figures 2.3,b and 2.4)
The carposporangia are ovoid to pyriform with a conspicuous central body and form the periphery of the short-celled gonimoblast filaments in chains.

**Nutritive filaments** (Figures 2.3 ,c)
Nutritive filaments connecting the gonimoblast parenchyma with the pericarp may or may not be present in different species or in the same species. These filaments are filiform, possess a base of greater or lesser width and haustorium-like ramification at the tips which pierce the pericarp cells.

**Carposporophyte**
Carposporophytes occur on the female gametophyte. This generation consists of a large irregular ramified fusion cell, bearing small or large gonimoblast parenchyma, with or without nutritive filaments and short-celled filaments which transformed into carposporangia in regular or irregular radial chains.

**Pericarp** (Figure 2.3,d)
The sterile cortical cells surrounding the developing carposporophyte, divide simultaneously and form the cortical layer of considerable thickness which becomes the cystocarpic wall (pericarp) enveloping the protruding carposporophyte.

**Cystocarp** (Figure 2.3)
The term cystocarp refers to the reproductive structure, found on the female plant, which forms as a result of the fertilisation of the carpogonium. The mature cystocarp consists of the carposporophyte and pericarp. They are either globose or dome-shaped, scattered or aggregated and protrude from the surface of the thallus.

**Ostiole**
The ostiole is an opening formed by successive divisions of cortical cells surrounding the developing carposporophyte. Carposporangia are liberated through the ostiole which are present in most species but absent in some species.

**Tetrasporangia** (Figure 2.5)
Tetrasporangia are cruciately divided, scattered on the surface of the thallus and surrounded by modified or unmodified cortical cells. Tetrasporangia initially may be cut off from any of the surface cortical cells.
4. LIFE CYCLE OF *GRACILARIA* (Figure 3)

*Gracilaria* shows an alternation of isomorphic generations between haploid gametophyte and diploid tetrasporophyte. The gametophyses are dioecious. The fertile male thallus produces spermatangia and the female thallus produces carpogonia. After fertilisation, a structure called a "cystocarp" is formed. The cystocarps are prominent, protruding, globose or hemispherical, with or without rostrum, scattered on the surface of the female thallus. Each cystocarp consists of pericarp, gonimoblast filaments and carposporangia, with or without absorbing filaments. Carpospores are liberated through a small hole or ostiole at the top of the cystocarp and germinate into tetrasporic thalli or tetrasporophytes. The mature tetrasporophyte produces tetrasporangia occurring generally in the cortex of the thallus. The tetrasporangium is cruciately divided and forms four spores or tetraspores which germinate into four gametophytic thalli or gametophytes, of which two are male and two are female thalli (Figure 3).
Figure 3: The life cycle of *Gracilaria*.

5. **LIST OF GRACILARIA SPECIES FROM SOME ASIAN COUNTRIES**

<table>
<thead>
<tr>
<th></th>
<th>BGD</th>
<th>CPR</th>
<th>IND</th>
<th>INS</th>
<th>JAP</th>
<th>ROK</th>
<th>MYA</th>
<th>MAL</th>
<th>PHI</th>
<th>THA</th>
<th>VIE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>G. arcuata</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td>G. armata</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3.</td>
<td>G. articulata</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4.</td>
<td>G. asiatica</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5.</td>
<td>G. bailinae</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6.</td>
<td>G. bangmeiana</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7.</td>
<td>G. blodgettii</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8.</td>
<td>G. bursa-pastoris</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9.</td>
<td>G. caudata</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10.</td>
<td>G. canaliculata</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>11.</td>
<td>G. changii</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>12.</td>
<td>G. chorda</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>13.</td>
<td>G. chouae</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>14.</td>
<td>G. coronopifolia</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>15.</td>
<td>G. corticata</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>16.</td>
<td>G. crassa</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>17.</td>
<td>G. cuneifolia</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>18.</td>
<td>G. cylindrica</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>19.</td>
<td>G. dactyloides</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>20.</td>
<td>G. debilis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>21.</td>
<td>G. denticulata</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>22.</td>
<td>G. disticha</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>23.</td>
<td>G. dura</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>24.</td>
<td>G. edulis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>25.</td>
<td>G. eucheumoides</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
6. DESCRIPTION OF THE MAIN SPECIES

6.1 G. articulata C. F. Chang et B. M. Xia (Figure 4)

Thallus erect, solitary or caespitose, arising from small discs, cylindrical throughout, usually 10-18 cm in length, alternately, secondly or irregularly dichotomously branched; common mango in colour, succulent, brittle, easily broken, adhering rather well to paper on drying; branches and branchlets conspicuously articulate-constricted to a sharp spine; articulations elongated club-shaped, arcuated, upwards, 10-20 times as long as broad, ending in blunt, almost equal in breadth, abruptly constricted at the base. Thallus in transverse section consisting of a large-celled medulla bordered by one to two layers of much smaller cortical cells; medulla composed of 4-6 layers of large roundish cells ca.
1030 µm in the greatest diameter, possessing a wall 3-7 µm thick; cortical cells roundish or elongated, 7-10x3-7 µm in dimensions, with cuticle 10 µm thick; transition from medulla to cortex strikingly abrupt.

Tetrasporangia scattered on the surface of thallus, circular or ovoid in shape as seen from surface, ca 33-36 µm in diameter, ovoid in transverse section, ca. 33 x 24 µm in dimensions, surrounded by somewhat modified cortical cells, cruciately divided. Antheridia borne in conceptacular pockets mostly with a broad opening, ca. 41 µm deep and 45 µm wide, well separated by modified cortex.

Figure 4: *Gracilaria articulata* Chang et Xia.
Cystocarps prominently protruding, semiglobose, 530-950 µm in diameter, non-rostrate, not constricted at base, the gonimoblast developing from a large fusion-cell and connected to the pericarp by numerous nutritive filaments; carpospores round or ovoid in shape, 25-30 µm in diameter, containing a conspicuous stellate central body; pericarp thin, 60-85 µm in thickness, consisting of 9-12 layers of cells, of which the outer cells of 1-2 layers are cuboid or oblong in shape while the inner layers of cells are horizontally elliptical with distinct cell wall, cells in distinct longitudinal rows, under low power.

Habitat: Grows on shells in the sublittoral region of low salinity.

6.2  **G. asiatica** Chang et Xia (Figure 5)

Thallus erect, solitary or caespitose, cylindrical throughout, habit variable, 3-50 (-200) cm in length, 1-3 mm in diameter, arising from a small discoid holdfast with 1-4 order of branches; purplish brown to dark brown, sometimes to greenish or yellowish, subcartilaginous in substance, adhering imperfectly to paper on drying; branches irregularly alternately, secundly or subdichotomously, sometimes with shorter or longer ramuli, 0.5-2. 5 mm in diameter, gradually tapering towards the apex and slightly or occasionally abruptly constricted or nonconstricted at the base. Thallus in transverse section consisting of a medulla of large parenchymatous cells, 165-365 µm in diameter with thicker walls, 8-24 (-40) µm, surrounded by 3-5 or more layers of cells, 7-10 x 5-7 urn in dimensions, pigmented and with surface jelly 10 µm thick; transition from medulla to cortex gradual; empty in central portion when old.

Tetrasporangia scattered among the surface layer of frond, subspherical or ovoid in shape in surface view, 40-46 µm in diameter, ovoid or oblong in transverse section, 49-69 µm x 39-49 µm in dimensions, surrounded by unmodified cortical cells, cruciately or occasionally tetrahedrally divided. Carpogonial branches two-celled; cystocarps prominently protruding, subspherical to hemispherical in shape, 660 x 750 µm in diameter nonrostrate or slightly rostrate, unconstricted or slightly constricted at base. Gonimoblast developing from a fusion-cell, parenchymatous, connected with the pericarp by rare (or without) absorbing filaments; carpospores round or ovoid in shape, 16-23 µm in diameter; pericarp 115-250 urn in thickness, consisting of 7-13 layers of cells; of which one layer of outermost pigmented cells and about 6-8 layers of cells with obscure cell walls except for 2-5 innermost layers of cells with clearer cell walls, the distinct stellate cell protoplasm of inner layer cells connected anticlinally and radially with each other by means of more distinct pit-connections. Spermatangial conceptacles scattered over the surface of the blade in well-separated, spherical to oblong in shape in surface view, 33-50 µm or more ovoid to long elliptical conceptacle in sectional view, 80-180 µm deep and 33-100 µm wide, well-separated by modified or unmodified cortex cells.

Habitat: Grows on gravel, pebbles and rocks which are often covered with sand and mud in the intertidal to upper subtidal.

Remarks: For more than two hundred years *Gracilaria verrucosa* (Hunds.) Papenfuss, (known as *G. confervoides* (L.) Greville) originally described in Devon, England by Hudson in 1762, has been reported from various places in the world from the arctic to the tropics and is regarded as a cosmopolitan species. In China, since Martens first reported the occurrence of this species from Macao, China in 1866, many other phycologists (Gepp in 1904; Cotton, 1915; Ariga, 1919; Tseng and Li 1935; Howe 1924; 1934 Setchell, 1931; Chang and Xia 1976; Zhang and Xia, 1984) have reported the same species from various places in China.
Figure 5: *Gracilaria asiatica* J. F. Zhang et B. M. Xia.
Determination of this species of *Gracilaria* is difficult because of their great variability and the lack of reliable characteristics in differentiating related species. Several years ago, Zhang and Xia found the structure of the pericarp to be quite constant with the species and valuable in differentiating species (Zhang and Xia, 1976). Although they identified the common Chinese species as *G. verrucosa* (Huds.) Papenfuss, they suspected the cosmopolitan distribution of the English species and that the Chinese species was different from the English species, but the lack of specimens from England and other places for comparative studies prevented them from dealing with this problem any further. After getting the specimens collected from Wemoury, Devon, England, comparative studies revealed that the Chinese species does differ specifically from the English *Gracilaria verrucosa* (Huds.) Papenfuss.

6.3 *G. changii* (Xia et Abbott) Abbott, Zhang and Xia (Figure 6)

Thalli 5-7 cm tall, with many branches arising from a small discoid holdfast or from a percurrent axis; branching alternate or irregular of two to four orders; branches cylindrical or inflated, 1.0-2.5 mm in diameter abruptly constricted at bases and tapering towards apices. Fronds in transverse section consist of large medullary cells with thick walls, two to three layers of cortical cells; transition of cells abrupt. Tetrasporangia ovoid, 13-17 x 28-36 µm in diameter, cruciately divided. Spermatangial conceptacles verrucosa type; adjoining ones frequently coalescing, forming polycavernosa-type spermatangia. Cystocarps conical or semiglobose, 1.0-1.4 mm wide, 0.5-0.8 mm high, some slightly rostrate, not constricted at base. Gonimoblasts consist of numerous small cells; absorbing filaments few, lateral and upper, carpospores round, 18-24 µm in diameter; pericarp thick, consisting of two kinds of cells, the outer five to six rows rounded, the inner six to nine rows compressed.

Habitat: Grows on gravel, shell, rock fragments, mangrove roots, or fish cages in sandy- muddy areas with high turbidity.

Remarks: This species has often been mis-identified as *G. cylindrica* (Doty et al. 1983, Santos and Doty, 1983, Doty and Fisher, 1987) and as *G. blodgettii* (Phang, 1986, Phang and Maheswary 1990) because of the constrictions at the branch bases. *Gracilaria changii* is quite widely distributed in Malaysian waters. The agar content and gel strength vary with local conditions.

Figure 6: *Gracilaria changii* (Xia et Abbott) Abbott, Zhang and Xia.
Figure 7: *Gracilaria chouae* J. F. Zhang et B. M. Xia.
6.4  **G. chouae** Chang et Xia (Figure 7)

Thallus erect, solitary or caespitose, arising from a small disc, cylindrical throughout, usually 15-20, but up to 40 cm long, 2-3 mm in diameter, with two to four orders of branches. Texture succulent; easily broken, and brittle when dry; adheres well to paper on drying. Light red when fresh. Branches irregularly alternate, occasionally furcate, but strongly mixed with unilateral or secund branching in whole or in part; gradually tapers toward apices and not constricted where attached.

In transverse section, medullar consists of large pseudo-parenchymatous cells, 232-598 µm in diameter, with walls 6.6-8.3 µm in diameter. Medullar surrounded by one to two layers of cortical cells, which are roundish and 23-33 µm in diameter; outermost layer pigmented and consisting of cells 10-17 µm by 7-10 µm in diameter. Transition from medulla to cortex abrupt. Tetrasporangia scattered among cortical cells, ovoid or subspherical in surface view, 31-43 µm in diameter, ovoid or oblong in transverse section, 26-63 µm by 17-33 urn in diameter, surrounded by slightly modified cortical cells; cruciately divided. Spermatangia conceptacles of "textorii-type", scattered over surface of frond in a small, shallow, well-defined depressions, 30- 53 µm deep and 30-43 µm in diameter; separated by modified, elongated cortical cells, characteristic of this type of conceptacle.

Cystocarps protrude prominently, conical or hemispherical, 664-913 µm by 650-880 µm, slightly rostrate, not constricted at base. Gonimoblast composed of some large pseudo-parenchymatous cells developing from a fusion cell, with many upper absorbing filaments connecting gonimoblast to pericarp. Carpospores round or ovoid, 20-36 µm by 10-20 µm in diameter. Pericarp 100-191 µm thick, consisting of 7-12 layers of cells; outermost layer pigmented cells ovoid-oblong; remaining pericarp cells 7-10 rows of rounded or horizontally elliptical cells, arranged in somewhat irregular vertical rows, with secondary pit connections inconspicuous; three to five innermost layers of small irregularly shaped cells.

Remarks: **G. bursa-pastoris** was characterised by Harvey (1849), and later by Ohmi (1958), as having succulent, brittle, alternate or subsecund branches, with broad insertions of the nonconstricted branches that had patent and rounded axils, numerous absorbing filaments in the cystocarp and shallow saucer-like spermatangial cavities. Chang and Xia (1976) thought that this characterisation matched the Chinese material rather well. However, the structure of the pericarp that Ohmi (1958) had depicted as having star-shaped cells with prominent secondary pit connections contrasted sharply with the Chinese material, which had pericarpic cells that were rounded to oval-obovate (Chang and Xia, 1976).

Recently, Abbott (1985), named a new species of *Gracilaria* for the Hawaiian material previously identified as *G. bursa-pastoris*, using as distinguishing features the small cells of the pericarp and their star-shaped contents, together with the large cells of the gonimoblast. This description of *G. parvispora* appears to include what has been called *G. bursa-pastoris* in Japan, and although Chang and Xia did not examine the Japanese material, they think that it is more like the Hawaiian than the Chinese material, particularly with respect to the structure of the pericarp.

In external appearance, particularly the unilateral branching pattern, *G. chouae* and *G. parvispora* look alike, although plants of *G. parvispora* tend to be more robust and shorter than the Chinese plants. The differences lie in some characteristics of the pericarp, such as thickness, shape, and arrangement of pericarp cells and contents, and in the sizes of the cystocarps. The cystocarp of *G. chouae* is conspicuously smaller than
that of *G. parvispora*. *G. parvispora* has the largest cystocarp, *G. bursa-pastoris* has an intermediate sized one, and *G. chouae* has the smallest of the three. In Chang and Xia's earlier described species and varieties, *G. asiatica*, *G. tenuistipitata var. liui*, *G. chouae* and *G. asiatica var. zhengii* are all mainly based on pericarp features. Because of a re-examination and critical study of Chinese specimens, the earlier reported 21 species of Gracilaria in China have been reduced to 18 species. This was accomplished by Xia (Xia, 1986), who placed *G. cacalia*, *G. crassa*, and *G. minor* into the synonymy of *G. salicornia*.

6.5. *G. coronopifolia* J. Agardh (Figure 8)

Plants are short, from 4-6 cm, irregular and sub-dichotomous, branching to form an entangled mass; purplish-red when fresh but red when dry. Tertiary branches (up to 1.5 m) are much thicker than branches (0.5 mm), probably because of anastomosing of the branches; ultimate branches have pointed apices. Cuticle is thick, one to two rows of small cortical cells before large medullary cells, and transition from cortex to medulla is abrupt. Only young cystocarps are found, rostrate at ostiole and constricted at the base. No distinct size variation is found in pericarp cells, gonimoblast cells are small and compressed, and upward growing absorbing filaments are present.

Remarks: This species resembles *G. edulis* in having subdichotomous branching, rostrate ostiole and constricted base in the cystocarp. However, unlike *G. edulis*, it has no "thick-walled brick-like cells" in the pericarp and no basal absorbing filaments (Xia and Abbott, 1987).

![Figure 8: Gracilaria coronopifolia J. Ag.](https://example.com/gracilaria-coronopifolia.png)
6.6. *G. cuneifolia* (Okamura) Lee et Kurogi (Figure 9)

Thallus erect, solitary or caespitose, flattened, 4-7 cm tall, arising from a small disc by a short, slender suberete stipe 2-6 mm long; purplish red; thin, membranous adhering imperfectly to paper on drying; two to three times dichotomously branched, segments 1.5-9.0 mm wide, with broad, round, patent axils, ending in blunt or notched apices, with entire margins. Thallus in transverse section consisting of a medulla of two to three layers of large parenchymatous cells, 112-224 µm x 66-120 µm in diameter, with walls 6 urn thick, surrounded by one to two layers of small cells, 10-33 µm x 17-43 µm in diameter. The outermost layer 4-7 µm x 3.3-6.6 µm in diameter, pigmented and surface jelly 10 µm thick; sterile thallus in transverse section 297-322 µm thick. Tetrasporangia cruciately divided, scattered among the surface layers of frond, circular or ovoid in surface view, 26-33 µm x 23-30 µm in diameter, ovoid or oblong in transverse section, 26-36 µm x 17-23 µm, surrounded by modified cortical cells. Cystocarps on both surface of frond, prominently protruding, hemispherical, dome-like in appearance, slightly rostrate, up to 0.7 mm x 1.23 mm in diameter, nonconstricted at base; gonimoblast consisting of branched pseudo-parenchymatous filaments developing from a fusion cell; gonimoblast cells polygonal, 46-92 µm x 26-46 µm, upper traversing filaments abundant, connecting gonimoblast to the pericarp; carposporangia round to ovoid, 13-23 µm x 10-13 µm. Pericarp of one kind of tissue, 191-198 µm thick, consisting of 13-16 layers of cells, the cells with obscure cell walls, the contents star-shaped. Spematangia textorii-type in cortical depressions, 23-30 urn x 20-33 µm deep.

Figure 9: *Gracilaria cuneifolia* (Okamura) Lee et Kurogi.
6.7  *G. edulis* (Gmelin) Silva (Figure 10)

Thallus growing from a disk-like holdfast, with prostrate rhizome forming a tuft or a cluster 6-27 cm tall; branching dichotomous or trichotomous, five to seven orders; branches 0.4- 1.0 mm in diameter. Two groups of plants can be distinguished. The first grows in fastigiate tufts with five to seven (up to 10) orders of branches with narrow-angle furcations, branch intervals gradually in length, the last order ending in bifurcate or trifurcate apices. This group is found growing on fish cages or on rocks in rather clear water. The second group grows on rocks or mud surfaces in sandy mud areas, forming an entangled mass or loose clump with hooks or root-like discs on branch apices; branching of five to seven orders with wide-angle furcation, lower branch intervals much longer than the last two orders; branches cylindrical, lower branches about 1 mm thick and becoming thinner, to 0.3 mm, for terminal segments with attenuate apices.

Frond in transverse section consist of roundish thin-walled medulla to cortex abrupt. Tetrasporangia ovoid in transverse section 20-60 x 32-38 µm, surrounded by elongated cortical cells. Spermatangia in groups of six to ten deep sac-like cavities (polycavernosa type), slightly elevated from the surface of thallus. Cystocarps globose, 0.7-1.2 mm in diameter with rostrate tips and constricted at bases; gonimoblasts consisting of elongated cells; carpospore roundish, 15 x 22 µm in diameter; pericarps thick consisting of 9 to 14 rows, cells of the outrows oval, inner cells horizontally compressed; basal absorbing filaments robust with many branches, lateral absorbing filaments rare.

Habitat: Growing in loose clumps on rocks or mud surface in muddy areas of intertidal zone, in dense tufts when growing on fish cages, and in loose fastigiate tufts on rocks in clear water.

Remarks: This species is often found with *G. changii*, sharing the same intertidal muddy substrate. The two can be easily distinguished because the plants of *G. edulis* are bushier than those of *G. changii* and are red when dry rather than black.

![Figure 10: Gracilaria edulis (Gmelin) Silva.](image)

6.8  *G. eucheumoides* Harvey (Figure 11)

Thallus compressed, prostrate, thick and succulent; branching pinnate to dichotomous; branches 1-3 cm long, 0.5-1.0 cm wide, 0.2-0.9 (up to 1.1) cm thick, with dentate margins, attached by discoid holdfasts. Fronds in transverse section consisting of many layers of cells with stellate plastids, transition of cells from medulla to cortex gradual, medullary cells 100-200 µm in diameter. Only a few specimens of tetrasporic plants found; tetrasporangia roundish on surface view and elongated in transverse section. Gametophytic plants not found.
Habitat: Grows on dead coral fragments.

Figure 11: *Gracilaria eucheumoides* Harvey.

6.9 *G. firma* Chang et Xia (Figure 12)

Thallus erect, caespitose or solitary, arising from a small disk-like holdfast, cylindrical throughout, usually 10-20 cm in length, whitish brown in colour, with slight green when fresh, firm in texture, adhering imperfectly to paper on drying; branches alternately or secundly, 1-2 mm, up to 3 mm in diameter, gradually tapering toward apex and abruptly constricted at the base. The structure of the fronds often having a somewhat more gradual transition from medulla to cortex; medulla composed of large parenchymatous cells which are 230-360 µm, up to 450 µm in diameter, with wall 10-20 µm thick, surrounded by intracortical layers of 3-5 layers roundish cells filled with globular floridean starch grains; outer small cortical cells are pigmented, 6.6-13.2 µm x 3.3-6.6 µm in dimensions and with surface jelly ca. 7 µm thick.
Tetrasporangia densely scattered over the greater part of the frond, cruciate, round in surface view, 33-37 µm in diameter, ovate in shape as seen in transverse section of the frond, 59-66 µm x 26-43 µm in dimensions, bordered by more or less modified cortical cells; tetraspore containing a conspicuous pigmented stellate central body. Cystocarps prominently protruding, conical or hemispherical in shape, 580-630 µm in diameter, with a large gonimoblast parenchyma of small, densely massed, thicker wall, richly protoplasmic cells, 23-46 urn x 16.5-23 µm in dimensions, producing dense, radiating chains of ovoid or elliptical carpospores filling the cystocarpic cavity; carpospore 23-40 µm x 16-20 µm in dimensions; without nutritive filaments. Pericarp thin, 83-95 µm in
thickness, consisting of 8-10 layers of cells, of which the outermost cells of 1-2 layers are pigmented and roundish square to oblong in shape, while the inner layers of cells are horizontally oblong with distinct cell wall, cells in distinct longitudinal rows, the innermost 2-3 layers of cells are elliptical to roundish in shape. Antheridia occur densely on the frond which are round in shape or sometimes irregularly as seen in surface view, elliptical cavities, 66-116 urn x 33-66 µm in dimensions in transverse view, bordered by modified cortical cells.

Habitat: Grows on shells, gravel and rock fragments in muddy areas of turbid water.

Remarks: The species resembles G. changii, and G. edulis. It can be separated from G. edulis on the basis of the abundance of basal absorbing filaments (few in G.firmd) and structure of pericarp cells. It has a less branched plant than G. changii, with gradual transition from cortex to medulla, cystocarp constricted at base and no horizontally oriented inner pericarp cells.

6.10  G. fisheri (Xia et Abbott) Abbott, Zhang and Xia (Figure 13)

Thallus bushy, 13-30 (up to 45) cm tall, with many branches coming from a short stipe or from percurrent axis; branching alternate, three to four orders; branches cylindrical, 0.6-2.3 mm in diameter, constricted at bases and tapering toward apices. Frond in transverse section consisting of medulla 220-620 µm in diameter, with thick layers of cortex; transition form medulla to cortex gradual. Tetrasporangia ovoid, tetraspores 20-25 urn in diameter. Spermatangia ovoid, single or in groups of two to three cavities. Cystocarps conical, rostrate, unconstricted at bases, 0.3-0.7 mm high, and 1.0-1.3 mm wide; gonimoblasts consisting of many small cells; absorbing filaments lateral and upper; pericarp thick with inconspicuous cell walls and star-shaped contents; carpospores rounded, 18-24 µm in diameter, or ovoid, 8-14 x 14-20 µm.

Habitat: Commonly found growing on living and empty shells (Cerithium sp.) and on broken rocks, gravel, polyethylene bags, and fish cages in sandy-muddy areas of turbid water.

6.11.  G. glomerata Zhang and Xia (Figure 14)

Plants 4.0-4.5 cm tall, complanate, dark purplish brown, attached below by a discoid holdfast, with a short and slender subterete stipe 1-2 mm long; frond 1-2 mm wide, irregularly dichotomously branched, to six to seven orders, usually branching one to three times subdichotomously below. Otherwise naked for 1.0-2.5 cm in basal and median parts, bearing toward their apices dense branches, often somewhat glomerate; texture cartilaginous, with entire margins and blunt apices. Frond in transverse section consisting of a medulla of large, thin-walled cells 79-145 urn x 73-112 µm in diameter, and one to two layers of small pigmented cortical cells 3.0-6.6 µm x 5-6 µm; transection 448-481 µm thick. Spermatangia textorii-type in cortical depressions, 23-30 µm deep. Tetrasporangial and female plants unknown.
Remarks: The dense branches in the upper parts together with rather thick segments are so distinctive as to separate this species readily from all others now reported in this genus. None of the other complanate species is reported as having glomerate branches. Although tetrasporophytes and female material have not been
seen, the habitat of these male plants is different from that of other species and the plants represent an independent species.

6.12. **G. hainanensis** Chang et Xia (Figure 15)

Thallus solitary or caespitose, arising from a small disc, cylindrical throughout, usually 15-35 cm, up to 45 cm in length, one to two orders of branches; chestnut purple or kelp green in colour, succulent in substance, becoming soft and adhering imperfectly to paper upon drying; branches generally supple, elongated, up to 30-40 cm, 2-3 mm in diameter, attenuated to a fine apex, flagelliform, irregularly alternately or secundly and sparingly branched, abruptly constricted at the base; thallus consisting of three kinds of tissues; medulla composed of large parenchymatous isodiametric or polygonal cells which are 0.5-0.6 mm, up to 0.95 mm in diameter, with walls 7-10 µm thick, merging rather abruptly into the subcortex of one to two layers of irregularly ovoidal cells, 23-33 µm in diameter, and one to three layers of small, pigmented, more or less anticlinally arranged cells, which are roundish, cuboid or ovate in shape, 6.5-9 µm x 5-6.6 µm in dimensions and with surface jelly ca. 7 µm up to 13 µm thick.

Tetrasporangia densely scattered over almost the entire surface of frond, round or ovate in shape in surface view, ca. 33 µm in diameter, ovoid or oblong in transverse section, ca. 37-39 µm x 21-26 µm in dimensions, surrounded by somewhat elongated ovoid cortical cells, cruciately divided. Antheridia borne in well-separated, deep, open, conceptacular pockets 37 x 39 µm in dimensions in the cortex. Cystocarps remote, scattered over the thallus surface above and below, rather small, mostly less than 450 µm diameter, conical, non-rostrate, not constricted at base, gonimoblast consisting of parenchymatous cells, connected to the pericarp by several nutritive filaments; mature carpospores round or ovoid in shape, 26-30 µm in diameter, containing a conspicuous stellate central body; pericarp thin, ca. 90-120 µm in thickness, consisting of 9-11 layers of cells, of which the outermost layer cells are pigmented and roundish square to oblong in shape, whereas inner layer cells are horizontally elliptical to horizontally roundish oblong with distinct cell wall, cells in distinct longitudinal rows, under low power.

Habitat: Growing on shells in the sublittoral region of low salinity.

6.13. **G. bailinae** (Zhang et Xia) Zhang et Xia (Figure 16)

Thallus erect, solitary or caespitose, arising from a small disc, cylindrical throughout, usually 10-50 (up to 70) cm long; main axis percurrent or not, 1-2 (up to 3) mm in diameter, with two to four orders of branches; succulent, brittle and easily broken; fleshy, light red to kelp (dark) green, dark brown and hardened when dry, adhering to paper on drying; branches irregularly alternate, secund or furcate, with second order long, third order short, sometimes spinose, 0.2-0.5 mm in diameter, gradually tapering towards the apex and nonconstricted at the base; branches and branchlets are distinguished clearly. Thallus in transverse section consists of a medulla of large parenchymatous cells, 200-531 µm in diameter, with walls 8-10 µm in diameter, surrounded by two to three layers of small roundish cells, 13-33 µm in diameter, the outermost layer 7-10 by 4-7 urn in diameter, pigmented and with surface jelly 3 urn thick; transition from medulla to cortex abrupt.

Tetrasporangia scattered among the surface layers of frond, ovoid or oblong in surface view, 33-36 by 16-26 µm in diameter, ovoid to cylindrical in transverse section 20-30 µm by 13-19 µm in diameter, surrounded by slightly modified cortical cells, cruciately or occasionally irregularly tetrahedrally divided. Cystocarps prominently protruding or subcornical, 500-780 µm by 830-1000 µm in diameter, nonrostrate or slightly rostrate,
nonconstricted at base; gonimoblast consisting of parenchymatous cells, the cell walls obscure and cell contents irregularly connected to each other, about 23-82 by 13-20 µm in diameter; pericarp thin, 76-100 µm thick, consisting of seven to eight layers of cells, of which the outermost layer is pigmented and cells roundish-cuboidal to oval, whereas the inner layers have cells that are horizontally oval-elliptical with rounded corners and obscure cell walls. Spermatangia superficial and continuous over the thallus surface.

Figure 15: *Gracilaria hainanensis* C. F. Chang et B. M. Xia.
Figure 16: *Gracilaria bailinae* (Zhang et Xia) Zhang et Xia.
The species shows a close morphological similarity to *G. bursa-pastoris* (Gmelin) Silva from China, but the latter has spermatangia in shallow depressions as opposed to being borne superficially and shows large gonimoblast cells and numerous absorbing filaments in the cystocarp. According to the spermatangial configuration, *G. bailinae* resembles *G. chorda* Holmes and *G. lemaneiformis* (Bory) Weber-van Bosse, but differs from *G. chorda* on the smaller medullary cells, by thicker cell walls and the structure of the pericarp; from *G. lemaneiformis* by the thin cuticle, thicker cell wall, small cystocarps, smaller size of carpospores, and by the large cells of the gonimoblast filaments that have numerous secondary pit connections. Gonimoblast cells of *G. lemaneiformis* from the type locality (Patia, Peru) are notably small, and the gonimoblast is stalked. Some elongated plants were found in a shallow ditch where there was more water motion than in other places.

6.14. *G. irregularis* Abbott (Figure 17)

Thalli erect, succulent, 2.5-13.8 cm tall, percurrent axes 1-5 mm in diameter; branching mostly secund; branches always smaller than main axes, the last order of branches sometimes clustered. Fronds in transverse section consisting of medulla of large cells, 300-775 µm in diameter, cortex one to two cells thick, sometimes three to five cells; transition of cells from medulla to cortex abrupt. Tetrasporangia cruciate, 23-36 µm in diameter, tetrasporic plants with many large "gland" cells. Spermatangia in superficial layer (chorda type); male plants pale. Cystocarps conspicuous, dome-shaped, 0.6-0.8 mm high and 0.9-1.6 mm wide, not constricted at bases; gonimoblast consisting of many small cells with obscure cell walls and star-shaped contents, giving a reticulate appearance; pericarps roundish or slightly compressed, 9 to 12 rows; absorbing filament lacking; carpospores oval to rounded, 21-25 µm in diameter.

Habitat: Growing on gravel, shells, and rock fragments in sandy-muddy areas together with *G. changii* and *G. firma*.

Remarks: Abbott (1988), described the male plants as having spermatangial conceptacles of the verrucosa type, oval to obovate, numerous, adjoining ones frequently coalescing. Lewnanomont has been unable, so far, to find any verrucosa type male plants, only the chorda type. In order to confirm the male type, gametophytic plants from tetraspores were cultured.

![Figure 17: Gracilaria irregularis Abbott.](image)
They showed the chorda type spermatangial arrangement. *Gracilaria irregularis* can be distinguished from other *Gracilaria* species in Thailand by its short and succulent axis and secund branching.

6.15  *G. lemaneiformis* (Bory) Weber-van Bosse (Figure 18)

Thallus solitary or caespitose, up to 46 cm tall, with few to several long branches 0.5-1.4 mm in diameter; branching irregular mostly from lower portion, branches simple, two to five branches frequently occurring from a single branch apex. Frond in transverse section consisting of medulla of large thin-wall cells, 130-260 µm in diameter, two layers of cells in cortex; transition from medulla

**Figure 18:** *Gracilaria lemaneiformis* (Bory) Weber-Van Bosse.
to cortex abrupt. Tetrasporangia ovoid 15-28 x 30-50 µm. Spermatangia superficial (chorda type). Cystocarps spherical, 0.7-0.9 µm in diameter, slightly rostrate or nonrostrate and constricted at bases; gonimoblasts consisting of filaments of many small cells with obscure cell walls and reticulate contents; carpospores roundish to ovoid, 18-25 µm in diameter; pericarp 10-14 layers; without absorbing filaments.

Habitat: Grows on fish cages.

Remarks: *Gracilaria lemaneiformis* resembles *G. tenuistipitata* var. *liui* and can be separated with certainty only on the basis of spermatangial configuration. Spermatangia are superficial in *G. lemaneiformis* (chorda type) and in shallow saucer-like depressions in *G. tenuistipitata* var. *liui*. The latter species is grown under mariculture conditions in Taiwan and South China.

6.16. *G. manilaensis* Yamamoto et Trono (Figure 19)

Fronds caespitose, up to 60 cm or more tall; main axes up to 1.5 mm thick, cylindrical throughout, usually percurrent; branching alternate, sometimes secund or irregular; branches abundant, long, similar to main axis, with lateral branchlets; bases of branches and branchlets sharply constricted at their point of attachment; purplish red or sometimes greenish; fleshy to somewhat cartilaginous. Cortical layer consisting of one to two rows of densely protoplasmic cells; outermost cells 7.2-12.8 µm high, 8.8-13.6 µm wide, with primary pit connections only; hair basal cells about 16 µm high, about 15 µm wide, scattered. Medulla consisting of polygonal cells, increasing in size toward centre, up to 570 µm. Outer layer of medulla more or less compressed parallel to frond surface; transition in cell size from cortex to medulla abrupt. Cystocarps globose, up to 1000 µm in diameter, gonimoblast cells elongated, up to 23 x 71 µm; absorbing filaments present, penetrating into pericarp. Spermatangia formed in conceptacles (verrucosa type); conceptacles cup-shaped, roundish, or oval, up to 71 µm deep, up to 50 µm wide, crowded but separated by vegetative tissue. Tetrasporangia 35-40 µm high, 24-27 µm wide, regularly cruciate, surrounded by several tiers of elongated vegetative cells. Life history is typical Polysiphonia type (Yamamoto 1991). Chromosome Number: n=24.

Habitat: This species grows on muddy bottom in shallow water.

6.17. *G. percurrens* (Abbott) Abbott (Figure 20)

Thallus erect, with percurrent axis, 12-21 cm tall; branching alternate to irregular, branches constricted at base and broadened distally, 0.5-2.2 mm in diameter, with blunt apices. Frond in transverse section consisting of large thin walled medullary cells, 123-370 µm in diameter, two layers of cells in cortex; transition of cells from medulla to cortex, abrupt. Tetrasporangia 15-18 x 25-29 µm. Spermatangial conceptacles sac-like, single (verrucosa type) and in groups of two to four cavities (polycavernosa type). Cystocarps conical, rostrate, not constricted at bases, 0.5-0.8 mm high, 0.7-1.0 mm wide; gonimoblast filaments consisting of small cells; basal absorbing filaments few, lateral and upper absorbing filaments few to many; carpospores roundish to ovoid, 13-20 µm in diameter; pericarps thick, 10-14 rows of cells.

Habitat: Growing on rock fragments in intertidal zone of turbid water.
Figure 19:  *Gracilaria manilaensis* Yamamoto et Trono.

Figure 20:  *Gracilaria percurrens* (Abbott) Abbott
6.18. **G. rubra** Chang et Xia (Figure 21)

Thallus erect, cylindrical, slender, 16-40 cm high, 0.5-1 mm thick, arising from a small disk-like holdfast, with 1-3 orders of remote, alternately or secundly, long attenuate branches, up to 15 cm or more in length, abruptly constricted at the base; thallus in transverse section consisting of a medulla of large parenchymatous cells, ca. 250-350 μm in diameter, with walls 3-7 μm thick, surrounded by a narrow, infracortical layer of one to two layers of roundish cells, 16.5-30 μm in diameter, and one to two layers of smaller cortical cells, pigmented, ovoid or cuboid, 7-10 μm x 6-10 μm in dimensions. Transition from medulla to cortex extremely abrupt; light purplish red in colour, membranous-cartilaginous in substance, adhering imperfectly to paper on drying.

Tetrasporangia scattered over surface of thallus, spherical of subspherical in shape as seen from surface, 26-33 μm in diameter; ovoid in transverse section, 26-30 x 20-23 μm in dimensions, surrounded by unmodified cortical cells, cruciately divided. Cystocarps scattered, prominent, protruding, globose or semiglobose, non-rostrate, unconstricted, 450-700 μm 700- 863 μm in dimensions; gonimoblast arising from a large fusion cell. Parenchymatous connected to the pericarp by nutritive filaments; carpospores about 20-33 μm diameter, with a conspicuous stellate central body; pericarp ca. 82-170 μm in thickness, consisting of 8-14 layers of cells, of which the outermost layer cells are pigmented and roundish to oblong in shape, whereas inner layer cells are roundish, and becoming horizontally oblong in mature with obscure cell wall, cells in distinct longitudinal rows, under low power. Antheridia borne in well-separated, deep, open, conceptacular pockets, 33- 43 μm deep, 23-36 urn diameter.

Habitat: Dredged at 10 m, growing on shells.

6.19. **G. salicornia** (J. Agardh) Dawson (Figure 22)

Thalli prostrate to semierect, branching of prostrate from dichotomous to irregular, branches cylindrical, 3-5 mm in diameter, partly constricted; forming a rough entangled mass of various sizes with root-like discs on branch apices. In semi-erect form, segments constricted throughout, with two to four branches at each node, 10-15 cm tall; yellow to bright orange. Fronds in transverse section consisting of many layers of thin-walled cells, 150-400 μm in diameter, cortical layer of two to four cells with abundant "gland" cells; transition of cells from medulla to cortex gradual. Tetrasporangia cruciately divided, 25-30 x 37-45 μm in diameter, scattered over the surface of thallus. Spermatangial conceptacles oval (verrucosa type), single or in groups of two to three cavities. Cystocarps globose, nonrostrate, slightly constricted at bases, 0.8-2.0 μm in diameter, gonimoblasts consisting of many small cells, pericarp thick, consisting of two kinds of cells, six to eight elongate cells in outer layer, five to eight rounded cells in inner layer, absorbing filaments lateral and upper; carpospores spherical, 16-24 μm in diameter.

Habitat: Growing on various kinds of substrates, rocks, gravel, shells and mangrove roots in clear to turbid water. Specimens growing in clear water are always orange, those growing in muddy areas are dark brown.
Figure 21: *Gracilaria rubra* C. F. Chang et B. M. Xia.
Figure 22: *Gracilaria salicornia* (Ag.) Dawson.
6.20  *G. sullivanii* Yamamoto et Trono (Figure 23)

Fronds prostrate, attached to substrate by hapters originating from ventral side of the fronds, up to 10 cm long. Main axes compressed, up to 5 mm wide and 3 mm thick; branching dichotomously in opposite manner upper portion of branches almost cylindrical, branch tips blunt; reddish brown; cartilaginous. Cortical layer consisting of one to two rows of densely protoplasmic cells; outermost cells 5.6-16 µm high, 4-8 µm wide, with primary pit connections only; hair basal cells about 20 urn high, about 18 µm wide, in groups of 20-40 cells. Medulla consisting of polygonal cells, increasing in size towards the centre, reaching up to 410 µm. Outer layer of medulla more or less compressed parallel to frond surface; transition in cell size from cortex to medulla abrupt. Tetrasporangia formed in upper half portion of nemathecium-like elevation on dorsal side of the frond, regularly cruciate, 44.5-50.5 µm high, 20-26.3 µm wide, surrounded by rows of elongated vegetative cells; nemathecium-like structure consisting of layers of up to eight cells, up to 140 µm thick. Cystocarps and spermatangia unknown.

Habitat: This species grows prostrate on rock at a depth of 2-3 m at the edge of coral reef.

Remarks: This species is characterised by regular dichotomous branching and prostrate habit. The several layers of elongated vegetative cells that surround the tetrasporangia form prominent and distinctive nemathecium-like elevated structures not present in other *Gracilaria* species. These features appear to be distinctive enough for separating this species from other taxa, notwithstanding the absence of cystocarps and male reproductive organs.

6.21  *G. tenuistipitata* var. *tenuistipitata* Chang et Xia (Figure 24)

Thallus from a small disk-like holdfast arising to a height of 20-40 cm, rarely up to 1 m, simple or moderately alternately branched near the base, the branches becoming like the main axis, terete throughout, very slender near the base of the plant, above expanding to 0.5-1.5 mm diameter, 1-2 times branched; structurally showing a broad medulla of large thick walled cells 225-390 urn, with walls, 13-16 µm thick, in the centre, toward the surface considerably smaller, the cortex of 1-2 layers of rounded cells 10-20 µm diameter, with cuticle 10-13 urn thick; fleshy red in colour, cartilaginous in substance, adhering rather well to paper on drying.

Tetrasporangia scattered among the surface layer of frond, round in shape in surface view, 30-33 µm in diameter, ovoid or oblong in transverse section, 30-46 µm x 18-30 µm in dimensions, surrounded by somewhat modified cortical cells, cruciately divided. Cystocarps prominently protruding, up to 830-950 µm high, globose, rostrate, constricted at base; gonimoblast composed of large parenchymatous cells, connected with the pericarp by very rare nutritive filaments; carpospores round or ovoid in shape, 33-49 µm in diameter; pericarp ca. 72-102 µm in thickness, consisting of 8-11 layers of cells, of which the outer cells of 1-2 layers are elliptical to roundish in shape with obscured cell wall, while the innermost few layers of cells are roundish to horizontally oval with distinct cell wall, cells not in distinct longitudinal series under low power. Antheridia are scattered over the surface of the blade in small, shallow, well-defined depressions 10-23 µm diameter and separated by modified, elongated cortical cells.

Habitat: Grows on gravel and shells in the sublittoral region of lower salinity.
1. Showing hapters (arrowed)
2. Showing hair basal cells (solid cells)
3. Showing tetrasporangial nemathecium-like structure (solid part), that is located on the dorsal side only (arrow).
4. Showing tetrasporangia formed in the upper half of nemathecium-like structure.
6.22. *G. tenuistipitata* var. *liui* Zhang et Xia (Figure 25)

Differing from the variety *G. tenuistipitata* by the slender thalli bearing numerous, delicate, short to long flagelliform lateral branchlets, frequently only 0.25 mm in diameter. Branching mostly from percurrent axes. Cystocarpic structure and shallow textorii-type spermangia similar to variety *G. tenuistipitata*. This new variety frequently occurs naturally in fish ponds and shallow intertidal areas on muddy substrate. When cultivated, as in Hepu, Guangxi Province and in Taiwan (as *G. verrucosa* of Chiang 1981 and Shang 1976), the thalli are usually detached and without holdfasts. They may then tumble about in fairly large masses.
6.23 G. textorii (Suringar) De Toni

Thallus foliose, attached below by a small discoid holdfast, 4.2-13.2 cm tall and 6.1-18.8 cm wide; blade branched dichotomously or sub-dichotomously in one plant, segments 0.7-2.0 cm wide, membranous when dried, with entire margins or proliferous, apices blunt, bifurcate; dark red to greenish red. Frond in transverse section 147-185 µm thick, consisting of a few layers of medullary cells, transition of cells from medulla to cortex abrupt. Gametophytic plants, both male and female, smaller than tetrasporic plants; male plants very rare, tetrasporic plants abundant. Tetrasporangia ovate, scattered on both surfaces 16-25 x 23-27 µm, surrounded by elongate cortical cells. Male frond pale, spermatangia in shallow or saucer-like depressions (textorii type). Cystocarps large and prominent. Semiglobose to globose, 0.7-1.0 mm in diameter, slightly rostrate, not constricted at bases; gonimoblast cells small, numerous, almost occupying the cystocarpic cavity; absorbing filaments upper and lateral.

Habitat: Grows on rocks and fish cages.

6.24 G. verrucosa (Hudson) Papenfuss (Figure 26)

Cylindrical thalli, extremely variable in size, colour and habitat. Branches gradually attenuating to blunt apex, constrictions taking place at the base of the branches. Fronds 1-2.5 mm in diameter (-3 mm or more), usually 20-50 mm in length. In transversal section the medulla cells connected with each other by numerous pits and composed of 4-5 layers of large roundish cells, 133-240 µm in diameter, bordered by 2-3 layers of much smaller and thinner cortical cells. The outermost cortical cells elongated more or less anticlinally, measuring 6-8 pan x 10-17 µm. Cystocarps hemispherical, globose, ostiolate scattered over the whole frond. Carpospores ovates or roundish, distributed radially, 13-47 µm thick, 23-67 high. Tetrasporangia ovoid or oblong, 14-42 µm x 45-65 µm in dimensions, cruciate, densely scattered on the surface of frond, surrounded by slightly modified cortical cells, Antheridia, in the bottom of a rather shallow conceptacle, 15-51 urn diameter, 18-43 µm depth, usually well separated by unmodified cortical cells. They occur with different densities according to the surface of the frond. Small globular antherozoids, 3-5 µm diameter.

This species has been studied and reported in detail from many locations throughout the world. Originally, this species was believed to be cosmopolitan. As early as 1976, Zhang and Xia suspected the cosmopolitan distribution of the English species and found the structure of the pericarp to be quite constant with the species. As they expected, the Chinese species does differ specifically from the English Gracilaria verrucosa (Huds.) Papenfuss. Meanwhile they also found that the "Gracilaria verrucosa" of Japan, at least the specimen collected by H. Yamamoto from Hokkaido, belonged to the same species as the Chinese species, now described as Gracilaria asiatica Zhang and Xia. The asiatica species may be readily distinguished from the Gracilaria verrucosa from Wemburg, Devon, England by the deeper spermatangia conceptacles, by the larger tetraspores, by the smaller carpospores and by the structure of pericarp, as already comparatively studied by Zhang and Xia.
Figure 25:  *Gracilaria tenuistipitata* var. *liui* Zhang et Xia.
Figure 26: *Gracilaria verrucosa* (Hudson) Papenfuss.
Habit of a cystocarpic plant.
6.25  \textit{G. yamamotoi} Zhang et Xia (Figure 27)

Plants 3-7 cm tall, foliose, dark purplish red, attached below by a discoid holdfast, with a short and slender subterete stipe 2-6 mm long; blade 1-3 mm wide, irregularly dichotomously branched in one plane, thick coriaceous to cartilaginous, with entire margins and attenuated apices; branches of one to two (up to three) orders; never adhering to paper on drying. Frond in transverse section consisting of a medulla of large, thin-walled cells, 99-106 µm in diameter, and one to two layers of small pigmented cortical cells, 7-10 µm x 3-7 µm; transection 310-320 µm thick.

Spermatangia (textorii-type) in individual to confluent saucer-like depressions, 30-33 µm deep, surrounded by modified cortical cells, distributed over thallus surface. Cystocarp nearly globose, 1.2-1.5 mm in diameter, prominently protruding, non-rostrate to only slightly rostrate, unconstricted at the base; gonimoblast consisting of many small cells, 20-36 µm x 10-17 µm, with several cells toward the apex of each filament developing into carposporangia; carpospores roundish, ovoid or oblong, 40-53 µm in diameter, traversing filaments absent; pericarp 264-304 µm thick, consisting of two kinds of tissue, the outer of six to seven rows of smaller dense, oblong shaped contents, the inner 10-11 rows of large, loose, horizontally oblong cells.

Remarks: This species is similar to slender forms of \textit{G. textorii} (Suringar) De Toni in external appearance. It is distinguishable, however, by the lack of traversing filaments in the cystocarp, by the small gonimoblast cells, and by two kinds of cell layers in the pericarp. In \textit{G. textorii}, the traversing filaments are conspicuous, and the pericarps are constricted of only one kind of cells.

7.  CULTIVATION

7.1  Selective criteria of species for culture

The species targeted for culture should have the following properties:

- It must be a fast growing species which can be propagated utilising vegetative propagules or spores. Species with large and robust thalli are preferred because these can produce large amounts of biomass within relatively short cropping periods.
- It must have a high, good quality, agar content.

The above considerations require that the available species be screened. Comparative studies on their productivity should be conducted to determine the species to be selected. Because the properties of agar differ among species it is terribly necessary that the correct name be applied. Thus, the taxonomy of the species should be clarified. The names are indices of the kind and quality of agar the \textit{Gracilaria} contains and are used as basis for determining the price.

7.2  Selection of culture sites

The selection of sites is a very important factor to consider in pond and field culture because this could be the primary factor determining success or failure of the farming venture. In general, areas that support natural stocks of species to be cultured are good sites. If culture is to be done in an area where no stock of the species exists, then one should select sites which have comparable ecological conditions as the sites where the stocks are found. This will entail the gathering of data on various parameters such as salinity ranges, nutrient levels, turbidity, water temperature and type of substrate, among which temperature and salinity are most important.
Figure 27: *Gracilaria yamamotoi* Zhang et Xia.
7.3 Production of seeds and sporelings

The term "seed" here refers to both the vegetative propagules and spores which can be utilised as planting materials. The sporelings are specially used as the young sporophytes and gametophytes, which are from carpospores or tetraspores, respectively.

The vegetative propagules are to select healthy thalli, which are fleshy and elastic in texture, dark reddish brown colour, robust well branched with smooth and shiny surfaces. In addition, these must be clear, free from dirt and epiphytes. The production of sporelings have two methods: natural spore-recruitment and induced spore-shedding in hatcheries.

- **Natural spore recruitment.** The use of spores as seeding materials for the production of sporelings for culture necessitates the availability of natural stocks. In the natural spore recruitment methods, artificial substrates such as ropes, rocks, shells and netting materials are used. Ropes and netting materials are generally preferred. These are left in the area for about two weeks to allow the naturally shed spores to settle on them. The sporeling developing from the spores visible after 3 to 4 weeks. The seeded material are then transferred to the culture sites for outgrowing.

- **Hatchery production of sporeling from spores.** The production of sporelings in hatcheries is quite similar to those in the seedling facilities for *Porphyra* and *Laminaria*, although less sophisticated. It consists of a seeding tank with provision for control of water depth with an adjustable seeding material support structure. This structure consists of a wooden frame with monofilament netting material which can fit into the seeding tank. It is provided with mechanisms, so that its height from the bottom of the tank can be adjusted.

Farmers collecting spores, either naturally or artificially, must possess knowledge of the reproductive habits and ecological properties of the culture species. A brief introduction to the biology of *G. asiatica* and *G. tenuistipitata* is given below.

7.4 Biology of *Gracilaria*

*Gracilaria asiatica* is the most common species in China. It grows rapidly and has a high agar content. A discussion on the influence of various environmental factors on the release and germination of spores growth of thallus of the spores is given below.

*Influence of environmental factors on spore release*

Both carpospores and tetraspores are released naturally into seawater after their maturation. Experiments have shown that the maximum quantity of spores is released at 8-10 am., gradually decreasing thereafter. The minimum quantity is released at 10 pm. to 6 am. the following day, after which the next maximum will take place. Besides the diurnal changes of releasing rhythmicity, the released quantity of spores also depends on ambient environmental factors.

*Desiccation*

In general, a mature *Gracilaria* is taken out of seawater and kept in a shady spot for 2 to 4 hours (air temperature 15-25 °C), the tetraspores or the carpospores will be released if the plant is dipped in seawater again. Table 1 gives the different quantities of tetraspores
or carpospores released at different durations of desiccation, or removal from the seawater.

Table 1: The relationship between the quantity of tetraspores and carpospores released and desiccation time.

<table>
<thead>
<tr>
<th>Time (hr.)</th>
<th>0.5</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetraspores</td>
<td>887</td>
<td>1011</td>
<td>3033</td>
<td>1921</td>
<td>1940</td>
<td>1904</td>
<td>782</td>
</tr>
<tr>
<td>Carpospores</td>
<td>201</td>
<td>226</td>
<td>686</td>
<td>683</td>
<td>458</td>
<td>477</td>
<td>421</td>
</tr>
</tbody>
</table>


Seawater temperature
Experiments have shown that the highest number of spores are released at 20-25 °C (Table 2). According to field surveys, the reproductive season of *Gracilaria* is between June to August in northern coastal provinces or between March to May in southern China. During these seasons, the natural seawater temperature is 20-25 °C. The experimental results coincide with the natural reproductive season (Zheng, 1987).

Table 2: The relationship between released quantity and seawater temperature.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>8–10</th>
<th>12–15</th>
<th>20–22</th>
<th>25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetraspore</td>
<td>88</td>
<td>518</td>
<td>2295</td>
<td>1525</td>
</tr>
<tr>
<td>Carpospore</td>
<td>522</td>
<td>998</td>
<td>1343</td>
<td>824</td>
</tr>
</tbody>
</table>

Source: Zheng, 1987

Specific gravity of seawater
When mature plants were kept in seawater of different specific gravity, those in seawater of lower specific gravity release spores earlier than those kept in water of higher specific gravity. If the plants are put in seawater with an obviously low specific gravity (< 1.005), the released spores will swell and even break up due to osmosis caused by the low salinity, so that most of them would not complete normal germination. In order to obtain good results in the collection of spores, an optimum specific gravity range of 1.015 (19.0-20.2 ppt) to 1.020 (25.5-26.9 ppt) should be observed (Zheng, 1987).

Influence of environmental factors on germination and growth of the spores
*Gracilaria* spores just released into seawater, measure around 30 µm in diameter and vary with species. Tetraspores are slightly bigger than carpospores. For example, the tetraspores of *G. tenuistipitata* have a diameter 24-56 µm, and carpospores 23-40 µm. Soon after release, the spores will attach to substrates and start first cleavage of cells. The germination and growth of spores are also influenced by ambient environmental conditions such as seawater temperature, light intensity, salinity, etc. (Zheng, 1987).

Seawater temperature
To examine the germination and the growth of the spores those attached to slides for 12 hrs were moved to containers with sterilised seawater enriched with nutrients and incubated with a light intensity of 400 lux, photoperiod of 10:14 (D: L) for different periods of time. The results are shown in Tables 3 and 4.
Table 3: The effect of temperature on carpospore disc size (m) after germination.

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Temperature (°C)</th>
<th>7</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>28</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>10</td>
<td>39.6×39.6</td>
<td>42.9×42.9</td>
<td>46.2×46.2</td>
<td>46.3×46.3</td>
<td>42.9×42.9</td>
</tr>
<tr>
<td>20</td>
<td>15</td>
<td>39.6×39.6</td>
<td>45.9×45.9</td>
<td>52.8×52.8</td>
<td>59.4×59.4</td>
<td>49.5×42.5</td>
</tr>
<tr>
<td>30</td>
<td>20</td>
<td>46.2×46.2</td>
<td>59.4×59.4</td>
<td>59.4×59.4</td>
<td>56.1×56.1</td>
<td>56.1×56.1</td>
</tr>
<tr>
<td>40</td>
<td>28</td>
<td>56.9×49.5</td>
<td>62.7×59.0</td>
<td>69.3×62.7</td>
<td>75.3×75.9</td>
<td>72.6×59.4</td>
</tr>
<tr>
<td>50</td>
<td></td>
<td>56.9×49.5</td>
<td>66.0×59.4</td>
<td>66.0×66.0</td>
<td>95.7×82.5</td>
<td>99.0×82.5</td>
</tr>
</tbody>
</table>

Table 4: The effect of temperature on tetraspores disc size (µm) after germination.

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Temperature (°C)</th>
<th>7</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>28</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>10</td>
<td>39.6×39.6</td>
<td>36.3×36.3</td>
<td>39.6×39.6</td>
<td>36.0×36.0</td>
<td>39.1×39.1</td>
</tr>
<tr>
<td>20</td>
<td>15</td>
<td>39.6×39.6</td>
<td>42.9×42.9</td>
<td>49.5×49.5</td>
<td>42.9×42.9</td>
<td>42.9×42.9</td>
</tr>
<tr>
<td>30</td>
<td>20</td>
<td>46.2×46.7</td>
<td>62.7×62.7</td>
<td>62.8×62.8</td>
<td>52.8×52.8</td>
<td>52.8×52.8</td>
</tr>
<tr>
<td>40</td>
<td>28</td>
<td>52.8×49.8</td>
<td>59.3×62.7</td>
<td>75.9×61.9</td>
<td>75.9×66.0</td>
<td>75.9×66.0</td>
</tr>
<tr>
<td>50</td>
<td></td>
<td>49.5×42.9</td>
<td>66.0×59.0</td>
<td>95.7×82.5</td>
<td>95.9×85.0</td>
<td>99.0×82.5</td>
</tr>
</tbody>
</table>

The results illustrate that both tetraspore discs and carpospore discs need a seawater temperature higher than 15 °C for their growth. If the temperature is below 15 °C, even if all other environmental conditions are suitable for their growth, they would survive but grow very slowly.

**Light intensity**

Light intensity is one of the most important factors influencing the germination and growth of spores. When the light intensity is stronger, the rate of germination and growth of spores are higher within 3000 Lux. If the spores attached to slides are kept in a dark place they will die in 20 days. The experimental results are shown in Table 5.

Table 5: The effect of light intensity on the spore disc growth (µm) after germination.

<table>
<thead>
<tr>
<th>Light intensity (Lux)</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>25</th>
</tr>
</thead>
<tbody>
<tr>
<td>3,000</td>
<td>69</td>
<td>78</td>
<td>93</td>
<td>112</td>
</tr>
<tr>
<td>1500</td>
<td>50</td>
<td>63</td>
<td>85</td>
<td>104</td>
</tr>
<tr>
<td>200</td>
<td>40</td>
<td>48</td>
<td>60</td>
<td>76</td>
</tr>
<tr>
<td>Dark</td>
<td>poor</td>
<td>poor</td>
<td>died</td>
<td>--</td>
</tr>
</tbody>
</table>

**Specific gravity of seawater**

Although *Gracilaria* plants prefer to inhabit estuarine areas, their spores are unable to withstand seawater with too low salinity. If the spores attached to substrate are in seawater with a specific gravity below 1.010 (12.5-13.7 ppt) their cells swell up due to the absorption of water into the cells from ambient environment. The colour of the pigments in the cells would change from red to pale, and the spores will die eventually. Experiments proved that the optimum specific gravity of seawater for germination and growth of spores ranges from 1.018 (23.0-24.2 ppt) to 1.025 (25.5-26.9 ppt).
The effect of environmental factors on growth of Gracilaria thalli

The effects of environmental factors on Gracilaria thalli are similar to that on their spores, but not entirely the same. For instance, as mentioned above, spores kept in seawater with a specific gravity below 1.010 will break up and die, but the thalli can grow very well in the same conditions. In view of this, ambient environmental factors required by Gracilaria thalli must be studied.

Specific gravity of seawater

Gracilaria is a group of euryhaline seaweeds. Under natural conditions, the specific gravity in which the plant can grow out ranges from 1.005 to 1.026 (5.2-38.1 ppt). Experiments and field surveys have shown that the optimum specific gravity is 1.010-1.020 (11.3-30.1 ppt) where fresh water regularly flows in.

Seawater temperature

Gracilaria is also an eurythermal plant which can grow at 5 to 30 °C. Optimum temperature varies with species. For example, the optimum temperature of G. asiatica is 15-25 °C. It can be found during May to August in northern China, but in south China only in winter and spring seasons. In summer, the growth of Gracilaria is almost completely stopped in the south until late autumn when the water temperature drops below 25 °C and the seaweeds will resuscitate again.

Field surveys have shown that the optimum seawater temperature for G. tenuistipitata, a species mainly distributed in Guangdong and Hainan Island, is identical with G. asiatica. When the water temperature is 30 °C, its diurnal growth rate measures 0.1-0.2 cm/day but as the temperature falls to 28 °C, the growth rate increases to 0.4-0.5 cm/day. When the temperature is from 15 to 25 oC, growth rates can be higher than 1 cm / day.

7.5 Farming

The farming of Gracilaria seaweeds is not as complicated as that of Porphyra, Laminaria and Undaria. Generally, three methods have been adopted in the world: intertidal culture, floating culture, and pond culture.

Intertidal culture

The main feature of this culture method is that the spore collection and farming are usually the same. In southern China, in late autumn or early winter, young Gracilaria can grow up to 5-6 cm as they enter a period of fast growth rates. At this stage, the substrate to which young Gracilaria are attached are kept in lines on the bottom at an interval of 30-40 cm which will serve as a walk way. The routine management practices involves removal of miscellaneous seaweeds, collection of herbivorous gastropods and so on. Women and children often do it. In a normal season, 1500 kg (dry weight) per ha of Gracilaria plants can be harvested.

Floating culture

The method has been adopted from kelp farming. In a suitable season, such as January in southern China or May in the northern part, young seaweeds, either collected from fields or reared in hatchery, are pulled up from substrate and inserted into ropes called "sporeling rope" which are made of palm thread or artificial fibres. In each rope of 20 m length, 200 pieces of seaweeds 10 cm apart can be inserted. The sporeling ropes are fixed on floating raft. Three months later, the seaweeds will reach a length of over 1 m and can be harvested. Yields can reach about 3,000 kg (dry) per ha. This culture method is suited to the culture of G. asiatica and G. lemaneiformis.
Pond culture

Pond culture of seaweeds has been adopted by Chinese phycologists and farmers since the 1960s. The stock seaweeds are cut into pieces and spread in the fish pond at a density of 5-6 tonnes (fresh thalli) per ha. Chemical fertiliser or pig manure is applied regularly. Every 30-45 days, most biomass of the seaweeds can be harvested. The rest is continually left in the ponds as stock. The harvesting period lasts six months from June to November in northern China and in southern parts, like Hainan Island, almost all year. It is used mainly to culture *G. tenuistipitata*, especially for the variety *G. tenuistipitata var. liui*, as it is hard to find their reproductive thalli, both tetrasporophytes and carposporophytes. In the lagoon area of Vietnam (Hue province), the method has been used successfully and it is believed that the method could easily be extended throughout the region, particularly in the south-east and south Asia.

REFERENCES


DELIMITATION OF SPECIES AND POPULATION GENETIC STRUCTURE IN
GRACILARIA VERRUCOSA: CONSEQUENCES FOR CULTIVATION.

Christophe Destombe, Remi Wattier, Davis Bulke and M. Valero,
Laboratoire de Génétique et Evolution des Populations Vegetates,
URA CNRS 1185, SN2,
59655 Villeneuve d’Ascq Cedex, France.

ABSTRACT
Species of Gracilaria are some of the most useful algae in the world, combining the production of valuable agar with a fast growth rate, ease of vegetative reproduction and other attributes favouring its cultivation. When planning to farm these red algae in new areas, it is essential to identify the species correctly and be aware of the biology of the species under consideration. In agarophytes, delimitation of species is very difficult using morphological characteristics and the biology of the species generally depends on local adaptation. In this study, molecular comparison of the Rubisco spacer allowed the identification of the species Gracilaria verrucosa. This method can be easily transferred to other species of Gracilariaceae.

The level and pattern of population genetic structuring and the possibility for local adaptation in Gracilaria verrucosa were analysed by RFLP methods of cytoplasmic DNA. Mitochondrial and plastidial DNA of Gracilaria verrucosa are polymorphic. The analysis of this polymorphism shows that genetic differentiation occurs between close populations of Gracilaria verrucosa even at short distances (about 100m). Consequently, it is very important before planning cultivation, to be aware of the origin of the Gracilaria verrucosa inoculum to use in breeding experiments and farming.

1. INTRODUCTION
When planning to farm red algae in new areas, it is essential to identify the species and to be aware of the biology of the species under consideration. In the economically important red algal agarophytes, the genera Gracilaria and Gracilariosis include over 170 species (Goff et al. 1994). Identification of genera and species in the family Gracilariaceae is problematic because of their great morphological similarities. Recent approaches to the taxonomy of the Gracilariaceae using traditional criteria such as morphological characteristics and reproductive anatomy led Fredericq and Hommersand (1989a; 1989b; 1990; and Hommersand and Fredericq, 1990) to conclude that Gracilariosis and Gracilaria are distinct. The determination of species remain, in some cases, very difficult or impossible because the diagnostic characters are absent (e.g. reproductive organs are absent in some strains of Gracilaria). The use of genetic molecular markers could be an additional tool to facilitate species recognition.

For optimal exploitation of a crop species, efficient seeding, a fast growth rate and genetic improvement are of prime importance to the farmer. Growth rates can be affected by the life history phase and the season, while genetic improvement and seeding from spores can be achieved only if the methods of reproduction are properly understood (for review of Gracilariaceae see Kain and Destombe, 1995). Moreover, the biology of the species generally depends on local adaptation therefore on genetic differentiation between populations. The amount of gene flow is a parameter of great evolutionary importance in determining the level and pattern of genetic differentiation of populations. Then, in the same species, different populations (with different growth rates, physiological or reproductive characteristics) can be found.
This paper investigates:

(i) the use of molecular tools to delineate the species *Gracilaria verrucosa* using the comparison of the RUBISCO spacer sequences; and

(ii) the level and pattern of population genetic structuring and the possibility for local adaptation in *Gracilaria verrucosa*. Genetic differentiation between populations was estimated using the analysis of cytoplasmic DNA by RFLP with the Southern probes method.

2. MATERIALS AND METHODS

2.1 Delimitation of the species

The sources of algae used in the interpopulation study are listed in Table 1. Except for the isolate from Hawaii, the algae is *Gracilaria verrucosa* (Hudson) Papenfuss (Gracilariales, Rhodopyhta) on the basis of the morphology and reproductive anatomy which have previously been ascribed to this species (Rice and Bird, 1990; Bird and Rice, 1990). For intrapopulation studies, the spacer was sequenced from five individuals from Cap Gris-Nez and Audresselles, Pas de Calais in the North of France.

Table 1: Sources of algal strains used in this study.

<table>
<thead>
<tr>
<th>Strain designation</th>
<th>Geographical location</th>
<th>Date of collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRZ</td>
<td>Brazil: Sao Paulo</td>
<td>1988</td>
</tr>
<tr>
<td>FR3</td>
<td>France: Cap Gris-Nez</td>
<td>Jan 1988</td>
</tr>
<tr>
<td>NOR</td>
<td>Norway: Oslofjord</td>
<td>Dec 1987</td>
</tr>
<tr>
<td>WAL</td>
<td>Wales: Haverfordwest, Dyfed</td>
<td>Aug 1987</td>
</tr>
<tr>
<td>ARG</td>
<td>Argentina: Puerto Madryn, Chbut</td>
<td>Jul 1986</td>
</tr>
<tr>
<td>NC</td>
<td>USA: Beaufort, North Carolina</td>
<td>Sept 1987</td>
</tr>
<tr>
<td>HI</td>
<td>USA: Oahu, Hawaii</td>
<td>May 1988</td>
</tr>
<tr>
<td>Five FR isolates</td>
<td>France: Cap Gris-Nez</td>
<td>June 1990</td>
</tr>
<tr>
<td></td>
<td>France: Audresselles</td>
<td>June 1990</td>
</tr>
</tbody>
</table>

Total DNA was isolated according to the method described by Rice and Bird, (1990) and Doyle and Doyle, (1990). Double-stranded DNA of the spacer region was amplified by polymerase chain reaction (PCR) (Saiki *et al.* 1988). The primers used were described by Destombe and Douglas (1991) and Destombe (1992). Both strands of the amplified DNA were directly sequenced using a modification (Bachman *et al.* 1990) of the dideoxy chain termination procedure developed by Sanger *et al.* (1977) and the DNA sequencing kit "Sequenase 2.0" (U.S. Biochemicals, Cleveland, Ohio).

2.2 Population genetic structuring

The alga used for this study was *Gracilaria verrucosa* from Europe. The individuals consist of a perennial holdfast fixed to the rock, bearing branched spaghetti-like thalli. Samples of *Gracilaria verrucosa* were taken from two European countries: France and Norway (Figure 1). Samples were taken from three French localities: Northern France, Brittany and Etang de Thau, a lagoon connected to the Mediterranean Sea. Each locality is about 1,000 km from the others. Within each locality individuals were sampled and mapped in four populations located at two sites. For example in northern France (Pas-de-Calais, on North Sea and Channel coasts), two sites were chosen: Audresselles and Cape Gris-Nez. At each of these two sites, two populations were chosen at the two extremes of the intertidal range of the species, to maximise their environmental difference.
High and low populations were noted as Gh and Gl in Gris-Nez, and as Ah and Al in Audresselles, respectively. Individuals of the same population are 50 cm to 25 m apart, high and low populations of the same site are approximately 100 m apart (50 to 70 m in Gris-Nez and 150 m in Audresselles due to the difference of slope of the shore in both sites), and the two sites (Audresselles and Gris-Nez) are 3 km apart. Within each population at least five individuals were sampled.

Ecological conditions
In France, ecological conditions differ among the three regions. In Northern France, populations were located in rocky pools. Individuals were fixed to the substratum and range from the lower to mid intertidal level of the shore (Destombe et al. 1989). High and low populations are subject to different tidal and light conditions. In Brittany, individuals from Roscoff are fixed to small pebbles and drift with strong tidal movements, thus experiencing different tidal level conditions during their lifetime and individuals from Island of Batz are fixed in rocky pools. In Etang de Thau, individuals are attached to the substrate just beneath the water surface in the lagoon. They are deprived of tidal fluctuations and are subjected to higher light intensity, temperature, and salinity levels than the two other sites. In Norway and England, individuals were fixed on rocky substrate.
**RFLP cytoplasmic methods**

The method of RFLP (Restriction Fragment Length Polymorphism) of cytoplasmic DNA (chloroplastic and mitochondrial) was used to study genetic population structuring. Total DNA was isolated essentially as described by Dellaporta *et al.* (1983) from fresh or frozen material. Total DNA was digested with the restriction endonucleases *Ecorl* and *EcorV* in single enzyme digests used according to the instructions of the manufacturers. In addition, the restriction reaction buffer was supplemented with spermidine to 2.5 mM. After separation of the restriction fragments on 0.8% agarose gels, the DNA was transferred to nylon membranes using the vacuum-blot system of Pharmacia. After transfer the DNA was UV cross-linked to the nylon support. The DNA was hybridised with two plastidial DNA probes (CpCh and Ep23) isolated from *Chondrus crispus* and one mitochondrial DNA probe (MtCh) isolated from *Chondrus crispus* according to the techniques previously described by Saumitou-Laprade *et al.* 1993. The RFLP technique allows the detection of structural rearrangements and restriction of site mutations in the region of DNA used.

To estimate the degree of differentiation between populations and regions the Fst method of Weir was applied to haploid data (Weir, 1990). This method gives a Fst coefficient and allows the estimation of genetic differentiation between groups.

3. RESULTS AND DISCUSSION

3.1 Delimitation of the species

The five individuals from the population from Northern France contain an identical Rubisco spacer region. Within the limitations of our sampling, this method does not reveal any variation among individuals of a population. In contrast, sequence comparisons of the spacer regions from seven isolates from different geographical locations demonstrate the occurrence of four different groups of algae. This finding is confirmed by phylogenetic analysis using parsimony (Figure 2) (Destombe and Douglas, 1991). The first group is composed of a mixture of populations of *Gracilaria verrucosa* from Western Europe (France, Norway, Wales) and Southern America (Argentina) in agreement with the RFLP analysis and interfertility study conducted by Rice and Bird (1990). The three other groups are distinct both from one another and from the European "core" group. The lengths of the Rubisco spacer vary, in *Gracilaria* sp. from Hawaii, the length is short (65 nucleotides) (Table 2). In contrast in algae designated originally as *Gracilaria verrucosa* the lengths of the spacer vary from 106 nucleotides (Brazil) to 131 nucleotides (North Carolina).

**Table 2:** Sizes (in bp) of Rubisco spacer in *Gracilaria*.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Country</th>
<th>Length</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Gracilaria verrucosa</em></td>
<td>Brazil</td>
<td>106</td>
</tr>
<tr>
<td></td>
<td>Europe and Argentina</td>
<td>115</td>
</tr>
<tr>
<td></td>
<td>North Carolina</td>
<td>131</td>
</tr>
<tr>
<td><em>Gracilaria sp.</em></td>
<td>Hawaii</td>
<td>65</td>
</tr>
</tbody>
</table>

Our results indicate that the sequence and the length of the Rubisco spacer are good additional characters that complete the morphological description of the species. These results were confirmed recently by Goffer *et al.* (1994) who separated *Gracilaria* and *Gracilariopsis* populations, species and genera using Rubisco spacer and flanking regions (Figure 3). In this study, the difference between *Gracilaria verrucosa* from Norway and France is due to only a single insertion/deletion that occurs in the rbcS region.
Figure 2: Phylogenetic tree of isolates of Gracilariaceae constructed using parsimony from aligned plastid Rubisco spacer. A single most parsimonious tree consisting of 89 steps was found. The isolate from Hawaii was used as the outgroup.

---

Figure 3: Single most parsimonious tree from aligned plastid Rubisco spacer and flanking regions sequence data (Goff et al. 1994). Gracilaria sp. are abbreviated "Gop". Values above branch points are bootstrap values (100 replicated). This tree required 82 steps. Saradiotkeca gaudichaudii was used as an outgroup.
A study of the length of the Rubisco spacer using PCR is a very easy and very fast method. The main advantage of this approach is that it requires only minute amounts of relatively unpurified template DNA (Goff and Moon, 1993). These authors amplified DNA from herbarium collections.

Population genetic structuring
Our results indicate that plastidial and mitochondrial DNA are polymorphic in *Gracilaria verrucosa*. Four enzyme-probe combinations appeared to be variable (Table 3). The polymorphism is low for the combination Ep23/EcoRI and for Mtch/Ecorl the polymorphism is higher. For a given enzyme-probe combination, there are different profiles. The different profiles obtained are visualised in Figure 4. For each combination, one profile is common and the others are more or less rare.

| Table 3: Polymorphism of the 4 enzyme-probe combinations |
|-------------|-------------|--------|--------|
| Probes      | Enzymes     | N      | Profiles frequencies |
| Plastidial DNA |
| Cpch        | EcoRV       | 89     | 0.89 0.11 |
| EP23        | EcoRI       | 73     | 0.95 0.04 0.01 |
| Mitochondrial DNA |
| Mtch        | EcoRV       | 96     | 0.88 0.11 0.01 |
| Mtch        | EcoRI       | 86     | 0.82 0.04 0.01 0.12 0.01 |


To estimate the degree of differentiation between populations and between regions, the Fst method of Weir (1990) was applied to haploid data. The results are given in the Table 4. Between populations, the F is significantly different to 0 for three combinations (Cpch-EcoRI, Mtch-EcoRV and Mtch/Ecorl). These results indicate that there is genetic differentiation between populations. Between regions, the Fst is significantly different to 0 only for one combination (Ep23-EcoRI). The result obtained for this combination could be due to the small size of the sample and to the low polymorphism of this combination.

| Table 4: Genetic differentiation between regions and populations measured by Fst (Weir, 1990). |
|---------------------------------|-------------|----------------|----------------|----------------|----------------|
|                                 | Cpch/EcoRV | Ep23/EcoRI | Mtch/EcoRV | Mtch/Ecorl | Mean over loci |
| Among populations               | Fst         | 0.67**      | 0.60ns      | 0.56**      | 0.46**        | 0.57           |
| N                               | 89          | 70          | 95           | 86           | 4              |
| Among Regions                   | Fst         | 0.06ns      | 16*          | 0.01ns      | 0.02ns        | 0.03ns         |
| N                               | 89          | 73          | 96           | 86           | 4              |

ns: not significant; *: p<0.05; ** p<0.01.
N: sample size.

For all the combinations (mean over loci), the Fst indicate that genetic differentiation occurs between populations, but that genetic differentiation is not significant between regions.

The Mtch/Ecorl enzyme probe combination is the more informative. The distribution of the 5 haplotypes of this combination between populations and regions were detailed in Table 5. The haplotype A is found in almost all the populations (excepted ROSII and BRI) and the haplotype D is found also in each region. The haplotype B is an endemic type of the island of Batz. The type D is not very frequent but when it is found in one
population, it is at a quite important frequency within the population. This explains why there is a high genetic differentiation between populations.

**Figure 4:** Enzyme-probe combination profiles of *Gracilaria verrucosa* obtained by RFLP.

<table>
<thead>
<tr>
<th>Types</th>
<th>Plastidial DNA Probe Ep23</th>
<th>Mitochondrial DNA Probe MtCh</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ECORI</td>
<td>ECORV</td>
</tr>
<tr>
<td>Kb</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Genetic differentiation occurs between close populations in this species. These results are in accordance with the previous dispersal and crossing experiments in *Gracilaria verrucosa* (Destombe *et al.* 1990, 1992 and Richerd *et al.* 1993). Contrary to the intuition that dispersal in the sea is large, data on recruitment (Santelices, 1990) dispersal potentialities (Destombe *et al.* 1992), fertilisation distances (Destombe, *et al.* 1990) and heterosis effects in crosses (Richerd *et al.* 1993) show that dispersal is leptokurtic and limited to the order of 100 m. This corresponds to the distance between high and low populations in each site that should then be able to diverge genetically. This divergence is linked to differential adaptation, since tidal level has important selective effects on algae.

In conclusion, in future aquaculture applications it is necessary to attempt to determine and study wild populations. This genetic differentiation between populations is important in breeding studies and genetic potential.
Table 5: Distribution of the 5 haplotypes of the Mtch/Ecorl combination between populations and regions.

<table>
<thead>
<tr>
<th>Populations</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gris Nez Site</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GNvl</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>GNI</td>
<td>6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td>GNh</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>GNvh</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>Audresselles site</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AU1</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>AUh</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>Northern region</td>
<td>27</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>29</td>
</tr>
<tr>
<td>Island of Batz site</td>
<td>GB</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>GBm</td>
<td>6</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>7</td>
</tr>
<tr>
<td>GBp</td>
<td>4</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>Bil</td>
<td>3</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>Roscoff site</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>ROSII</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>3</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>Brittany Region</td>
<td>21</td>
<td>3</td>
<td>1</td>
<td>4</td>
<td>-</td>
<td>29</td>
</tr>
<tr>
<td>Le Barrou site</td>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>BR1</td>
<td>9</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>9</td>
</tr>
<tr>
<td>Bouzigues site</td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>&amp;</td>
<td>5</td>
</tr>
<tr>
<td>BZII</td>
<td>7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>7</td>
</tr>
<tr>
<td>Languedoc Region</td>
<td>18</td>
<td>-</td>
<td>-</td>
<td>5</td>
<td>1</td>
<td>24</td>
</tr>
<tr>
<td>Norway</td>
<td>4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4</td>
</tr>
</tbody>
</table>

REFERENCES


ABSTRACT

Research in seaweed from 1988-1994 focused on the agar producing Gracilaria. Studies in the following areas were also conducted: inventory of the seaweed resources; production ecology; farming systems; and agar characterisation. Six species of Gracilaria and one Gracilariopsis abound in the Western Visayas. There were monthly variations in biomass and agar quality of G. blodgettii, G. manilaensis and G. heteroclada collected at different places.

The bulk of the studies were carried out on Gracilariopsis heteroclada because of its wide distribution, fast growth characteristics and good quality agar. Its reproductive state was seasonal with tetrasporophyte abundant in May and carposporophyte in January. Seventy-five percent harvesting of the available biomass was sufficient for the next cropping season. Harvesting the seaweed using "arafia" was not appropriate in places where the biomass was exposed to air during the lowest tide. The addition of nutrients to the stock increased the growth rate of the seaweed and gel strength of the agar. G. heteroclada grown at lower stocking density in hapa nets, both in floating cages and in ponds, gave higher growth rates and production than at higher stocking densities. This species, when polycultured with P. monodon at lower stocking density combinations, gave the highest growth rate and income. Likewise, this species when cultured vertically in ropes inside a floating cage showed encouraging results. G. heteroclada, when grown at 24-25 ppt under tank conditions, produced the highest gel strength.

1. INTRODUCTION

During the Aquaculture Development in Southeast Asia (ADSEA) Seminar-Workshops in 1987, 1991 and 1994, the member countries of the Southeast Asian Fisheries Development Center (SEAFDEC) recommended Gracilaria as the number one priority for seaweed research in the following areas: (1) basic biology and production ecology; (2) refinement of culture techniques; (3) screening and characterisation of natural products; (4) product utilization; and (S) biofiltration. The Seaweed Unit of the Department was re-activated in 1988 and studies along these lines have been conducted.

1.1 Taxonomy

In order to determine the existing resources of the region, an inventory of the macrobenthic algae of Panay was conducted. Among the species identified, seven were Gracilaria, namely: Gracilaria arcuata Zanardini; G. changii (Xia et Abbott) Abbott, Zhang and Xia; G. coronopofolia J. Agardh; G. eucheumoides Harvey; G. manilaensis (= G. "verrucosa") Yamamoto et Trono sp. nov.; G. salicornia (C. Agardh) Dawson; and Gracilariopsis heteroclada (= Gracilaria heteroclada) (Zhang et Xia) Zhang et Xia (Hurtado-Ponce et al 1992a). Occurrence of these species ranges from full seawater on rocky-coral substrate to brackishwater on sandy-muddy substrate.

Among the species identified in western Visayas, G. heteroclada was the most widely distributed from mudflats, estuarine, rivers, drainage canals to ponds. Consequently, several studies were conducted on this particular species.
1.2 Production ecology

Reproductive state
Studies on production ecology are necessary to have a better understanding of the basic biology and ecology of an organism so that management schemes may be formulated for its commercial farming. One study was done on the reproductive state of *G. heteroclada* to determine the monthly occurrence of fertile plants. Plants originating from spores are more viable sources of new plants. Results showed that tetrasporophyte (64%) and carposporophyte (48%) were more abundant in May and January, respectively. Percentage occurrence of tetrasporophyte and carposporophyte did not significantly correlate with water temperature, salinity, and turbidity (Luhan, unpubl. data).

Stock assessment
Studies on the monthly biomass of wild *Gracilaria* species were assessed to determine the amount of available biomass that could possibly support an industry. Variations due to culture months and geographical locations were factors which influenced differences in biomass (Figure 1) (de Castro *et al* 1991, Pondevida unpubl. data). Among the species studied, *G. heteroclada* gave the highest dry biomass and it is available throughout the year (Figure 2).

Regeneration capacity
The amount of harvest left after the first cropping is important in determining the amount of biomass available for the next cropping season. A 75% harvest provides an appropriate amount of "seeds" for the next cropping season compared with 25, 50, and 100% (Hurtado-Ponce, 1993). The amount of biomass to be harvested during each harvest regime should not exceed the amount of biomass available for cropping.

Harvesting tools
The use of harvesting tools in the gathering of wild *Gracilaria* in places where it is convenient and effective improves the regeneration capacity of the seaweed and increases the harvest (Santelices *et al*. 1984). Such tools (araña) were not proven to be effective in places where the seaweed is exposed to air during the lowest tide (Hurtado-Ponce, 1994). Raking the seaweed as reported by Hurtado-Ponce *et al*. 1992) brings excessive disturbance on the substrate and to the population (Luxton, 1981). Gathering with bare hands is a much better means although it is tedious.

The addition of nutrients to the seaweed is one of the management schemes to increase its growth characteristics. This was substantiated when *G. heteroclada* was cultured in tanks at different stocking densities. The addition of nutrients was significant at lower stocking density (500 g m\(^{-2}\)) than at higher density (1000 and 2000 g m\(^{-2}\)) (Chavoso and Hurtado-Ponce, unpubl. data). The enriched stock gave higher growth rates than the unenriched ones.

Salinity tolerance
Salinity tolerance studies on 3 species of *Gracilaria* was done to formulate a management scheme for cultivation. Each species tolerated a wide range (15-35 ppt) of salinity, however, it is species-specific. Optimum growth was obtained at 15 ppt in *G. tenuistipitata*, 24-25 ppt in *G. changii* and *G. heteroclada*, and 35 ppt in *G. firma*.

Environmental parameters
Production ecology of the seaweed is incomplete without considering environmental factors that influence the growth of the species both cultivated and in natural beds. The single and interactive effect of the different factors were analysed and correlated with
one another to determine the relationship of the seaweed with its environment. Correlation analysis showed that temperature and pH had no effect on biomass; salinity is negatively correlated with biomass; while rainfall is inversely correlated with the biomass of wild G. heteroclada (de Castro et al 1991, Pondevida, unpubl. data). The same environmental factors had no effect on the growth rate and production of G. heteroclada when grown in net cages inside a brackishwater pond (de Castro and Guanzon, 1993).

Figure 1: Stock assessment of wild G. blodgetti and G. manilaensis.

Figure 2: Stock assessment of wild G. heteroclada.
1.3 Farming systems

Indiscriminate or uncontrolled gathering of *Gracilaria* from the wild leads to the depletion of the resources. Gathering of agarophytes (like *G. blodgettii*, *G. coronopifolia*, *G. eucheumoides*, *G. salicornia* and *Gracilaripsis heteroclada*) during the dry season has been reported (Hurtado-Ponce *et al.* 1992b). Although commercial cultivation of *G. heteroclada* both in ponds and drainage canals has been reported (Hurtado-Ponce 1992c), other farming techniques need to be developed both land-based and in open sea, while existing techniques need to be refined. These are all necessary in order to have a sustainable industry.

Attempts were made to cultivate *G. heteroclada* using different systems to develop an economically feasible technique.

*Cage culture*

A study was done on *G. heteroclada* using vegetative fragments to determine the effect of spacing interval on growth. Results show that significant differences in growth and monthly yields were observed when the seaweed was cultured on vertical ropes at 3 spacing intervals (10, 15 and 20 cm) inside a floating cage. Maximum yields were obtained in April at 10 and 15 cm and in February at 20 cm (Hurtado-Ponce, 1990).

Another study was carried out to test the effect of the presence of sea bass as biological control to grazers of *G. heteroclada*. The interactive effect of the presence of sea bass, water depth and month of culture significantly affected the growth *G. heteroclada* (Hurtado-Ponce 1994a). Highest and lowest growth rates were recorded with seabass at 25 and 100 cm below the water surface, respectively.

Manipulating the stocking density of a species will yield different growth rates and production (Santelices *et al.* 1993). To confirm this statement, a study was done on *G. heteroclada* using hapa nets installed to a floating cage. The results showed that lower stocking rates (400 and 500 g m\(^{-2}\) ) yields higher growth rates and production than higher stocking densities (600 and 700 g m\(^{-2}\)) (Guanzon and de Castro 1992). Highest production was obtained during the dry season.

*Pond culture*

When the same stocking densities were tried in ponds using the same species, similar results were obtained when *G. heteroclada* was cultured in net cages inside a brackishwater pond. Higher growth and production was obtained at lower stocking densities (400 and 500 g m\(^{-2}\)) than at higher stocking densities (600 and 700 g m\(^{-2}\)). Higher production was obtained during the dry season than during the wet culture months (de Castro and Guanzon, 1993). To ascertain whether *G. heteroclada* and *Penaeus monodon* can be cultured together in brackishwater ponds using the "rice planting" and long line methods, a five-month study was done at different stocking combinations. A lower stocking density combination of *G. heteroclada* (250 g m\(^{-2}\)) and *P. monodon* (2,500 pcs ha\(^{-1}\)) gave higher growth rates and production of the seaweed but a lower production of shrimp. Income above variable cost was also highest at this stocking density combination. Growth rates and production of the seaweed was significantly affected by the culture month and stocking density (Hurtado-Ponce, 1995). Higher growth rates and production were obtained at 24-29 ppt salinity confirming the results of an earlier study by Hurtado-Ponce (1994b) in tanks.
Figure 3: Gel strength of wild seaweed populations.

a. *G. heteroclada*

b. *G. blodgettii* and *G. manilaensis*
Drainage canal
Maximising use of drainage canals for the cultivation of seaweeds will increase production. A study on *G. heteroclada* using long-line, a deviation from the existing practice, revealed a 4.5% day\(^{-1}\) growth, a yield of 3,440 kg/ha, an annual production cost of 4,120 Pesos, annual net returns of 17,552 Pesos, a return on investment of 566% and a pay back period of 2 months (Hurtado-Ponce *et al*, unpubl. data).

1.4 Agar characterisation
Attempts to screen several species of *Gracilaria* as source of good quality agar were made both from the wild (Figures 3a-b) and cultivated strains. The effect of pre-alkali (NaOH) treatment (concentration and time) to the seaweed was species-specific (Table 1). *G. heteroclada* cultured at 24-25 ppt under tank conditions gave the highest gel strength (807-850 g cm\(^{-2}\)). Among the species tested for agar both wild and cultured, *G. heteroclada* is the best source.

2. RECOMMENDATIONS
As mandated during ADSEA 94, the Aquaculture Department of SEAFDEC will continue to conduct research studies on *Gracilaria*. As the number one priority commodity among the seaweeds, studies on the following areas will be conducted:

- inventory of other species, selection and development of highly productive cultivators with high quality agar;
- bioremediation (biofilter) for water quality control in aquaculture;
- monoculture and polyculture in ponds;
- refinement of production methods;
- product utilization;
- village municipal processing (semi-refined);
- socio-economic studies;
- development of hatchery technology; and
- genetic studies and creation of seedbank.

REFERENCES


AN OVERVIEW OF SEAWEED PROCESSING TECHNOLOGY FOR GRACILARIA WITH REFERENCE TO AGAR YIELD AND QUALITY

Suwalee Chandrkrachang,
Asst. Professor, Biopolymer Research Unit Department of Chemistry, Faculty of Science, Srinakarinvirot University, Prasanmitre, Sukhumvit, Bangkok.

ABSTRACT

Seaweeds are a major coastal resource which are valuable to both the economy and environment of many countries. The uses of the seaweeds as raw materials for phycocolloid production has become a major world-wide industry involving food, cosmetics, drugs and biotechnology. The most valuable product of phycocolloids is the agar, which is produced from red seaweeds of the family of Gracilaria, involving different species. Agar consists of heterogeneous biopolymers containing galactose units and their derivatives. The different market prices of the agar results from different grades, which depend on the qualities of gel strength, clarity and the quantities of charged particles in the agar composition. Different techniques of agar extraction are reviewed in this paper. Samples of seaweed and agar from NACA member countries participating in the regional study were sent for analysis at BRU. The results will be used as guidelines for the development of both quality and quantity of the raw seaweed materials and their agar products, which are essential information for research study and public services.

1. INTRODUCTION

Seaweeds, or marine macroalgae, are valuable coastal resources with respect to marine ecology, environment and human consumption. The utility of seaweed has been known since ancient times, varying with time and geographic location. There are so many variations in seaweed species, their habitats, availability, methods of harvesting and processing, chemical properties and product utilization, it is quite impossible to consider the seaweed industry as a homogeneous entity. Processing is dominated by relatively few major commercial enterprises who keep their production methods and figures confidential as the market for seaweed colloids is highly competitive.

Presently, the extraction of valuable products is the most rapidly developing area of seaweed utilization, which will lead to world-wide commercialisation of seaweeds.

1.1 Phycocolloids

Phycocolloids are chemical polysaccharides or carbohydrates which have the ability to give viscosity, gel strength and stability to aqueous mixtures, solutions and emulsions. With such properties, they play increasingly important roles in the production of processed foods, dairy products, cosmetics, drugs, fertilisers, animal feeds and other valuable commodities. Agar, a high-priced phycocolloid, is a galactan-type biopolymer produced from red seaweeds of different families in the Class Rhodophyceae. Other strongly negatively charged polymers of galactan, derived from different families of red seaweeds, are carrageenans and furcellarans. The most important colloid product from brown seaweeds is the alginate which is widely used in the textile, printing, paint and pharmaceutical industries.

1.2 Agar

Agar, a traditional gelling agent, may be defined as a hydrocolloid of ancient origin. Chemically, agar consists of neutral and negatively charged polymers of galactan. Agar
is soluble in hot water but becomes water-insoluble at room temperature. One of its unique characteristics is gel formation at very low concentration. With the substitution of the negative charge in the polymer chain of galactan, agar is considered as one of the anionic biopolymers from marine resources.

The traditional concept of agar being comprised of neutral agarose and negatively charged agaropectin, is now regarded as an over simplification. Many research results indicate that agar is a complex mixture of polysaccharides all with the same backbone of linkages but substituted to a variable degree with negatively charged groups of some sulphate, pyruvate and methoxy substitutions. The agarose has been suggested as the polymer containing the agarobiose structure of 1,3-linked β-D-galactopyranose and 1,4-linked 3,6-anhydro-a-L-galactopyranose. However, the naturally-occurring material is variously substituted with half sulphate groups, pyruvic acid, ketal and methyl esters which refer to the negative charged agaropectin in the composition of the agar. Dockworth and Yaphe (1971), recommended a practical definition of agarose: "— not a neutral polysaccharide obtained by fractionating it away from the charged polymer agaropectin, but rather that mixture of agar molecules with lowest charge content and, therefore, the greatest gelling ability fractionated by a whole complex of molecules, called agar, all differing in their extent of masking with charged groups."

The basic repeating unit of agarose, agarobiose, is shown in Figure 1.

Figure 1: Chemical structure of "agarobiose" unit

![Chemical structure of "agarobiose" unit](image)

The chemical nature of agar which, affects its quality, varies according to the seaweed raw materials, the environment where the seaweeds grow and on the methods of extraction of the agar. Originally, Gelidium was the major source of agar, because it gave superior quality of agar gel even by the traditional simple method of extraction. However, availability of Gelidium is now limited. Since 1950, Gracilaria spp, which is abundant along coastal areas particularly in the Asia-Pacific region, have become important sources of agar production. However, further modification of alkali treatment of Gracilaria spp. to improve the gelling ability needs to be done during the extraction process. Different alkali treatment techniques are now commonly used in agar factories. The most common concentration (pre-alkali treatment) is between 3 to 5 % NaOH at a temperature of 80-90°C for about one hour.

An alternative technique developed by the Biopolymer Research Unit (BRU) research team is strong alkali treatment of agar at relatively low temperatures after traditional extraction. With this technique, the low grade crude agar can be refined to obtain high quality agar.
2. AGAR EXTRACTION TECHNIQUES

Since seaweed raw materials are different depending on the species and environmental conditions during growth, optimum conditions for extraction vary from source to source. Furthermore, the purity of the harvested seaweed as well as storage and handling conditions prior to processing, also affect the quality and quantity of the agar product. Laboratory tests are essential to optimise suitable conditions for industrial agar production as the seaweeds need to be carefully evaluated for their agar quality. Samples of the seaweed are taken and tested for moisture content, purity, agar yield and other qualities. The most common method involves the following steps:

1. Cleaning and washing to remove impurities.
2. Chemical treatment - some hard seaweeds need acid modification but most of the *Gracilaria* species require the alkali treatment for desulphation to improve agar gelstrength. Some seaweeds do not need any treatment.
3. The extraction step is carried out after washing out the acid or alkali.
4. Filtration and gelation. Filtration techniques depend on the scale of extraction. In small scale production, the simple pressing tool and the pressure filter column can be used. Most industrial scale productions need plate and flame pressure filter units. During filtration, a hot and clear solution should be obtained and then cooled to set the gel at room temperature or below.
5. Dewatering of the gel - the hydraulic pressing machine is the most common piece of equipment used in the agar industry. The freezing and thawing processes are done naturally during winter time for strip-agar production in some countries.

In order to obtain a good quality agar product, the seaweeds are generally sorted by hand and then thoroughly washed with fresh water, followed by sun-drying for natural bleaching. The industrial methods of agar extraction by traditional and pressure techniques, are shown below:

<table>
<thead>
<tr>
<th>Traditional method (Gelidium + Gracilaria)</th>
<th>Pressure method (Gracilaria)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hard Seaweed</td>
<td>Seaweed</td>
</tr>
<tr>
<td>→ Boiling with water</td>
<td>→ Treating with alkali</td>
</tr>
<tr>
<td>→ Adding soft seaweed</td>
<td>→ Washing</td>
</tr>
<tr>
<td>→ Extraction</td>
<td>→ Extraction in open system</td>
</tr>
<tr>
<td>→ Filtration</td>
<td>→ Filtration</td>
</tr>
<tr>
<td>→ Coagulation</td>
<td>→ Coagulation of agar</td>
</tr>
<tr>
<td>→ Cutting to Square or Strip</td>
<td>→ Mechanical dehydration or Freeze - Thawing</td>
</tr>
<tr>
<td>→ Dehydration by natural freezing and thawing for 7–8 days</td>
<td>→ Drying by heat</td>
</tr>
<tr>
<td>→ Packing as square or string agar</td>
<td>→ Crushing</td>
</tr>
<tr>
<td></td>
<td>→ Packing as powder agar</td>
</tr>
</tbody>
</table>
### Small-scale agar production

The sequence of processes involved in the extraction of agar-agar from seaweed on a small-scale is summarised in the following flow diagram. The resultant product is classified as crude agar. It is normally used in the preparation of desserts, or sold as a raw material to be refined into higher-grade products. The yield is about 20-25% by weight of the initial dried seaweed.

**Small-scale processing of seaweed for the extraction of agar**

1. **Dry seaweed**
2. ↓
3. **Wash with water**
4. ↓
5. **Sun dry**
6. ↓
7. **Repeat the wash/dry process several times**
8. ↓
9. **Cleaned dry seaweed**
10. ↓
11. **Soak in water**
12. ↓
13. **Boil**
14. ↓
15. **Grind**
16. ↓
17. **Boil**
18. ↓
19. **Add filter aid**
20. ↓
21. **Boil**
22. ↓
23. **Filter by pressing**
24. ↓
25. **Cool to set**
26. ↓
27. **Crude agar-agar gel**
28. ↓
29. **Cut into smaller pieces**
30. ↓
31. **Press to dewater**
32. ↓
33. **Sun dry**
34. ↓
35. **Grind to powder**
36. ↓
37. **Crude agar powder**
3. APPLICATIONS OF AGAR RELATED TO ITS QUALITY

Agar has wide ranging applications in many areas of the food, Pharmaceuticals and biotechnology related industries, as detailed below:

1. **Food industry**: Agar is used in the food industry as a stabilising, thickening and gelling agent. It is used directly in different kinds of desserts in Thailand. It functions as a texture-improving, setting and stabilising agent for the bakery and candy industry. Agar of high gel strength is always preferred for food preparation in order to reduce the quantity required and costs.

2. **Tissue culture**: The use of agar as a medium for tissue culture is very important to improve the consistency and standard methods for propagation of orchids and other ornamental plants, vegetables and other agricultural crops. Agar with high gel strength is preferred because of the low concentrations of agar required and low cost.

3. **Microbiology and pharmaceutical uses**: An important application of agar is as a culture medium for micro-organisms, bacteria and fungi. Agar has the unique characteristic of gel firmness and stability and it is not easy to metabolise. Agar for bacterial culture needs to be clean, clear and low in contamination. The temperature for gel setting should be equal or lower than human body temperatures (37°C). Agar with low gel setting temperatures are preferred for bacteriological culture mediums.

4. **Preparation of agarose**: Agar is an essential source for agarose which is defined as a neutral biopolymer. Agarose is the high value added bio-product used as a medium in biotechnology. Agarose gel media are used in electrophoresis for separation of nucleic acids, specific proteins, virus and other genetic materials. Agarose is a biologically inert material with low ionic properties. Agar for agarose production needs to have a low sulphate content and relatively high gel strength. Agarose beads, prepared from agarose gel, are used in gel filtration for molecular weight determination and separation of specific protein mixtures and other biological materials. Agarose is used extensively in immunology techniques of identification such as electroimmunoassay, immuno-electrophoresis and counter electrophoresis, including the modern techniques of separation and identification of genetic materials.

4. **INTERNATIONAL AGAR SPECIFICATIONS** (Chandrkrachang and Chinadit, 1988)

The most important properties to be considered when determining the quality of agar for food, tissue culture or bacteriological use are gel strength, gelation and melting temperatures, sulphate and methoxyl content, clarity of the solution and ash content (Tables 1 and 2). An agar of high gel strength is always preferred for food. For bacteriological media, a gel strength of 300-400 g/cm at a 1% concentration is acceptable. Agar, when dissolved in hot water, should form a clear solution, the clarity of which can be determined by measuring the transmittance of the solution.
Table 1: Major parameters of the Japanese specification for processed agar.

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Special</td>
</tr>
<tr>
<td>Gel strength (g/cm²)</td>
<td>≥600</td>
</tr>
<tr>
<td>Water content (%)</td>
<td>≤22</td>
</tr>
<tr>
<td>Crude protein content (%)</td>
<td>≤1.5</td>
</tr>
<tr>
<td>Solids insoluble in hot water (%)</td>
<td>≤0.5</td>
</tr>
<tr>
<td>Crude ash (%)</td>
<td>≤4</td>
</tr>
</tbody>
</table>

Table 2: Agar specifications of the United States Pharmacopeia (USP) and Food Chemical Codex (FCC)

<table>
<thead>
<tr>
<th>Criteria</th>
<th>USP</th>
<th>FCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microbial limit (Salmonella)</td>
<td>negative</td>
<td>-</td>
</tr>
<tr>
<td>Maximum water (%)</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Maximum total ash (%)</td>
<td>6.5</td>
<td>6.5</td>
</tr>
<tr>
<td>Maximum acid-insoluble ash (%)</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Maximum foreign inorganic matter (%)</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Maximum foreign insoluble matter (%)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Arsenic (ppm)</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Lead (ppm)</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Heavy metals (ppm)</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Foreign starch</td>
<td>negative</td>
<td>negative</td>
</tr>
<tr>
<td>Gelatin</td>
<td>negative</td>
<td>negative</td>
</tr>
<tr>
<td>Maximum water absorption (ml)</td>
<td>75</td>
<td>75</td>
</tr>
</tbody>
</table>

Higher grade agar is white in colour, while light yellow is allowed for lower grades. Consistent quality and size are also important criteria.

6. ANALYSIS OF AGAR QUALITY FROM GRACILARIA OF PARTICIPATING COUNTRIES

The participating countries used the same methods of agar extraction together with some analysis of seaweed quality to allow an evaluation to be made of their local Gracilaria species. Some of the seaweed and agar samples were sent to BRU for analysis. The results of the analysis are shown in Table 3. Some important properties which reflect the quality of agar and the process of agar extraction are gel strength, sulphate content and the clarity of the agar solution. Since the seaweeds are of many variations in different geographic locations and seasons, it is impossible to consider and make an evaluation in the same, or similar, directions. Each country needs to develop and improve extraction techniques which are suitable to their seaweed raw materials. The analytical results are important guidelines which will lead to the improvement of the process as well as product development.

REFERENCES


<table>
<thead>
<tr>
<th>Country</th>
<th>Agar</th>
<th>Seaweed</th>
<th>% Yield</th>
<th>Clarity at 520 mm (%)</th>
<th>Gel strength gm-cm</th>
<th>Sulphate content (%)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>China</td>
<td></td>
<td>G. verrucosa</td>
<td>12.75</td>
<td>26</td>
<td>233</td>
<td>2.89</td>
<td></td>
</tr>
<tr>
<td>China</td>
<td>Strip</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>India</td>
<td></td>
<td>G. edulis</td>
<td>26.5</td>
<td>50</td>
<td>245</td>
<td>2.57</td>
<td>IND(MA)06</td>
</tr>
<tr>
<td>India</td>
<td>MA-01-06</td>
<td></td>
<td>-</td>
<td>82</td>
<td>357</td>
<td>1.55</td>
<td></td>
</tr>
<tr>
<td>Indonesia, West Java</td>
<td>-</td>
<td>Gracilaria sp</td>
<td>19.75</td>
<td>78</td>
<td>174</td>
<td>2.64</td>
<td>SW010/IND</td>
</tr>
<tr>
<td>Indonesia, Kalianda</td>
<td>-</td>
<td>Gracilaria sp</td>
<td>23.8</td>
<td>62</td>
<td>99</td>
<td>2.36</td>
<td>SW 001/IND</td>
</tr>
<tr>
<td>Indonesia, Kalianda</td>
<td>-</td>
<td>Gracilaria sp</td>
<td>13.8</td>
<td>45</td>
<td>267</td>
<td>0.76</td>
<td>SW/002/IND</td>
</tr>
<tr>
<td>Malaysia, Midale</td>
<td>MSW-007</td>
<td>G. fastigiata</td>
<td>-</td>
<td>2.0</td>
<td>89</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Malaysia, Kedah</td>
<td>MSW-008</td>
<td>G. changii</td>
<td>-</td>
<td>10.0</td>
<td>174</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Philippines, SW-0050</td>
<td>-</td>
<td></td>
<td>-</td>
<td>66.0</td>
<td>139</td>
<td>0.12</td>
<td>Two people trained in BRU for 1 month under FAO support</td>
</tr>
<tr>
<td>Philippines, SW-0078</td>
<td>-</td>
<td></td>
<td>-</td>
<td>87.0</td>
<td>214</td>
<td>0.23</td>
<td></td>
</tr>
<tr>
<td>Thailand, Songkhla</td>
<td>No. 5</td>
<td>G. tenuistipitata</td>
<td>-</td>
<td>72</td>
<td>768</td>
<td>1.81</td>
<td>The extraction of agar and analysis were done at BRU</td>
</tr>
<tr>
<td>Thailand, Pattani</td>
<td>No. 4</td>
<td>G. fisheri</td>
<td>-</td>
<td>57</td>
<td>758</td>
<td>2.01</td>
<td></td>
</tr>
<tr>
<td>Thailand, Pattani</td>
<td>No. 3</td>
<td>G. firma</td>
<td>-</td>
<td>66</td>
<td>692</td>
<td>2.04</td>
<td></td>
</tr>
<tr>
<td>Vietnam, Haiphong</td>
<td>-</td>
<td>G. verrucosa</td>
<td>25</td>
<td>60</td>
<td>298</td>
<td>2.18</td>
<td></td>
</tr>
<tr>
<td>Vietnam, Haiphong</td>
<td>-</td>
<td>G. blodgetti</td>
<td>21</td>
<td>32</td>
<td>157</td>
<td>2.33</td>
<td></td>
</tr>
<tr>
<td>Vietnam, Haiphong</td>
<td>No. 1</td>
<td></td>
<td>-</td>
<td>76</td>
<td>813</td>
<td>0.91</td>
<td>High quality agar, Haiphong</td>
</tr>
</tbody>
</table>
ABSTRACT

The research currently carried out in co-operation with Dr. Suwalee Chandkrachang from Bangkok (Srinakariniroj University) deals with the protein content of different samples of *Gracilaria* spp. growing or cultivated in Thailand. Amino acids are analysed to select the best species for preparation of proteins on an industrial scale from these algae. Such proteins could be used in human and animal food with the same advantages as vegetal proteins, at low cost to countries lacking in animal proteins in Asia. The extraction of proteins has to be done after the extraction of agar, adding value to the production of cultivated *Gracilaria* spp. Research and extraction of lipid with polyunsaturated fatty acids essential in children's nutrition, are also planned from *Gracilaria* spp. Further, the preparation of enriched endocytic vesicles, cell organelles previously isolated and studied in other algae, has to be set up from these algae to develop their use in pharmacology particularly with reference to using such materials as drug carriers.

1. INTRODUCTION

The most widely used material obtained from algae such as *Gracilaria*, is agar. Extraction of good quality agar has been set up in different countries of the region. In the laboratory of Dr Suwalee Chandkrachang, Biopolymer Research Unit, Sri Nakarinviroj University, the procedure used allows agar to be obtained on an industrial scale with well defined characteristics. Such a product may permit the use of the phycocolloids for a large variety of applications.

1.1 Biotechnology

Recently, algal polysaccharides have found new uses in the field of biotechnology which must be considered as important directions of research in Asian countries because of their high value on the world market. Agars have been used for a long time as solid media for the growth and identification of all types of microorganisms. Agar derived from *Gracilaria* spp. has functional properties which need to be defined for the different types of uses. One of the new uses is as material for immobilisation of biocatalysts. Immobilisation of an enzyme in its soluble state can be achieved by either cross linking or binding the protein to the polysaccharide. This technique, however, presents some problems and more recently whole cells immobilised on the same material have been used. The procedure should differ from the one used for enzymes. Gel entrapment or micro-encapsulation are the most widely used techniques for immobilising living cells, which can be animal or plant cells. The gel-entrapped cells could then be used as biocatalysts for a lot of commercial processes going from production of ethanol by yeast cells to the production of monoclonal antibodies from hybridoma cells. Since high temperatures have to be avoided for the handling of living cells, the characteristics of each agar have to be well known. Agars with low setting and melting temperatures are the ones to be used.

Techniques to obtain spherical gel beads from agarose/agar to encapsulate living cells have been described, but they have to be studied in each country according to the type of agar obtained from each *Gracilaria* spp. Immobilisation of living cells in gel beads has
found numerous applications due to the easier handling of the beads compared to fragile cells. Plant protoplasts have been entrapped in agarose gels for use in plant cell studies or reproduction, as well as pregerminated seeds or somatic embryos for agricultural uses.

These new uses of agar and agarose in the field of biotechnology make the production, processing and study of agar/agarose from *Gracilaria* spp. grown and cultivated in south east Asia a process of high economic value. *Gracilaria* spp, like many other red algae, contain (besides the phycocolloids) proteins which can enter human or animal food.

1.2 Source of protein

A procedure to prepare protein extracts without destroying the colloids has been set up and a study of the content of these proteins in the different amino acids has been undertaken on *Gracilaria* sp. obtained from Dr Chandrkrachang's lab. Preliminary results suggest that *Gracilaria* can be an important source of vegetal proteins for the different countries of Asia which lack animal proteins. Like any other protein extracted from vegetal origin they are rich in the essential amino acids and can be obtained on an industrial scale by low cost processing. The proteins are lipo-proteins associated with cell membranes, chlorophyll pigments and soluble proteins from the cytoplasm. The procedure used for extraction of proteins from plant leaves, mainly "luzerne" has to be adapted to the algal extract. Because of the similarity between the luzerne protein extracts and the one obtained from different red algae, namely *Gracilaria*, the studies recently published by the European Association "Leaf extract and nutrition" can be applied to algae.

Projects to supplement children’s food with vegetal proteins in India, Sri Lanka and different countries in Africa have been very successful, with the children showing weight increases and regular growth. The suppression of anaemia and improvements in vitamin A carency and good tolerance are other advantages. Since red algae like *Gracilaria* are commonly used in the food of the Asian population, the use of algal protein extracts for human food should not bring any problems and may greatly help the nutrition of children in all countries with low resources in animal proteins.

The project for using *Gracilaria* as a source of vegetal proteins should encompass: research on protein supplementation techniques; the training of local people; and a follow-up of the project among the population receiving supplementation, with a series of control tests.

1.3 Food supplements

Further advantages of algal extracts for food supplementation are their content of different vitamins and minerals. It has also been shown that some lipids with polyunsaturated fatty acyl chains which are essential in child nutrition, are present in algae. Future research on *Gracilaria* should therefore bring more details on the lipid content.

2. FUTURE RESEARCH

In a more distant future, research on *Gracilaria* should deal with the use of some cell organelles as drug carriers, according to the results obtained with unicellular algae *Dunaliella*. This would be a very important field of research and development for the aquaculture and processing of red algae. Pharmacology could benefit from research on the use of algae which are naturally rich in useful molecules and could be the source of cell organelles with low antigenicity. These cell organelles can be enriched in different
molecules used as drugs and further used directly as drug carriers since they are natural liposomes with more specificity. Such a field of research requires:

• the training and education of personnel, possibly in foreign laboratories;
• the establishment of small scale laboratories for biotechnology in different countries;
• the regular exchange of information; and
• continuous follow up of the progress in each country.

NACA should be the co-ordinator of the research and applications and should be able to control future development, taking into consideration environmental protection.
Annex IV-9

PHYTOSANITATION - UTILIZATION OF GRACILARIA IN RECLAMATION OF SHRIMP POND EFFLUENTS

Mr. Kanit Chaiyakam,
National Institute of Coastal Aquaculture,
Songkhla, Thailand, 90000.

ABSTRACT

Experiments on using seaweed, *Gracilaria fisheri* for biological wastewater treatment from shrimp pond effluent were conducted by static bioassay with indoor tanks 200 litres in size. The first trial, used 168 ± 2 g of seaweed and the second trial used 340 ± 2 g.

The results of the first experiment (Table 1) showed decreases in the measured parameters, especially after 48 hours had elapsed.

<table>
<thead>
<tr>
<th>Hours</th>
<th>BOD</th>
<th>COD</th>
<th>Total Ammonia</th>
<th>Nitrate</th>
<th>Suspended solids</th>
<th>Chlorophyll a</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>-</td>
<td>20.4</td>
<td>12.5</td>
<td>12.5</td>
<td>9.1</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>-</td>
<td>23.4</td>
<td>14.4</td>
<td>14.4</td>
<td>9.1</td>
<td>6.7</td>
</tr>
<tr>
<td>24</td>
<td>5.1</td>
<td>17.3</td>
<td>3.6</td>
<td>3.6</td>
<td>6.1</td>
<td>13.7</td>
</tr>
<tr>
<td>48</td>
<td>36.8</td>
<td>36.0</td>
<td>54.9</td>
<td>54.9</td>
<td>35.6</td>
<td>42.4</td>
</tr>
</tbody>
</table>

The second experiment showed more pronounced results (Table 2) due to the higher biomass of seaweed.

<table>
<thead>
<tr>
<th>Hours</th>
<th>BOD</th>
<th>COD</th>
<th>Total Ammonia</th>
<th>Nitrate</th>
<th>Suspended solids</th>
<th>Chlorophyll a</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>41.2</td>
<td>25.6</td>
<td>-</td>
<td>42.1</td>
<td>8.1</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>68.7</td>
<td>52.1</td>
<td>-</td>
<td>100</td>
<td>5.1</td>
<td>-</td>
</tr>
<tr>
<td>24</td>
<td>39.5</td>
<td>55.8</td>
<td>-</td>
<td>97.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>48</td>
<td>73.9</td>
<td>20.1</td>
<td>66.7</td>
<td>100</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

The utilization of the seaweed *Gracilaria* requires aeration to encourage water movement and stop sediment attachment to the thallus of seaweed, which otherwise inhibits thallus growth.

Experiments have also been conducted using *Gracilaria* in combination with green mussel (*Mytilus* sp.) for biological water treatment. There have also been some field trials in Lake Songkhla for reducing nutrient loads in proximity to finfish cages. The results are very encouraging and confirm the phytosanitation value of *Gracilaria*.
INTERNATIONAL AND REGIONAL TRADE IN SEAWEEDS AND SEAWEED PRODUCTS, WITH SPECIAL REFERENCE TO GRACILARIA AND AGAR QUALITY STANDARDS

Suchart Wongwai,
Port Authority of Thailand,
Bangkok, Thailand.

ABSTRACT

*Gracilaria* is the raw material for the agar industry world-wide. Chile is the largest producer of *Gracilaria* and Japan is the largest producer of agar. World agar production is currently estimated at 10,000 tonnes per annum, about half of which is from *Gracilaria*. Japan is the largest producer and consumer of agar. Thailand, Malaysia and Indonesia are major importers of agar as shortages of raw materials and technological expertise are a constraint to agar production in these countries. Vietnam can produce a large quantity of seaweeds (*Gracilaria*) and has an agar industry, but agar quality is not up to standard. As the price of *Gracilaria* increased year after year, agar also became expensive, both commercial and bacterial grade. In Japan, South Korea and Taiwan, which are top agar producers, labour and land costs were the reason for the increasing the price of agar. These countries switched production to other Asian countries to increase profits.

1. INTRODUCTION

The annual world production of agar stands at between 7 and 10,000 tonnes per annum, approximately half of which was produced from *Gracilaria* and the remainder came mainly from *Gelidium* (Coppen, 1989). The world agar industry basically uses the following seaweeds:

1. Different species of *Gelidium* harvested mainly in Spain, Portugal, Morocco, Japan, Korea, Mexico, France, USA, People's Republic of China, Chile and South Africa.
2. *Gracilaria* of different species harvested in Chile, Argentina, South Africa, Japan, Brazil, Indonesia, Philippines, People's Republic of China (including Taiwan Province), India and Sri Lanka.
3. *Pterocladia capillace* from Azores (Portugal) and *Pterocladia lucida* from New Zealand.
4. *Gelidiella* from Egypt, Madagascar and India.

Other seaweeds used include: *Aphelitia plicata* from North Japan and the Sakhalin Islands; *Acanthopheltis japonica*, *Ceramiun hypnaeordes* and *Ceranium boydenii* (Armisen and Galatas, 1987). A country breakdown of world agar production is given in Table 1.

Japan is the largest producer of agar in the world and, in 1987, Japan exported 3,729 tonnes of agar to the countries shown in Table 2.
Table 1: World production of agar, 1984 (tonnes).

<table>
<thead>
<tr>
<th>Country</th>
<th>Agar Production (tonnes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Japan</td>
<td>2,440</td>
</tr>
<tr>
<td>Spain</td>
<td>890</td>
</tr>
<tr>
<td>Chile</td>
<td>820</td>
</tr>
<tr>
<td>S. Korea</td>
<td>600</td>
</tr>
<tr>
<td>Morocco</td>
<td>550</td>
</tr>
<tr>
<td>Portugal</td>
<td>320</td>
</tr>
<tr>
<td>Taiwan</td>
<td>275</td>
</tr>
<tr>
<td>Argentina</td>
<td>200</td>
</tr>
<tr>
<td>Indonesia</td>
<td>150</td>
</tr>
<tr>
<td>China</td>
<td>140</td>
</tr>
<tr>
<td>Others</td>
<td>300</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>6,685</strong></td>
</tr>
</tbody>
</table>

Source: Coppen, 1989

Table 2: Japanese agar exports, 1987.

<table>
<thead>
<tr>
<th>Country</th>
<th>Agar (tonnes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chile</td>
<td>873</td>
</tr>
<tr>
<td>S. Korea</td>
<td>749</td>
</tr>
<tr>
<td>Japan</td>
<td>430</td>
</tr>
<tr>
<td>Spain</td>
<td>715</td>
</tr>
<tr>
<td>Portugal</td>
<td>347</td>
</tr>
<tr>
<td>Morocco</td>
<td>615</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>3,729</strong></td>
</tr>
</tbody>
</table>

Source: Coppen, 1989

Japan is highly dependent on imported raw material for its agar production and accounts for most of the world trade in agarophytes (Table 3). The total raw material requirement to produce 7,000 tonnes of agar is around 35,000 tonnes of seaweed, which means that almost a third of the seaweed used enters world trade. The dominant position of Chile as a supplier of *Gracilaria* means mat, including their own domestic production of agar, they are the world's largest source of *Gracilaria* derived agar. Chilean sources estimated the 1985 harvest to be almost 16,000 tonnes (dry weight). The majority of this was collected from the wild but 400 tonnes was cultivated.

The need for greater quantities of agarophytes has encouraged *Gracilaria* cultivation. Seaweed cultivation has only had limited success, however, and there are still problems to be solved before it can be generally adopted. At present, cultivation is used for industrial purposes in the People's Republic of China and its Taiwan Province and it is now being initiated in Chile. (Armisen and Galastas, 1987).

The production, utilisation and international trade of commercial seaweeds and seaweed products are important for the countries of Asia-Pacific, especially *Gracilaria* and agar. In the case of *Gracilaria* the problem is more difficult to solve. The enzymatic hydrolysis of agar occurs spontaneously even at relatively low moisture contents, but at variable rates depending on the *Gracilaria* species and its origin.
Table 3  
Agarophyte \(\textit{Gracilaria}\) imports to Japan (tonnes).

<table>
<thead>
<tr>
<th>Country</th>
<th>1986</th>
<th>1987</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chile</td>
<td>3,036</td>
<td>2,728</td>
</tr>
<tr>
<td>Philippines</td>
<td>859</td>
<td>1,006</td>
</tr>
<tr>
<td>S. Africa</td>
<td>1,124</td>
<td>850</td>
</tr>
<tr>
<td>Argentina</td>
<td>235</td>
<td>193</td>
</tr>
<tr>
<td>Brazil</td>
<td>55</td>
<td>176</td>
</tr>
<tr>
<td>Sri Lanka</td>
<td>88</td>
<td>10</td>
</tr>
<tr>
<td>Others</td>
<td>1,146</td>
<td>2,143</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>6,543</td>
<td>7,106</td>
</tr>
</tbody>
</table>

Source: Coppen, 1989

\(\textit{Gracilaria}\) harvested in India, Sri Lanka, Venezuela, Brazil and generally in warm waters, contains an agar less resistant to enzymatic hydrolysis than the Chilean \(\textit{Gracilaria}\) which is the most stable one known. Nevertheless, the stability of agar contained in \(\textit{Gracilaria}\) is less than that of \(\textit{Gelidium}\).

The world production of red seaweeds was 1,256,918 metric tonnes in 1992 (Table 4).

Table 4:  

<table>
<thead>
<tr>
<th>Country</th>
<th>Quantity (tonnes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canada</td>
<td>10,388</td>
</tr>
<tr>
<td>France</td>
<td>6,812</td>
</tr>
<tr>
<td>Portugal</td>
<td>5,000</td>
</tr>
<tr>
<td>Spain</td>
<td>5,300</td>
</tr>
<tr>
<td>Saint Lucia</td>
<td>2</td>
</tr>
<tr>
<td>Morocco</td>
<td>7,783</td>
</tr>
<tr>
<td>Ukraine</td>
<td>6,500</td>
</tr>
<tr>
<td>Argentina</td>
<td>2,300</td>
</tr>
<tr>
<td>South Africa</td>
<td>1,000</td>
</tr>
<tr>
<td>Madagascar</td>
<td>285</td>
</tr>
<tr>
<td>Tanzania</td>
<td>100</td>
</tr>
<tr>
<td>China</td>
<td>158,990</td>
</tr>
<tr>
<td>Japan</td>
<td>383,773</td>
</tr>
<tr>
<td>Korea Rep.</td>
<td>38,408</td>
</tr>
<tr>
<td>Russian Fed</td>
<td>4,741</td>
</tr>
<tr>
<td>Other Asia</td>
<td>11,758</td>
</tr>
<tr>
<td>Fiji</td>
<td>48</td>
</tr>
<tr>
<td>Indonesia</td>
<td>188,218</td>
</tr>
<tr>
<td>Philippines</td>
<td>350,554</td>
</tr>
<tr>
<td>Kiribati</td>
<td>400</td>
</tr>
<tr>
<td>Mexico</td>
<td>5,283</td>
</tr>
<tr>
<td>Chile</td>
<td>69,145</td>
</tr>
<tr>
<td>Peru</td>
<td>130</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>1,256,918 tonnes</td>
</tr>
</tbody>
</table>

Source: FAO Yearbook of Fishery Statistics, 1992

Marketing of industrial agar is done through trading companies operating from Japan, Europe or the United States, where the most important trading companies are located in the area close to New York. There are, however, different standard specifications as shown in Table 5.
Table 5: Standard specifications of agar for FCC, USP, EEC and FAO (* negative).

<table>
<thead>
<tr>
<th>Quality requirements</th>
<th>FCC</th>
<th>USP</th>
<th>EEC</th>
<th>FAO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microbial limit, Salmonella</td>
<td>-</td>
<td>*</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Moisture, % max.</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Ash, % max.</td>
<td>6.5</td>
<td>6.5</td>
<td>6.5</td>
<td>6.5</td>
</tr>
<tr>
<td>Acid-in solution ash, % max.</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Foreign organic matter, % max.</td>
<td>-</td>
<td>1.0</td>
<td>-</td>
<td>1.0</td>
</tr>
<tr>
<td>Insoluble matter, % max.</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>-</td>
</tr>
<tr>
<td>Arsenic, ppm max.</td>
<td>3</td>
<td>3</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>Lead, ppm max.</td>
<td>10</td>
<td>10</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>Heavy metals (as Pb), ppm max.</td>
<td>10</td>
<td>40</td>
<td>-</td>
<td>40</td>
</tr>
<tr>
<td>Foreign starch and dextrins</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Gelatin and other proteins</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Water absorption, ml max. per 5.0g agar</td>
<td>75</td>
<td>75</td>
<td>75</td>
<td>75</td>
</tr>
</tbody>
</table>

Source: Tengtein Y., and Wattanaoran P., 1989

FCC - Food Chemicals Codex
USP - The United States Pharmacopoeia
EEC - European Economic Countries
FAO - Food and Agriculture Organisation of the United Nations

It is difficult to get an idea of the prices of commercial agar because the usual trade statistics list agars with different specifications and applications and therefore with different prices, as shown in Tables 6 and 7.

Table 6: Agar Imported and Exported by Japan in 1986 (January - October).

<table>
<thead>
<tr>
<th>Exports</th>
<th>Quantities (tonnes)</th>
<th>Value (Yen)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Natural Agar</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Strip</td>
<td>6,664</td>
<td>21,651,000</td>
</tr>
<tr>
<td>- Square</td>
<td>3,430</td>
<td>34,127,000</td>
</tr>
<tr>
<td>b. Industrial Agar</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Powder</td>
<td>447,559</td>
<td>1,194,436,000</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>457,653</td>
<td>1,240,214,000</td>
</tr>
</tbody>
</table>

| Imports                  |                   |             |
| a. Natural Agar          |                    |             |
| - Strip                  | 103,078            | 212,693,000 |
| b. Industrial Agar       |                    |             |
| - Powder                 | 227,231            | 750,114,000 |
| **Total**                | 330,309            | 962,807,000  |

Exchange rate 1 US$ = 154.23 Yen

Source: Armisen and Galatus, 1987
Table 7: Japanese export/ import, average price for agar in 1986.

<table>
<thead>
<tr>
<th></th>
<th>Price/kg (Yen)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Export</td>
<td></td>
</tr>
<tr>
<td>Agar, natural, strip</td>
<td>21.07</td>
</tr>
<tr>
<td>Agar, natural, square</td>
<td>45.61</td>
</tr>
<tr>
<td>Agar, Industrial, powder</td>
<td>17.30</td>
</tr>
<tr>
<td>Import</td>
<td></td>
</tr>
<tr>
<td>Agar, natural, strip</td>
<td>13.38</td>
</tr>
<tr>
<td>Agar, industrial, powder</td>
<td>21.40</td>
</tr>
</tbody>
</table>

Exchange rate: 1 US$ = 154.23 Yen
Source: Armisen and Galatas, 1987

The major parameters of the Japanese Specifications of Processing agar to show the different of qualities of agar, is shown in Table 8. Table 9 shows the chemical composition of *Gracilaria*.

Table 8: Major parameters of the Japanese Specifications of Processing Agar.

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Special</th>
<th>Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pinh Strength (gm cm-1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water content</td>
<td>600 or more</td>
<td>350 or more</td>
</tr>
<tr>
<td>Crude protein content</td>
<td>22% or less</td>
<td>22% or less</td>
</tr>
<tr>
<td>Solids insoluble in hot water</td>
<td>1.5% or less</td>
<td>1.5% or less</td>
</tr>
<tr>
<td>Crude ash content</td>
<td>0.5 or less</td>
<td>4% or less</td>
</tr>
</tbody>
</table>

Source: Chandrkrachang and Chinadit, 1988

Table 9: Chemical composition (%) of *Gracilaria*.

<table>
<thead>
<tr>
<th>Item</th>
<th>Content in 100 gram</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water (g)</td>
<td>83.5</td>
</tr>
<tr>
<td>Crude protein (g)</td>
<td>2.3</td>
</tr>
<tr>
<td>Fat(g)</td>
<td>0.2</td>
</tr>
<tr>
<td>Carbohydrates (g)</td>
<td></td>
</tr>
<tr>
<td>sugar</td>
<td>11.0</td>
</tr>
<tr>
<td>fibre</td>
<td>0.5</td>
</tr>
<tr>
<td>Ash(g)</td>
<td>2.5</td>
</tr>
<tr>
<td>Minerals (mg)</td>
<td></td>
</tr>
<tr>
<td>Ca</td>
<td>514</td>
</tr>
<tr>
<td>Fe</td>
<td>56</td>
</tr>
<tr>
<td>P</td>
<td>12</td>
</tr>
<tr>
<td>Vitamins (mg)</td>
<td></td>
</tr>
<tr>
<td>A(iu)</td>
<td>260</td>
</tr>
<tr>
<td>B2</td>
<td>0.03</td>
</tr>
<tr>
<td>Niacin</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Source: Arasaki, S. and Arasaki, T. 1983
The Asian nations which would participate in the expansion of *Gracilaria* production and other agar-bearing seaweeds are Thailand, Malaysia and Indonesia. Vietnam can produce a great quantity of *Gracilaria* and agar, but they are still problems reaching quality standards for agar in the world market. Vietnam will be developing their *Gracilaria* production and processing in the near future. Japan, South Korea and Taiwan are now looking for joint ventures in other Asian countries to transfer funding and technology for the agar industry because the labour and land costs in their countries were very high. Vietnam would be especially good for investment because of their high production of *Gracilaria*.

**Exporters of seaweed and seaweed products**

Marcel Trading Corp., P O Box 241, Manila, Philippines.

**REFERENCES**


Socio-Economics of a Coastal Community in the Philippines with Gracilaria Seaweed Production as an Alternative Livelihood

Nyan Taw\(^1\),
Chief Technical Adviser, Seaweed Production Development Project,
PHI/89/004, BFAR/UNDP/FAO,
Philippines.

Abstract

The estimated income level of coastal households in Sorsogon, which is situated approximately 600 km south of Manila, ranges from 2,000 to 3,560 Pesos\(^2\) per month. About 39\% of the population are engaged in fishing as their main source of income. Studies in coastal areas of Sorsogon have revealed that the area is rich in *Gracilaria* resources, both in quality and quantity. Three types of areas with their specific environmental conditions were found for *Gracilaria* fanning purposes: (i) open sea coralline flats with high salinity; (ii) bays along the coast with sandy-muddy substrates and high salinity; and (iii) brackishwater ponds with muddy substrates and low salinity. Three methods of fanning have been developed, namely: fixed bottom line; floating raft line; and pond culture. Simple family-sized processing technology to produce one kg per day of food agar powder was also developed. Using the technologies developed, coastal communities have initiated *Gracilaria* farming and processing activities, with local Government support, as an alternative livelihood. The unit cost to produce one kg of dried *Gracilaria* was estimated at 3.20 Pesos per kg with a selling price of between 6.00 to 10.00 Pesos, while the unit cost to produce one kg of agar powder was estimated at 406.20 Pesos with the selling price of between 750.00 and 1,000 Pesos. Previously, apart from gathering *Gracilaria* from natural beds and selling it to buyers from Manila, the potential of farming and processing *Gracilaria* had not been fully realised.

1 Correspondence: Pt. Puteri Cendana Prima, Intergraded Shrimp Aquaculture Industry
Jl Sumatra 136, Surabaya 60281, Indonesia.

2 1 US$ = 25 Pesos

1. Introduction

The Seaweed Production Development Project (PHI/89/004) aimed to develop farming and processing technologies of species of seaweeds other than *Euchewna*. The seaweed *Gracilaria* is one of the major sources of raw material for the manufacture of agar. It is used in crude form by the food processing industry, while more sophisticated forms are utilised in pharmaceutical and biomedical applications.

One of the main objectives of the project was to improve the socio-economic conditions of fishing communities dependent on coastal fisheries for livelihood, by developing alternative employment and income opportunities through an extended and diversified seaweed fanning and processing industry.

The project is financed by the United Nations Development Programme (UNDP) and executed by the Food and Agriculture Organization of the United Nations (FAO). The Bureau of Fisheries and Aquatic Resources of the Department of Agriculture (BFAR/DA) is the Government counterpart agency.
2. COMMUNITY BASED GRACILARIA PRODUCTION DEVELOPMENT TECHNOLOGIES

2.1 Gracilaria resources

Studies have revealed that Sorsogon, which is situated approximately 600 km south of Manila (Figure 1), is rich in Gracilaria resources both in quantity and quality (Liana, 1992; 1994). Naturally rich Gracilaria beds have been found both on the east coast and in Sorsogon Bay, most notably in Magallanes where approximately six hectares have been reserved for conservation purposes (Nyan Taw, 1993c).

Taxonomic studies of Gracilaria species from Sorsogon, revealed eleven species of which five have been previously unrecorded in Filipino waters (Trono, 1994). One Gracilaria species appears to be unknown and has yet to be described and named, while three others require verification. Five Gracilaria species were found to have good potential for farming purposes in Sorsogon and other parts of the Philippines (Nyan Taw, 1993a; 1993b; 1994a), namely: G. firma; G. fastigiata; G. changii; G. heteroclada; and G. tenuistipitata var liui.

2.2 Farming

Generally, three types of areas with specific environmental conditions can be classified for farming purposes (Nyan Taw, 1994a):

- **Open Sea**: Open sea coralline flats protected by reef breakers at the fringes of the coast. High salinity (33-35 ppt), clear water with firm coralline substrate.
- **Bays**: Protected bays and mangrove channels along the coastline, channels within coastal mangrove islands and river estuaries with little freshwater influence. High salinity (25-35 ppt), semi-clear water with sandy-mud substrate.
- **Brackishwater ponds**: Unused fish ponds, supply/drainage canals of fish ponds or mangrove ponds. Low salinity (10-25 ppt), turbid water with muddy substrate.

Three methods of farming have been developed, namely: fixed bottom line, floating raft line and pond culture (monoculture and polyculture) using the five identified species in their preferred area and environment. Five demonstration farms were in operation utilising the areas identified and the methods developed: three on the East Coast (Prieto Diez, Gubat and Bulusan) and two in Sorsogon Bay (Juban and Magallanes).

The average daily growth rate recorded over 45 to 50 days of culture, at optimum environmental conditions, for the five Gracilaria species were as follows: G. changii = 9.0%; G. firma = 8.7%; G. fastigiata = 9.0%; and G. heteroclada/ G. tenuistipitata = 6.2%. Growth rates compare favourably with the estimated average daily growth for Eucheuma (5%) in commercial farming (Trono et al. 1988). Generally, based on this daily growth rate and production from line farming trials, a production of 4.0 tonnes of (dried) seaweed can be expected from a one hectare area after 45 to 50 days of culture in an optimum environment. A maximum production of 12.0 tonnes of dried seaweed can be obtained from one hectare of pond with monoculture.
Figure 1: Eastern Sorsogon and Sorsogon Bay, Philippines.
2.3  Processing and agar quality

The agar quality (average gel strength) of the five *Gracilaria* species are: *G. changii* = 963 g/cm²; *G. firma* = 765 g/cm²; *G. fastigiata* = 890 g/cm²; *G. heteroclada* = 968 g/cm²; and *G. tenuistipitata* = 433 g/cm² (Santos, 1993). In terms of gel strength, the quality of *Gracilaria* from Sorsogon can be considered very good. Quality standards for bacteriological media and plant tissue culture medium (bacto-agar), are set at 600-800 g/cm² for gel strength. For agarose, a gel strength of 800-1,000 g/cm² is required (Anonymous, 1990).

Village level processing plants utilising available local materials have been developed (Whitaker, 1994; Pena, 1994). Training programmes, attended by members of co-operatives from different barangays, municipal agriculture officers and academe from Sorsogon, were conducted at the Field laboratory in Cabid-an, Sorsogon. With some basic village level processing facilities provided by the project, three co-operatives started operating. These co-operatives were able to come-up with the required counterpart facilities and operational expenses and are now acting as model village-level processing plants in their respective areas. The small scale agar production flow diagram is in Figure 2.

Figure 2: Small scale agar production flow diagram (from de la Pena, 1994).
With additional funds from UNDP, DA-BFAR and contributions from the local government of Sorsogon, a prototype processing plant to produce five tonnes of food agar annually was completed in December 1994.

3. SOCIO-ECONOMICS

3.1 Coastal communities of Sorsogon

A comprehensive benchmark study on socio-economic aspects of the project areas (Prieto Diaz, Bagacay, Gubat, Barcelona, Bulusan, Santa Magdalena and Matnog at East coast of Sorsogon and Castilla, Juban and Magallanes at Sorsogon Bay) has been completed (Tagarino, 1992). The assessment was carried out in 89 out of the 169 coastal communities in six municipalities in Eastern Sorsogon, namely: Barcelona, Bulusan, Gubat, Matnog, Prieto Diaz and Sta. Magdalena. There were at least 13,905 households in these areas, with an average size of 5.2 persons per household. There were at least 4,528 fishermen of which 2,908 were full-time fishermen, who were equipped with a total of 3,352 fishing boats. About 7.0% of the boats were motorised. The estimated income level of the coastal households in the area ranged from 2,006 to 3,556 Pesos per month. About 75% of their income was derived from primary sources. About 39% of the population were engaged in fishing as their main source of income. The fishermen normally used conventional fishing methods but other methods, such dynamite/cyanide fishing, were also known to be common in the area. About 50-70 % of the households had temporary housing with an average floor area of 22 to 28 m$^2$. Only a few of the households (about 5-15 %) owned basic household appliances such as stoves, refrigerators or sewing machines. A majority of the households (more than 50%) did not own basic household production tools and implements.

3.2 Economics of Gracilaria farming and processing for food agar

In Sorsogon, seaweed farmers have initiated farming and production of Gracilaria in the areas identified and using methods disseminated by the project. An economically viable farm size for a farming/ fishing family (a family of four to five) was calculated to be one hectare (Orogo, 1994b). The cost of production at a farm varies depending on the locality and availability of farm input. Major production costs were seedlings, stakes and lines. The operational costs would be much reduced if the seedlings were collected by the farmer's family to start the operation. The unit cost of production would be less from year two onwards. A family-sized processing plant that can produce one kg of food agar per day (288 kg/year) is an ideal size. However, with extra help, production can be increased to a maximum of 3 kg/day using the same facilities with additional minor equipment. Orogo, (1994b) studied the economics of a simple seaweed farming and processing farm (Table 1).

Table 1: Simple economics of Gracilaria farm and processing plant (Year 1).

<table>
<thead>
<tr>
<th>Description</th>
<th>Farming</th>
<th>Processing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type/size of operation</td>
<td>Family type 1 ha farm</td>
<td>Family type plant</td>
</tr>
<tr>
<td>Method</td>
<td>Fixed bottom line 4 crops</td>
<td>Dewatering by press</td>
</tr>
<tr>
<td>Production per year</td>
<td>16,000 kg (dried)</td>
<td>288 kg agar powder</td>
</tr>
<tr>
<td>Sales income</td>
<td>160,000 Pesos (10.00 Pesos/kg)</td>
<td>288,000 Pesos (1,000 Pesos/kg)</td>
</tr>
<tr>
<td>Cost of production</td>
<td>51,000 Pesos</td>
<td>117,000 Pesos</td>
</tr>
<tr>
<td>Unit cost</td>
<td>3.2 Pesos/kg</td>
<td>406.2 Pesos/kg</td>
</tr>
<tr>
<td>Net income</td>
<td>109,000 Pesos/year (9,083 Pesos/month)</td>
<td>171,000 Pesos/year (14,250 Pesos/month)</td>
</tr>
</tbody>
</table>
3.3 Co-operatives and Marketing

A number of community-based groups/ co-operatives have been organised in the project area (Orogo, 1994a). These co-operatives/ associations have attained strong marketing and communication linkages and have formed the Sorsogon Federation of Seaweeds and Aqua-based Co-operatives. The co-operatives deposited 50,000 Pesos with the Philippine National Bank for safekeeping and registration purposes with the Co-operative Development Authority. Sorsogon Marine Product Development Cooperative, Gubat was a recipient of a 50,000 Pesos grant from the local government to develop 24 hectares of ponds for Gracilaria farming.

The co-operatives and associations from the east coast and Sorsogon bay had set target activities for expansion of at least 35 hectares pond culture. The farmer/ fishermen families were processing Gracilaria seaweed into gulaman and making local jelly sweets for sale within their villages to acquire additional income for the family.

Aside from pre-organisational and pre-membership training for newly organised co-operatives, selected co-operative officers were sent to a number of National Seminars and Workshops organised by CDA. The major constraint reported by the seaweed farmers/ gatherers in Sorsogon is the marketing of dried seaweeds. The seaweeds could be processed into a dried gulaman bar or powdered agar, a more durable form of product, which would be easier to market.

The volume of Gracilaria being harvested from the wild and traded within the project area was about 67.5 tonnes/year. The range of prices of the Gracilaria being traded between gatherers and middlemen was from 3.0 to 10 Pesos per kg (dried) depending on the trading level of middlemen (Guanio, 1994). It is apparent that there is a ready market for Gracilaria, but the problem is the low price the gatherers/farmers were obtaining. A federation of co-operatives are now being formed to cope with this situation.

According to studies by Guanio, 1994, the Philippines has imported approximately 20 tonnes of bacto-agar and 185 tonnes per annum since 1986. There are ten large food processing companies in the Philippines which use agar as an ingredient in their products. This indicates that there is a ready domestic market for food and bacto agar. According the Philippine Seaplant Corporation which exports dried Gracilaria, the price received varied between US $ 300 to 400 per tonne depending on the quality.

The farming and processing technology developed has also been utilised by cooperators (NGOs) from other regions of the Philippines such as: Butuan and Cagayun in Mindanao. In Quezon, commercial gathering from the wild and farming in ponds in areas identified by the project, is developing rapidly. The dried seaweeds are exported directly to Taiwan and other countries. The project also assisted farmers/ fishermen from Bataan, Quezon, Batagas and Binmaley in Pagasinan on Gracilaria farming and processing technology.

ACKNOWLEDGEMENTS

I sincerely wish to thank the following: Mr F. Taclan, Project Leader and technicians from Sorsogon for their assistance; Mr G. L. Morales (National Project Director), Ms. Purita Dela Pena (National Project Coordinator) and members of BFAR staff; and Governor Juan G. Frivaldo of Sorsogon Province for their continued support throughout the project period. The wholehearted support of Mr Kevin Mc Grath, Resident Representative UNDP Manila, Mr. Peer Hijnams, FAOR Manila and Mr A. P. Isarankura, FAO HQ is also highly appreciated.
REFERENCES


GRACILARIA PRODUCTION AND TRADE

INFOFISH,
Kuala Lumpur, Malaysia.

In 1992, the total world production of agar was estimated at 7-10,000 tonnes, half of which originated in Japan and the Republic of Korea. Major agar producing countries were Japan, Spain, Chile and the Republic of Korea and several of the leading agar producers exported most of their production. Emerging producers in the Asia-Pacific region were Indonesia, the Philippines and Thailand.

Today, the agar industry probably represents a market value well in excess of US $ 200 million and agarophytes command a higher price than other colloid-bearing seaweeds. An estimated 10,000 tonnes of raw agar and 3,500 tonnes of final product enter the world market each year. The raw material goes principally to Japan, which is also the main exporter of the final product.

Japan is the main agar consuming country (about 2,000 tonnes a year) and almost all of its consumption comes from domestic production. The USA, another major consumer (1,000 tonnes per year), imports more than 80% of its supply. Its main suppliers are Chile, Morocco, Spain and, more recently, the Philippines. The demand for agar in the EEC is approximately 1,300 tonnes per year.

Demand also exists, in newly developed and developing countries for feed grade and bacteriological agar. Thailand, Indonesia, Singapore and Malaysia each import about 200 tonnes each year. The main suppliers for the region are the Republic of Korea, Japan and, more recently, Chile. Thailand, Malaysia, Indonesia and India have also produced modest amounts of agar.

Product forms that are marketed are seaweed powder, dried/treated agarophytes and strip, flake, powdered and granular agar. Prices for dried seaweed or alkali-treated (colagar) vary depending on agar quantity and quality.

3 INFOFISH faxed in this information and attached tables. The invited Resource Person could not participate.

REFERENCE

Table 1:

World-wide production of red seaweed (tonnes) FAO Fishery

Area
1983
1984
1985
1986
1987
1988
1989
1990
1991
1992
Canada
18430 20387 22610 17240 29550 17230 19420 14707
9537
10388
USA
4062
…
66
166
Area total
18482 20554 22610 18828 29550 21312 19420 14773
9703
10388
France
2564
2223
2479
1900
4028
3152
5059
4023
4351
6812
Portugal
4935
6512
7906
5955
6349
5385
6876
4833
5000
500
Russian Fed. 3100
2609
2649
3173
3583
2223
2280
1871
3442
457
Spain
3603
4107
4063
4754
5548
5309
5300
5500
5300
5300
Area total
14202 15451 17099 15782 19508 16069 1951S 16227 18093 17569
Saint Lucia
4
4
4
4
2
Area total
4
4
4
4
2
Morocco
5170
4590
4500
4500
4500
3875
5895
6000
7489
7783
Area total
5170
4590
4500
4500
4500
3875
5895
6000
7489
7783
Ukraine
15569 14730 12074 19847 13927 14379 16856 14258
7168
6500
Area total
15569 14370 12074 19847 13927 14379 16856 14258
7168
6500
Argentina
10740
7001
12584
5947
2575
2400
2264
2233
2300
2300
Area total
10740
7001
12584
5947
2575
2400
2264
2233
2300
2300
South Africa
590
460
1180
1200
2000
3632
842
1000
1000
1000
Area total
590
460
1180
1200
2000
3632
842
1000
1000
1000
Madagascar
50
50
50
45
76
167
63
100
220
285
Tanzania
0
0
0
0
102
486
276
96
100
100
Area total
50
50
5O
45
178
653
339
196
320
385
Indonesia
834
493
427
384
324
602
551
597
841
862
Thailand
6
2
2
1
1
0
1
Area total
840
495
429
385
325
602
552
597
841
862
China
99870 123740 123670 135860 122850 155790 92550 88230 116840 158990
Japan
371185 409360 361808 412171 330549 452755 412619 395311 404407 383773
Korea Rep
95399 143462 114783 149132 93140 125841 150818 109834 150781 38406
Russian Fed 8694
9594
10805 13338 15291 11538 12159
7884
11025
4284
Other Asia
10723 10548 10754
9824
5722
6653
9387
10727
9474
11758
Area total
585871 696704 621820 720325 567552 752577 677S33 611986 692104 597213
Fiji
15
173
217
100
120
126
128
48
Indonesia
87720 85940 55250 72421 85092 85359 86311 118679 96974 187356
Philippines 132650 145036 184410 170483 222003 257305 270165 292471 285233 350554
Thailand
664
653
4231
1154
1666
823
605
Area total
221034 231629 243906 244231 281978 343587 357201 411276 382335 537958
Kiribati
1500
324
411
324
350
400
400
Mexico
7459
8572
7542
5479
7140
8110
5683
10737 10792
5283
Area total
7459
8572
7542
6979
7464
8521
6007
11087 11192
5683
New Zealand
31
0
0
5
1
0
0
0
0
0
Area total
31
0
0
5
1
0
0
0
0
0
Chile
136762 123833 146377 95874 83643 95466 106710 137956 110439 69145
Peru
103
144
245
437
256
267
412
269
127
130
Area total
136865 123977 146622 96311 83899 95733 107122 138225 110566 69275
1016903 1124213 1090416 1134385 1040457 1263344 1213580 1227862 1243115 1256918
SPECIES
TOTAL


Figure 1: Total production of aquatic plants (FAO, 1994).

- by Country, 1992
  - Chile: 2%
  - Indonesia: 3%
  - Norway: 3%
  - Philippines: 6%
  - Rep. of Korea: 10%
  - Japan: 13%
  - Others: 5%
  - China: 57%

- by Groups of Species, 1992
  - Tonnes ($\times 10^3$)
  - Year
  - Total aquatic plants
  - Brown seaweeds
  - Red seaweeds
  - Aquatic plants nei
  - Green seaweeds