Recent Technological Innovations in Aquaculture

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Introduction

Aquaculture faces many challenges over the next decade, notably, combating diseases and epizootics, broodstock improvement and domestication, development of appropriate feeds and feeding mechanisms, hatchery and grow-out technology, as well as water-quality management. These all present considerable scope for biotechnological and other technology interventions. Aquaculture biotechnology can be described as the scientific application of biological concepts that enhance the productivity and economic viability of its various industrial sectors (Liao and Chao, 1997). The Convention on Biologicial Diversity defines Biotechnology as, "*any technological application that uses biological systems, living organism, or derivatives thereof, to make or modiy products or processes for specific use*". Biotechnology encompasses a wide range of approaches that can improve subsistence and commercial aquaculture production and management. Although some biotechnologies are modern and novel, others have a long history of application, e.g. fermentation and fertilization of ponds to increase feed availability. Many modern biotechnologies are based on rapidly evolving knowledge of molecular biology and genetics. The major biotechnology sectors involved in aquaculture are similar to those for agricultural sectors. Development of the knowledge required to optimise safe biotechnological innovation in aquaculture is of particular significance, and presents a unique set of challenges, due mainly to the diversity of species cultured and production systems used. A key consideration behind all technology transfer to the aquaculture sector is that it should be used with due consideration to the protection of wild aquatic diversity and potential impacts on the autonomy and economy of rural and subsistence populations. The emphasis on biotechnology and its contribution to food security, poverty alleviation, and income generation is increasing and we need to be prepared to address the challenges this will bring, and develop these technologies in a responsible manner.

Reproduction Innovations

The application of genetic principles to increase production from aquatic animals currently lags far behind that of the plant and livestock sectors. Only a small percentage of farmed aquatic species have been subject to genetic improvement programmes (Gjedrem 1997); however, biotechnology and genetics have great potential to increase production and enhance ecological sustainability. Biotechnology can be applied to enhance reproduction and early development success of cultured organisms, as well as expand periods of gamete and fry availability. Genetics also have the potential to satisfy new markets for farmed products, e.g., for specific market tastes or aesthetics. Likewise, biotechnology may provide avenues for improving the reproductive success and survival of endangered species, thereby helping to identify and conserve aquatic biodiversity. Transgenic technologies can enhance growth rates and market size, feed conversion ratios, resistance to disease, sterility issues and tolerance of extreme environmental conditions. In the shrimp aquaculture sector, transgenic shrimp have been reported (Mialhe *et al.,* 1995), but there has been no successful development to date for commercial culture (Bachère *et al.,* 1997; Benzie, 1998). However, the use of transgenic organisms in aquaculture (as in other sectors) is controversial and issues of consumer education and acceptance must be addressed.

Carp and tilapia culture in Asia is benefiting from genetics research in a number of areas, including genetic sequencing and the development of specific genetic markers. Markers are short unique pieces of genetic code that can help locate genes that are important for growth, sex determination factors or disease susceptibility (Kocher *et al.,* 1998). Such techniques have already resulted in genetic improvements in some fish being cultured. The traditional technique used for many generations by farmers throughout Asia has been selecting fish by desirable phenotypic traits for breeding, on an *ad hoc* basis. This has led, in many cases, to in-breeding and suppression of optimum production performance (Chen Defu and Shui Maoxing, 1995). Improving genetic understanding across millions of small-scale farms in the Asia region is a difficult challenge, especially since traditional approaches have focussed on improvement of core stocks that can then be distributed to farmers.

The GIFT (Genetic Improvement of Farmed Tilapia) project in Asia is an example of a programme aimed at examining the genetics of an important farmed fish species. The GIFT project has been working with Nile tilapia hybrids and strains in culture around the region, with a view to development of pure-bred lines and the distribution of strains of improved performance to farmers. The programme is a collaborative effort between ICLARM (International Centre for Living Aquatic Resources Management) headquartered in Malaysia and research institutions in Malaysia, Philippines, UK and USA. The programme has not yet reached the fully commercial phase and the 'improved' tilapia in most of the participating countries are still under evaluation by fisheries scientists. The program has, however, shown considerable potential for improving farm production (http://www.iclarm.org/resprg_1f.htm. Similar breeding programmes for commercially important carps could bring comparable benefits; indeed, because carp fry production is typically more centralised than is the case for tilapia, the spread of improved stocks could occur more readily.

For many species of farmed freshwater fish, there are differences in growth rate between the sexes. Consequently, the development of techniques to produce monosex populations continues to be important. Historically, farmers have mainly depended on the use of hormones to trigger sex-reversal, or on the use of particular hybrid crosses that give skewed sex distributions in their offspring, in order to produce fish of all one sex, e.g., in tilapias. However, these techniques both have drawbacks. The use of hormones in food animals is increasingly being questioned by consumers and hybrid crosses that give skewed sex distributions may not be the best hybrids for farm productivity. Alternative methods for producing monosex populations include cloning by nuclear transplantation and gynogenesis. Cloning has been possible for carp for more than thirty years (Zhu *et al.,* 1985) and can form a useful basis for producing all female fry. In several

commercially important carp species, females grow faster than males for the first years of life, so farmers prefer all-female populations. All-female offspring can be produced from certain carp species, such as the silver crucian carp (*Carassius auratus gibelio*), which can reproduce gynogenetically (monosex female reproduction). Artificially induced gynogenesis has also been used successfully for many years in China to produce pure lines of common carp, silver carp and ornamental colour carp (*Cyprinus carpio*) (Jian-Fang Gui and Qi-Ya Zhang, 2000).

In the case of tilapia, males are preferred for culture as they grow faster than females. Recently all male populations of tilapia fry have been produced through use of YY chromosome male fish, sometimes termed 'supermales'. These are the offspring of a normal male, bred with a female produced by hormonal sex reversal of genetic male. A quarter of the offspring from such a mating typically have a YY configuration of their sex chromosomes, instead of the normal XY. When a YY male is crossed with a normal XX female it produces a high percentage of XY (male) offspring (Figure 1).

Figure 1. Production of all male offspring via YY males.
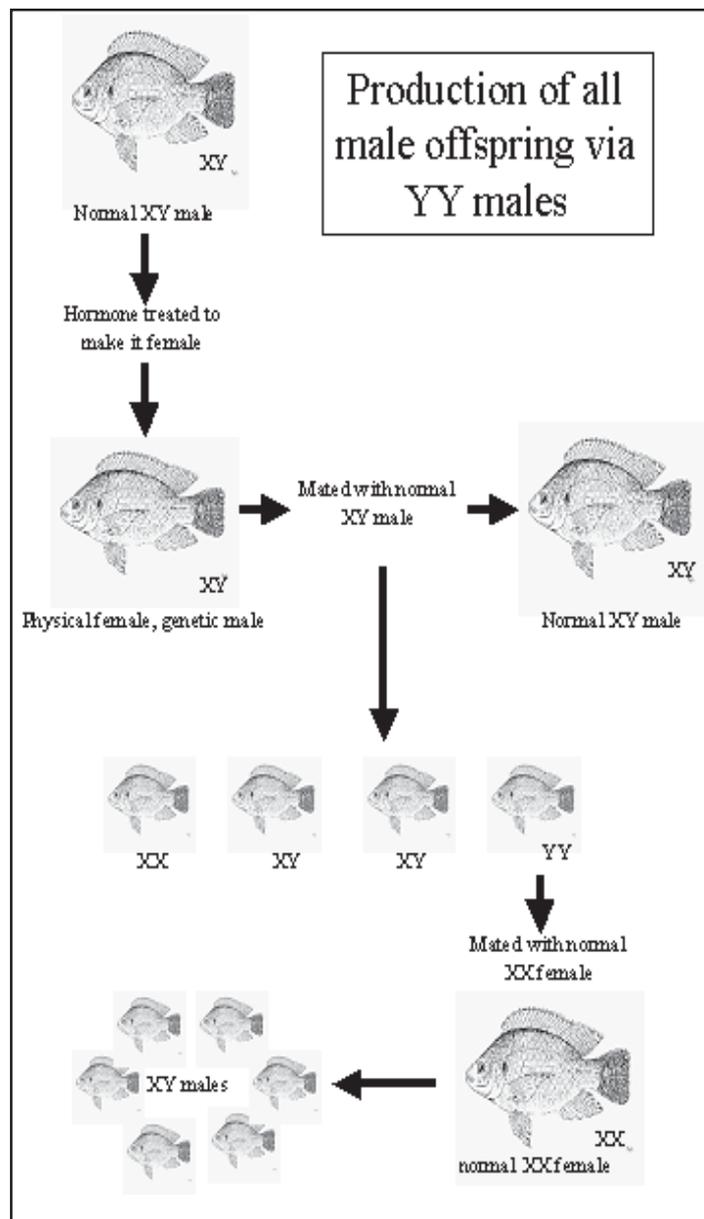
Sex differentiation does not depend entirely on the XY/XX chromosomes, so a small percentage (normally less than 5%) of the offspring are female, but this technique allows breeders the freedom to work with the best culture species and to avoid the use of hormones in the production of food fish (Mair *et al.*, 1999). This technology is now well developed for tilapia and research is ongoing for a number of other fish species. Since consumer resistance to 'hormone' treated fish is unlikely to disappear, technologies such as that of the 'supermale' will become increasingly important, especially for fish produced for export markets. The production of 'superfemales' by a complimentary technique to that used for 'supermales' may also be feasible.

In some farmed fish species, early maturation and breeding before attaining market size is a significant constraint on production. Energy is used for egg production at the expense of growth and, in some cases, such as with tilapia, ponds can become filled with undersized fish. This is a notable problem in Africa with the Nile tilapia. In such cases, stocking with sterile fry would be useful. Techniques used to achieve this include the production of

fish with extra sets of chromosomes, i.e., polyploid (triploid and tetraploid) fish (Thorgaard, 1986), or shock treatment using temperature or pressure during early embryo development to cause retention of multiple sets of chromosomes in each cell and, in most cases, sterility. Artificial triploids and tetraploids have been induced in many farmed fish species, including crucian carp (*Carassius auratus*), bighead carp (*Aristichthys nobilis*), silver carp (*Hypophthalmichthys molitrix*) and common carp (*Cyprinus carpio*) (Jian-Fang Gui & Qi-Ya Zhang, 2000).

Snapper (mainly *Lutjanus spp.*) culture has been limited by the availability of supplies from wild fisheries. Researchers in the southern USA, however, have made considerable recent advances in hatchery production of one species, the mutton snapper, *L. analis*, and are proceeding to ongrowing trials (Benetti *et al.,* 2001). At the University of Miami egg production by this species has been achieved for the first time using environmental manipulation, rather than hormone injection. It is hoped that this technique will allow year-round egg production. A similar advance has been reported by the Hawaii-based Oceanic Institute with the red snapper *L. campechanus* (Oceanic Institute News, 2000).

Molecular techniques also show significant promise for aquaculture application, in that they help provide more accurate information on the genetic diversity of natural stocks and allow genetic tagging of animals in breeding programmes (Subasinghe *et al.,* 2000). Effective breeding programmes need to identify and track the pedigrees of individual organisms. Physical tagging of early life-history stages of many aquatic species is difficult, thus, non-invasive, genetic markers using microsatellite DNA, and AFLP's (amplified fragment length polymorphisms) have been developed to track pedigrees and provide linkage maps to identify quantitative trait loci (QTL's – genes coding for characters that have production value, such as growht rates, disease resistance or cold tolerance) (Garcia *et al.,* 1996, Benzie, 1998, Moore *et al.,* 1999; Agresti *et al.,* 2000).

Increased attention is being directed towards domestication of shrimp species. In order to minimise environmental impacts and optimise use of genetic diversity, shrimp culture must, however, break its current dependence on wild post-larvae for stocking (Wang 1998). Wild larvae may currently be more economical and perform better than some hatchery produced post-larvae, but there is a constant (and inevitable) risk of introducing pathogens into the culture environment. Furthermore, there is a significant by-catch of other aquatic organisms. Recent improvements in husbandry, larval rearing, and larval nutrition, as well as genetic improvement of farmed shrimp, all have the potential to significantly reduce dependence on wild-caught postlarvae in the future. For example, considerable success has already been achieved with shrimp species, such as *Penaeus vannamei,* in development of specific pathogen free (SPF) broodstock and some of these broodstock are now becoming commercially available. Similar work is being undertaken for domestication of giant tiger shrimp (*P. monodon*) but only preliminary progress has been made to date.

Endocrine regulation of reproduction has been effectively applied across a broad range of cultured fish species, however, advancements have been slow with shrimp and molluscs. Recent research has shown that there is potential for chemical treatment of shrimp gonad inhibiting neurohormone (GIH) that could promote reproduction without the negative side effects of eye stalk ablation (Keeley 1991; Wang *et al.,* 2000). Research on shrimp GIH isolation is still ongoing but elucidation of the structure and function of shrimp GIH, using peptide biotechnology-based approaches, shows promise for countering the reproductive inhibitory effects of GIH. Further research in this area is needed, and collaboration between researchers, shrimp culturists, and resource providers from different regions could expedite its achievement.

Disease Management

Production of specific pathogen free (SPF) and specific pathogen resistant (SPR) stocks are two complementary objectives being developed through shrimp broodstock management programmes. The specific pathogens for these programs are those listed as 'notifiable' by the OIE, representing direct trade concerns, as well as, significant threats to optimal production (OIE, 2000, 2001). SPF shrimp are produced by selecting animals shown to be free of specific pathogens, using them as broodstock, and raising their offspring under strictly controlled sanitary conditions. SPF shrimp are of value for trade to countries or areas that are free of the specific disease agent, or for restocking ponds following a disease outbreak and disinfection. By contrast, SPR shrimp are developed through selective breeding of individuals that have survived challenges/infections by specific pathogens. These, therefore have great potential to enhance production in waters endemic for the specific diseases, but are inappropriate for use in non-endemic waters, as they may carry sub-clinical infections of the pathogen in question. These specific pathogen approaches are now being applied to shrimp stocking in countries like the USA, Venezuela and French Polynesia using shrimp species such as *P. vannamei* and *P. stylorostris* (Bedier, 1998). Both approaches produce 'high health' (HH), however, many SPF stocks perform poorly when challenged by other pathogens , since their production under sterile conditions impedes development of acquired resistance to common, but normally less significant, pathogens (Browdy, 1998). If the immune or physiological traits of SPR strains are heritable, this has the potential to confer significant performance improvement at the farm level. Taking this technology beyond specific pathogens, there is exciting potential for this approach to be adapted to sselection of lines with high non-specific immunity or high tolerance of physiological stresses that facilitate opportunistic infections or other pathology (Bedier, 1998). Considering the major contribution of *P. monodon* to the global shrimp production and the economic losses encountered due to both facultative and opportunistic disease outbreaks; it is appropriate and timely to concentrate further research to develop specific and non-specific resistant broodstock – especially for *P. monodon.*

Infectious disease is currently the single most devastating problem in shrimp culture and presents ongoing threats to other aquaculture sectors. In addition, there is increasing concern over the consequences of newly emerging diseases in aquaculture. Conventional methods of controlling such diseases, such as chemotherapeutants, are ineffective for many new pathogens (notably viruses), thus, molecular techniques are receiving increasing attention for pathogen screening and identification. In addition, these techniques are providing significant insights into pathogenesis (disease development), showing strong potential for disease control and prevention programs, as well as for treatments of diseases (e.g., DNA vaccines). The increased sensitivity and specificity conferred by nucelic acid (DNA or RNA) based probes has provided significant inroads for early detection of diseases and identification of sub-clinical carriers of infections. This has had a direct effect on enhancing preventative management and control of disease in cultured species. Concomitant with this has been a decrease in the need for reactive treatments using traditional methodologies such as antibiotics, or culling and disinfection. This has been particularly successful for shrimp broodstock selection and breaking the infection cycle perpetuated for years by accidental broodstock transmission of viral pathogens to developing offspring.

In shrimp aquaculture, commercially available molecular probes have been developed for IHHNV and type-A baculovirus (Durand *et al.,* 1996), whereas commercial probes for other viral pathogens, such as white spot, SEMBV, MBV, TSV, HPV, YHV are still under development. As noted above, nucleic acid probes are extremely sensitive and can detect microbial infections before they progress to produce clinical signs. In addition, such probes can be designed to be highly specific, thereby allowing more accurate identification of pathogens than was possible with many non-molecular techniques (Walker and Subasinghe, 1999). This clearly helps with differentiation between significant and closely related infectious agents, which in turn helps focus disease management intervention and reduce control costs. Increased efficiency in detecting

early stages of pathogen development also reduces reliance on prophylactic and active use of antibiotics to control disease under culture conditions.

In vitro tissue culture for detection and isolation of pathogenic viruses and intracellular bacteria are currently available for many fish species (FAO and NACA, 2001; OIE, 2000; Groff and La Patra, 2000; Chi *et al.*, 1999), although these still require specialised maintenance and quality assurance to ensure optimum application to fish health needs (Lorenzen *et al.*, 1999; Ariel and Olesen, 2001). No self-replicating cell-lines currently exist for aquatic invertebrates. Considerable research has gone into the development and maintenance of crustacean cell cultures but success has, so far, been marginal (Shimizu *et al.*, 2001; Wang *et al.*, 2000; Walton and Smith, 1999; Ghosh *et al.*, 1995; Toullec, 1995). Many researchers have managed to develop primary cell cultures, but most have failed to subculture or maintain them (Le Groumellec *et al.*, 1995). The situation is similar for molluscan cell-lines (Buchanan *et al.*, 1999, 2001; Cheng *et al.*, 2001; LaPeyre and Li, 2000). Further research efforts to develop and maintain crustacean and molluscan cell cultures is required, in order to provide equitable culture options for study of intracellular infectious agents to those possible for many finfish pathogens.

Transboundary movements of aquatic animals have in some cases lead to the spread of aquatic animal diseases. Reliable and sensitive diagnostic techniques and standards are required to ensure such movements of live aquatic animals does not also include the dispersion of their pathogens. Once DNA probes are field validated and refined for non-specialist use, these will be particularly valuable tools for this purpose (FAO 2000). For example, once appropriate DNA probes are developed for specific shrimp pathogens, live and processed shrimp could be certified to be free of specific pathogens, thus promoting confidence in the shrimp culture industry, and facilitating access to wider international markets.

Besides screening for pathogens, biotechnological methods can be used to ascertaining other health parameters, including haematocrits, leucocrits, blood cell differentials, neutrophil oxidative radical production, myeloperoxidase activity and phagocytic functions. Such techniques can be applied to quantitative protein, immunoglobulin, lysozyme, cortisol and ceruloplasmin analysis from plasma samples. Methods such as agglutination tests to assay antibody after immunisation can now be supplemented with immunoassays, such as fluorescent antibody test (FAT) and enzyme-linked immunosorbent assay (ELISA) (e.g., Bachère *et al.*, 1995; Noel *et al.*, 1996; Austin 1998; Mishra, 1998; Crawford *et al.*, 1999; Romalde, 1999; Pernas *et al.*, 2000; Meloni and Scapigliati, 2000; Munoz *et al.*, 2000; Nadala and Loh, 2000: Shelby *et al.*, 2001). Also leucocyte samples from fish blood or haematopoietic organs can be assayed by haemolytic plaque assay or an enzyme labelled tag (ELISPOT) to determine levels of antibody (plaque-forming cells). ELISPOT can be used to accurately quantify numbers of immunoglobulin or non-specific antibody-secreting cells (Anderson, 1995) and are used in immunodiagnosis.

One of the most urgent needs for aquaculture health management is establishment of standards for quantitative assessment of health status in the broad range of species under culture. Progress in this regard is being made for certain finfish, however, knowledge of shrimp and molluscan health (and stress) are still relatively undeveloped. The above mentioned techniques could be used to develop simple and rapid predictive health tests for use under field conditions by field technicians, veterinarians and the farmers themselves. Bearing in mind the extensive literature applied to physiological indices of aquatic animals (especially molluscs) as indicators of environmental quality (Handy and Depledge, 1999), such tests could provide an invaluable early warning of stress under hatchery production conditions, where disease losses are usually acute and catastrophic (e.g., Weirich and Reigh, 2001).

Harnessing the host's specific and non-specific defence mechanisms in an effort to control aquatic animal diseases has considerable potential for reducing the impact and losses from diseases. Immunostimulants and non-specific immune-enhancers are being incorporated into diets to boost protection. Such methods, however, are still very limited, especially for shrimp, however,

the large number of commercial immunostimulants available on the market clearly reflects the interest in this area as an alternative method to enhance survival from disease challenges. To date, however, results from biological trials of these commercial products have been highly variable, thus, further research is required to determine the precise mechanisms of their action and assess their cost-benefit value (Flegel, 1996; Subasinghe *et al.,* 1998).

Probiotics are generally administered as live microbial feed supplements which affect the host animal by improving the intestinal microbial balance to optimise the presence of non-toxic species. A stable gut microflora helps the host resist pathogenic invasions, particularly via the gastro-intestinal tract. Antibiotics reduce specific or broad-spectrum gut microflora and probiotics may have post-antibiotic treatment potential for restoring the microbial balance. Probiotics are widely used in animal husbandry but their use in aquaculture is still relatively new. However, there are increasing reports of potential probiotics for shrimp aquaculture, which has been plagued by opportunistic bacteria, such as the luminescent *Vibrio harveyi,* and, in some cases, probiotics have been reported to significantly reduce antibiotic use in shrimp hatcheries. Suppression of proliferation of certain pathogenic bacteria (e.g., *Vibrio* spp.) in shrimp hatcheries has been achieved by introducing (inoculating) non-pathogenic strains or species of bacteria, that compete for microbial metabolite resources. This procedure shows promise to be effective and economical, however, further refinement of administration and concentration loads required for effective pathogen suppression is required. Effective and economically viable probiotics also require greater research into optimal strains of probiotic micro-organisms and stringent evaluation under field conditions of their economic feasibility.

Beside producing aquatic organisms for food, aquaculture has other important purposes which help human well-being. Aquatic organisms are often adapted to extreme environments, and can, therefore, provide unique models for research on biological and physiological processes. Furthermore, studies of the developmental, cellular, and molecular aspects of aquatic organisms could provide insights into the basis of disease mechanisms and pathogenesis in humans (Wright *et al.,* 2000).

Feed Technologies

Currently, one of the most heated debates concerning aquaculture development is the use of fishmeal and other animal proteins in aquafeeds (Naylor *et al.,* 2000; Forster and Hardy, 2001). Although fishmeal is used for its high quality protein content, it has several disadvantages, including high cost and instability of supply. Wild fish catches are on the decline and there are increasing environmental concerns (eutrophication, pollution associated with excess nutrient waste), ethical concerns over feeding fish to non-piscivorous fish, and social concerns over using aquatic protein to feed fish that could be used for human nutrition (especially in nutritionally-deficient areas of the world) . Although, the major users of fishmeal is terrestrial agriculture, and the salmon, bream, bass and shrimp farming sectors are using species that would not normally be used for human consumption, the concerns of consumers provide a strong impetus to find ways to replace fish meal with vegetable protein from more sustainable sources. Biotechnology offers opportunities for development of alternatives to fishmeal, especially plant-based protein sources, by enhancing production and processing techniques. Other technologies also offer potential for enhancing the efficacy of feed delivery.

Plant protein has significant potential for addressing the problem of phosphorus pollution, since plants do not contain the high levels of phosphorus found in animal protein. The use of plant protein in aquafeeds also helps reduce pressure on wild fish stocks. Research in this area is focusing on the investigation of various plant species and plant-animal protein mixes, as new sources for protein for aquafeeds for shrimp (Mendoza *et al.,* 2001); molluscs (Shipton and Britz, 2000) and finfish (Ogunji anf Wirth, 2001). In addition, brewers yeast is another protein source under investigation for finfish (Oliva-Teles and Goncalves, 2001), along with plant lipid

substitutes for fish oils (Ng et al. 2000). One of the difficulties in using plant proteins in aquafeeds is the need for proper processing to destroy *anti-nutritional compounds* which may harm the fish once fed. Researchers are looking at the possibilities of dealing with *anti-nutritional factors* by producing feed *enzymes* to counteract them. *Phytase* is one example. This enzyme helps fish make optimal use of the phosphorous available in plant-protein based feeds (Papatryphon and Soares, 2001; Vielma *et al.,* 2000; Van Weerd *et al.,* 1999; Papatryphon *et al.,* 1999; Storebakken *et al.,* 1998).

Dependable availability of quality fry to stock grow-out production systems has been one of the most critical factors affecting commercial success of fish and shellfish production (Sorgeloos, 1995). Although nutritional and dietary requirements of most fish and shellfish species have been identified, large-scale hatchery production of most aquatic species still depends on live feeds, such as selected species of microalgae, the rotifer *Brachionus* and the brine shrimp *Artemia*.

More than 15 species of diatoms and green algae are used for first-feeding of hatchery produced fish fry and shrimp larvae. Selection of these species has been done mainly by trial and error, rather than on a nutritional scientific basis. The live feed production systems used in most developing countries are still labour intensive. This lowers cost efficiency and poses many problems for consistent mass production, including optimal nutritional quality and prevention of microbial contamination. These problems have created a whole new area of biotechnological research aimed at finding cost effective and efficient supplements to live microalgae, commercial production of freeze-dried algae, microencapsulated diets, and manipulated yeasts. Results of much of this work have shown significant success (Garcia-Ortega *et al.,* 2001; Oliva-Teles and Goncalves, 2001). This area requires further research and shows considerable potential for reducing reliance on live microplankton in fish and shrimp hatchery production.

Artemia nauplii are the most widely used live feed in shrimp aquaculture (Sorgeloos and Leger, 1992). Considerable progress has been made in improving the dietary value of this planktonic crustacean through selection of traits and batches, efficient cyst disinfection and decapsulation (Garcia-Ortega et al. 2001), nauplius hatching, and enrichment and cold storage (Sorgeloos, 1995). Improvement of the nutritional quality of *Artemia* through bioencapsulation (enrichment), especially with highly unsaturated fatty acids and vitamins, has improved larviculture outputs in terms of quality, survival, growth, and stress resistance (Merchie *et al.,* 1995). Bioencapsulation has also been applied for oral delivery of vaccines, vitamins, and chemotherapeutants (Lavens *et al.,* 1995; Robles *et al.,* 1998), notably for hatchery developmental stages of finfish (Majack *et al.,* 2000; Touraki *et al.,* 1996, 1999) and shrimp ((Uma *et al.,* 1999). Research into bioencapsulation and use of live feed as a means of oral delivery for compounds to enhance survival and fitness of larval stages of aquatic organisms merits further research priority.

Future aquaculture development ultimately depends on the ability of farmers and processors to produce a product acceptable to consumers. Increasing consumer demands for quality and safe products have to be recognised and addressed. Biotechnology also shows promise in this area, especially for assessing and improving safety, freshness, colour, flavour, texture, taste, nutritional characteristics, and shelf-life of cultured food products. Tools are already under development, or commercially available, that can detect and assay toxins, contaminants, and residues in aquatic products (Jellet *et al.,* 1999; Quilliam, 1999; Marr *et al.,* 1992, 1994; Pleasance et al. 1992).

Biotechnology tools can also be used to identify and characterize important aquatic germplasm resources, including those of endangered species. The genetic make-up of aquatic species can now be analysed, characterised and quantitative trait loci identified that code for phenotypic characters that are beneficial for culture (e.g., fast growth, disease resistance and cold tolerance). The study of biotechnology can also improve understanding of gene regulation and expression, sex determination and definition of species, stocks, and populations (Alcivar-Warren, 2001; Agresti *et al.,* 2000; Davis and Hetzel, 2000; Ward *et al.,* 2000; Moore *et al.,* 1999; Sakamoto *et al.,* 1999; Liu *et al.,* 1999Cross *et al.,* 1998; Poompuang and Hallerman, 1997). This can be achieved

by marker-assisted gene selection techniques, transgenic manipulations and improved cryopreservation of gametes and embryos.

Progress in this arena will require sophisticated molecular biology technologies to be adapted to aquatic organisms, in order to enhance understanding of their biological processes. For example, approaches for gene transfer into eggs have been developed for many terrestrial organisms and many freshwater species, but not for most marine species. This technology is needed for analyses of gene regulatory systems and gene expression. In addition, methods need to be developed for culturing tissues from marine organisms. Cultured cell lines will provide opportunities for gene transfer and gene expression studies and enhance the usefulness of marine species as biomedical research models.

Bioremediation is another promising biotechnological approach for degradation of hazardous waste to environmentally safe levels using aquatic microorganisms, or other filtering macro-organisms (Srinivasa Rao and Sudha, 1996). Although this procedure has been used in various situations, such as sewage treatment, application to shrimp and other aquaculture wastes is fairly novel. There are a lot of commercial products on the market, mainly bacterial preparations, but the mode of action and efficacy of many of these have yet to be scientifically measured. In addition to microbes, bivalves, seaweeds, holothurians (sea cucumbers), etc., have been tested to assess their ability to reduce organic loading, or reduce excess nutrients produced during culture production. Various bioremediation preparations have also been developed with the view to remove nitrogenous and other organic waste in water and bottom sludge, to reduce chemically-induced physiological stress, e.g., in pond-reared shrimp. More products will undoubtedly emerge with continued research in this field, however, controlled field trials are urgently needed to determine the cost-benefit and effectiveness of these products under culture conditions.

Concomitant with bioremediation is enhanced feed delivery. Aquaculture development in recent years has, therefore, included investigation into methods for more efficient feeding. Underwater closed circuit television is in use to record when fish are satiated (no longer feeding), so feeding can be halted, and also to monitor the accumulation of wastes under moored cages. More recently, research organisations, such as IFREMER[1], have been looking into the use of demand feeders, where the fish trigger feeding by learning to push a lever. This method has shown some success and may have potential for many farmed fish species. IFREMER reported a notable variation in feed demand by European seabass on a daily and monthly scale (IFREMER, 2000). Training fish to trigger feeding when hungry offers strong potential to lower feed costs, raise conversion efficiency and reduce wastage and pollution. IFREMER are also looking to develop faecal stabilisers for species such as turbot and seabass that have rather liquid waste. Feed additives that would stabilise the faecal matter would benefit surrounding water quality in sea-cage rearing situations.

Holding Systems

A notable technology-based development in the US freshwater farming sector has been a significant expansion tilapia production using indoor closed-recirculation systems. This American production, however, is still dwarfed by imports from countries such as China, Costa Rica, Ecuador and Honduras, where production can be achieved using less capital investment. Although this makes long-term sustainablility of tilapia farming in the US uncertain, there is ongoing interest in diversifying to other species, such as carp, bass and perch that can take advantage of lower ambient temperatures.

Recent technological advances in the salmon farming have been particularly in sea-cage design. In the past, the industry had typically used steel-framed rectangular support structures for the net cages, with walkways around them as work platforms. With the exception of the pond rearing of some marine species practised in Asia, this general cage design has also typified the

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commercial culture methods used for most other sea fish, including Asian grouper and snapper, and the Mediterranean seabass and bream. In recent years, however, there has been a move in the salmon farming industry towards the use of circular cages with plastic support structures and incorporating no walkway. Instead the cages are dependent on boats for maintenance. The feeding of the fish, instead of being carried out by hand or by cannon/blower, is by automatic cage-mounted machines with a capacity of up to 100 mt of feed. Visits by farm staff can thus be reduced, lowering costs. As salmon prices have slipped lower, these technology shifts, and a move towards amalgamation of companies, are allowing the industry to cut operating costs and retain profitability. The change towards boat-maintained circular cages with plastic support structures has not yet been seen in Europe's seabream or seabass industry but it may be a trend that will develop in these sectors in coming years.

If the commercial aquaculture of marine finfish is to continue expanding, it will likely take place in more offshore locations than have traditionally been used. Atlantic salmon have been farmed almost exclusively in sheltered nearshore waters, but this has been linked to production, environmental and aesthetic problems. At offshore sites, removal/dilution of wastes is facilitated by greater water exchange and volumes. In addition offshore sites offer greater salinity stability. Cages developed specifically for offshore culture, such as the Ocean Spar® rectangular Sea Cage design and the innovative double cone SeaStation™ Sea Cage, have been put into commercial use in recent years. The Oceanic Institute of the US, in Hawaii, has developed a similar bi-conical offshore cage design to that of Ocean Spar®, named the SeaStation 3000™ (Oceanic Institute 2001).

The double cone shaped net is suspended on a central floating vertical support column and can be permanently submerged, with feed administered through a pipe from the surface. Access is via zippered doors underwater and daily net cleaning is carried out by divers. In times of severe storms, the structures can be sunk below the high-energy surface waves. An Oceanic Institute cage 24m in diameter and 15m deep, moored 10m down and 3 km offshore in 30m of water, has been used to grow batches of 70,000 Pacific threadfin or 'moi' (*Polydactylus sexfilis*) fingerlings to 3-400g size in 4-5 months. In the USA, a recently formulated national aquaculture policy has specifically identified open ocean aquaculture as one of two main areas for research and development. The second being closed system (or "urban") aquaculture, including research on recirculating technologies for inland facilities (NOAA, 2001).

Technology from the oil industry has supplied some of the background for the design of such offshore cages. Another cross-over with the oil industry has been interest in conversion of disused oil platforms for use as offshore fish farms. The high cost of decommissioning oil drilling and pumping platforms at the end of their service life makes this an interesting proposition, though to date, the costs and problems of conversion have proven to be formidable obstacles (Bugrov *et al.*, 1994; Osborn and Culbertson, 1998). In the USA, recently formulated national aquaculture policy has specifically identified open ocean aquaculture as one of two main areas for research and development. (The second being closed system (or "urban") aquaculture, including research on recirculating technologies for inland facilities (NOAA, 2001)). This emphasises the likely future direction of aquaculture.

Another recent development in holding technology has been in closed-circulation systems. These systems have shown great potential for reducing fishmeal consumption compared with open-field farming. Although experiments rearing shrimp without water exchange date back to the 1970's in Tahiti, and to the 1980s in Hawaii and South Carolina, USA, pilot projects have not moved to commercial realisation. A commercial shrimp farming project in Belize in 1998 - initially aiming to isolate the farm from the danger of disease introduction - took the technology to a new level by keeping particulate matter aerobic and in suspension in the growout pond. This facilitated nitrification of waste products (essential to a healthy rearing environment) by the bacteria in the pond. As long as the system is aerated, pond conditions can be kept suitable for shrimp to thrive and the flocculent bacteria and organic matter that form in the water contribute

directly to the food of the shrimp. As a result, protein and fishmeal contents of the feed can be considerably reduced. Closed systems of this kind can also be housed inside buildings and there are currently numerous projects at early stages of testing in the Americas and Asia to develop this technology further.

The higher dissolved oxygen requirement of many finfish culture species makes it more difficult to use similar systems to reduce protein demand in fish feeds but the production of catfish in closed ponds is one example where closed systems can maximise the feed use and reduce the need for outside protein inputs (Boyd and Tucker, 1995; Tucker *et al.,* 1996).

Aquaculture Related Enhancement Technology

Sea ranching, where juveniles are produced in hatcheries and then released to the sea to grow, dates back over one hundred years. There have been some notable successes, for instance with the Japanese flounder (*Paralichthys olivaceus*), but there have also been failures where factors affecting recruitment and loss from the fishery were not properly understood (Howell *et al.,* 1999). As understanding of the factors affecting the success of ranching programmes has improved, interest in this technique has spread to new areas and targeted at new species. Countries such as Norway, USA, Australia and China have all launched stock enhancement projects. on a variety of species. To promote a more effective exchange of information, the First International Symposium on Stock Enhancement and Sea Ranching was arranged in Norway in 1997 (Howell *et al.,* 1999) and a second symposium is to be held in Kobe, Japan in January 2002. Sea-ranching can be one useful approach to increasing overall landings, provided habitat is adequate and that fishing is prudently regulated (Welcomme and Bartley, 1998)

Pre-Market Conditioning

An interesting sector which has opened up in recent years is the temporary holding of bluefin tuna (*Thunnus thunnus*) to improve meat quality. The early development of this activity began in Australia with the southern bluefin tuna (*Thunnus maccoyii*)*,* in response to falling catches from the South Australian wild fishery. Australian landings of this migratory species reached a peak of 21,500 mt in 1982 but increasingly lower quotas had to be introduced, dropping to 5,265 mt by 1989. The quality of the product being landed was poor which diminished export value, thus, fishing/farming companies began holding 2-4 year old fish in cages for 3-5 months for conditioning. This enhanced meat quality and enabled them to sell to high-value sushi markets in Japan at prices of around US$18 per kg, or up to US$620 per fish. By 1997, tuna 'fattening' had become Australia's most valuable single aquaculture sector (Brown *et al.,* 1997).

Similar techniques were adapted by fishermen in the Mediterranean (Malta, Croatia and Turkey) over the last few years, holding Atlantic bluefin tuna captured during the limited fishing season (May-July). The fish are on a spawning migration at this time, thus flesh quality is poor, and meat prices are depressed. The fish are held in floating cages until November or December and fed on mackerel and herring. By then end of the holding period the fish improve in condition and meet high market price quality for export to Japan. The cages used to hold and transport the fish are large structures of up to 100 m in circumference and it can take a week or more to tow them as much as 300 km from the fishing grounds to the holding area.

The capture of bluefin tuna from the declining, and possibly threatened, stocks in the Atlantic has caused some controversy, thus, there interest in further development of this technology for true farming – and reduce reliance on wild captured stocks. In addition, there is controversy over the amount of fish required to feed the tuna during the 'fattening' process, especially since these are species also used for human consumption in the Australian and Mediterranean (cf discussion under "Feed Technologies" above). This presents a significant challenge, however for this highly piscivorous migratory species.

Conclusion

Aquaculture biotechnology and other technological innovations are showing a positive impact on aquaculture diversification success, investment potential, and international technology exchange. The development of biotechnology in aquaculture should provide a means of producing healthy and fast growing animals, through environmentally friendly means. However, this development will largely depends on the desire and willingness of the producers to work hand-in-hand with scientists and the international donor community to assist developing counties in related research, capacity building and infrastructure development. Improved exchange of information and discussion between scientists, researchers, and producers from different regions on their problems and achievements will undoubtedly help this important sector to further develop with the view to increasing sustainable aquatic animal production globally.

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