SECTION 1- INTRODUCTION

I. INTRODUCTION

I.1 Background

The FAO Regional Technical Cooperation Programme (TCP) Project ***"Assistance for Responsible Movement of Live Aquatic Animals"*** (TCP/RAS/6714-A and 9605-A), was implemented in January 1998 by NACA , in cooperation with the OIE[1] , regional and international agencies (*e.g.* AAHRI[2] , AusAID/APEC[3] , AFFA[4] , and others), representatives (designated National Coordinators and focal points for disease reporting) of 21 governments/territories in the Asia-Pacific region (Australia, Bangladesh, Cambodia, China PR, Hong Kong SAR China, India, Indonesia, Iran, Japan, Korea (DPR), Korea (RO), Lao PDR, Malaysia, Myanmar, Nepal, Pakistan, Philippines, Singapore, Sri Lanka, Thailand and Vietnam) and many regional and international aquatic animal disease experts. The over-all objective of the program was to provide guidance to countries in undertaking responsible movement (introductions and transfers) of live aquatic animals through appropriate strategies that minimize potential health risks associated with live aquatic animal movements. The program took into account the need for concordance with existing international agreements/treaties (*e.g.* WTO's SPS Agreement and OIE health standards) along with the need for the strategies to be practically applicable to the Asia region and in support for FAO's Code of Conduct for Responsible Fisheries (CCRF). This TCP became the focal point for the development of a strong, multidisciplinary Asia-Pacific Regional Programme on Aquatic Animal Health Management which is a major element of NACA's Five Year Work Programme (2001-2005). The ***"Asia Regional Technical Guidelines on Health Management for the Responsible Movement of Live Aquatic Animals and the Beijing Consensus and Implementation Strategy (TGBCIS)"*** or ***'Technical Guidelines'*** (FAO/NACA 2000) and the corresponding ***"Manual of Procedures (MOP)"*** (FAO/NACA 2001) were developed over a period of three years (from 1998-2001) of awareness and consensus building in consultation (through various national level and regional workshops, FAO/NACA/OIE 1998) with government representatives, representatives of collaborating organizations and aquatic animal health experts. The *'Technical Guidelines'* was finally adopted in principle during a Final Workshop of the TCP held in Beijing, China PR in June 2000 (FAO/NACA 2000). The **Asia Diagnostic Guide to Aquatic Animal Diseases** or ***'Asia Diagnostic Guide'*** is a third of a series of documents produced under the TCP that will support the implementation of the ***'Technical Guidelines'*** particularly with respect to the component on disease diagnosis, surveillance and reporting.

The ***'Asia Diagnostic Guide'*** is a comprehensive diagnostic manual for the pathogens and diseases listed in the NACA/FAO/OIE Quarterly Aquatic Animal Disease Reporting System[5] . It was developed from technical contributions from members of the Regional Working Group (RWG) and Technical Support Services (TSS) of the TCP and other aquatic animal health scientists in the Asia-Pacific region and outside who supported the regional programme.

Many useful aquatic animal health diagnostic guides and manuals and others in CD-ROM format already exist in the literature. Some are in

[1] Office International des Epizooties
[2] Aquatic Animal Health Research Institute of the Thai Department of Fisheries
[3] Australian Agency for International Development/Asia-Pacific Economic Cooperation
[4] Agriculture, Fisheries and Forestry of Australia
[5] The quarterly reporting system was developed as one of the four major components of the TCP, developed based on the OIE International Aquatic Animal Health Code – 1997, in cooperation with the OIE Regional Representation for Asia and the Pacific.

the language of individual countries. In the Asia-Pacific region, more recent ones include Indonesia's Manual for Fish Disease Diagnosis - II (Koesharyani *et al.* 2001, GRIM[6]/JICA[7]); the Philippines' Diseases of Penaeid Shrimps in the Philippines (Lavilla-Pitogo *et al.* 2000, SEAFDEC-AQD[8]); Thailand's (a) Diagnostic Procedures for Finfish Diseases (Tonguthai *et al.* 1999, AAHRI), (b) Health Management in Shrimp Ponds, Third Edition (Chanratchakool *et al.* 1998, AAHRI), and (c) Epizootic Ulcerative Syndrome (EUS) Technical Handbook (Lilley *et al.* 1998, ACIAR[9]/DFID[10]/AAHRI/NSW[11]-Fisheries/NACA); Australia's Australian Aquatic Animal Disease – Identification Field Guide (Herfort and Rawlin 1999, AFFA); and a CD-ROM on Diagnosis of Shrimp Diseases (Alday de Graindorge and Flegel 1999). Some more are listed and appear as an Annex in the different sections of the *Asia Diagnostic Guide.*

The ***'Asia Diagnostic Guide'*** supplements these existing manuals/guides and provides relevant information on diseases in the ***NACA/FAO and OIE Asia-Pacific Quarterly Aquatic Animal Disease Reporting System,*** which commenced during 3rd quarter of 1998 (NACA/FAO 1999, OIE 1999). The information in the *Asia Diagnostic Guide* is presented in a format that spans from gross observations at the pond or farm site (Level 1), to guidance for information on technologically advanced molecular or ultrastructural diagnostics and laboratory analyses (Levels II and III, and OIE 2000a, b), thus, taking into account international, regional, and national variations in disease concerns, as well as varying levels of diagnostic capability between countries of the Asia-Pacific region.

I.2 Objectives and Scope

The objective of the ***"Asia Diagnostic Guide"*** is to produce a manual/guide of specific use for both farm and laboratory level aquatic animal disease diagnostics in the Asia region that complements the *'Manual of Procedures'* and that which will serve as a supplement to the implementation of the *'Technical Guidelines'.* The *Asia Diagnostic Guide* is aimed at providing a tool that can be used to expand national and regional aquatic animal diagnostic capacities and the infrastructure required to meet the OIE aquatic animal health standards (OIE 2000a, b). This guide aims to improve aquatic animal health awareness as well as provide knowledge on how to access the diagnostic resources required to help prevent or control disease impacts.

The ***Asia Diagnostic Guide*** focuses on the NACA/FAO and OIE listed diseases, but also includes some which are significant in parts of the Asia-Pacific region.

I.3 Guide for Users

The ***Asia Diagnostic Guide*** is divided into four sections: **Section 1** on Introduction, Background, Scope and Purpose, Guide for Users, Health and Aquatic Animals, Role of Diagnostics and Levels of Diagnostics; **Sections 2 to 4**, divided into host groups, *i.e.* **Finfish Diseases (Section 2)**, **Molluscan Diseases (Section 3)** and **Crustacean Diseases (Section 4)**, each commences with a chapter on *"General Techniques"* which covers the essential "starting points" that will enable prompt and effective response(s) to disease situations in aquatic animal production. This chapter is not disease-specific, providing information applicable to a wide range of both infectious and noninfectious disease situations. It emphasizes the importance of gross observations (Level 1), and how and when they should be made. It also describes environmental parameters worth recording, general procedures for sampling and fixation and the importance of record-keeping. Each **General Techniques** section is divided as follows:

Gross Observations
- *Behaviour*
- *Surface Observations*

Environmental Parameters

General Procedures
- *Pre-collection Preparation*
- *Background Information*
- *Sample Collection for Health Screening*
- *Sample Collection for Disease Diagnosis*
- *Live Specimen Collection and Shipping*
- *Dead or Tissue Specimen Collection and Shipping*
- *Preservation of Tissues*
- *Shipping Preserved Specimens*

[6] Gondol Research Institute for Mariculture of the Central Research Institute for Sea Exploration and Fisheries, Indonesia's Department of Marine Affairs and Fisheries
[7] Japan International Cooperation Agency
[8] Aquaculture Department of the Southeast Asian Fisheries Development Center
[9] Australian Centre for International Agricultural Research
[10] Department for International Development of the United Kingdom
[11] New South Wales (Australia)

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Table I.2.1. NACA/FAO and OIE listed diseases and other diseases[12] covered in the *Asia Diagnostic Guide.*

DISEASES PREVALENT IN SOME PARTS OF THE REGION
Finfish diseases
1. Epizootic haematopoietic necrosis* (EHN)
2. Infectious haematopoietic necrosis* (IHN)
3. *Oncorhynchus masou* virus disease* (OMVD)
4. Infectious pancreatic necrosis (IPN)
5. Viral encephalopathy and retinopathy (VER)
6. Epizootic ulcerative syndrome (EUS)
7. Bacterial kidney disease (BKD)
Mollusc diseases
1. Bonamiosis * (*Bonamia* sp., *B. ostreae*)
2. Marteiliosis * (*Marteilia refringens, M. sydneyi*)
3. Microcytosis * (*Mikrocytos mackini, M. roughleyi*)
4. Perkinsosis * (*Perkinsus marinus, P. olseni*)
Crustacean diseases
1. Yellowhead disease (YHD)
2. Infectious hypodermal and haematopoietic necrosis (IHHN)
3. White spot disease (WSD)
4. Baculoviral midgut gland necrosis (BMN)
5. Gill associated virus (GAV)
6. Spawner mortality syndrome ('Midcrop mortality syndrome') (SMVD)
DISEASES PRESUMED EXOTIC TO THE REGION, BUT REPORTABLE TO THE OIE
Finfish diseases
1. Spring viraemia of carp* (SVC)
2. Viral haemorrhagic septicaemia* (VHS)
Mollusc diseases
1. Haplosporidiosis* (*Haplosporidium costale, H. nelsoni*)
OTHER DISEASES WITHIN THE REGION, NOT CURRENTLY LISTED
Finfish diseases
1. Lymphocystis
Mollusc diseases
1. Marteilioidosis (*Marteilioides chungmuensis, M. branchialis*)
2. Iridovirosis (Oyster velar virus disease)
Crustacean diseases (the following diseases are so far presumed, but not proven, to be exotic to this region)
1. Taura Syndrome (TS)
2. Nuclear Polyhedrosis Baculovirosis (*Baculovirus penaei*)
3. Necrotising hepatopancreatitis
4. Crayfish plague

*OIE Notifiable Diseases (OIE 1997)

[12] The diseases listed in the Asia-Pacific Quarterly Aquatic Animal Disease Reporting System were agreed through a process of consultation with the National Coordinators, members of the Regional Working Group (RWG) and Technical Support Services (TSS), FAO, NACA and OIE based on the OIE International Aquatic Animal Health Code – 1997, including some diseases which are deemed important to the Asia-Pacific region.

Record-keeping
Gross Observations
Environmental Observations
Stocking Records
References

The "General Techniques" section is followed, under each host group, by a number of chapters aimed at specific diseases (*e.g.* viral, bacterial, fungal) listed on the current NACA/FAO and OIE quarterly reporting list (Table 1.2.1). These are recognised of being of regional importance, as well as of international trade significance. Those diseases listed as "Notifiable" or "Other Significant Diseases" by the OIE are cross-referenced to the most up-to-date version of the OIE *Diagnostic Manual for Aquatic Animal Diseases* (OIE 2000b, also available at http://www.oie.int). The diagnostic techniques described in the *Asia Diagnostic Guide* are consistent with those recommended by OIE. Since it is recognised that disease diagnostics is a dynamic field, it is highly recommended that **anyone using this manual for the purpose of health certification for international transfers of live aquatic organisms refer to the OIE diagnostic manual prior to performing diagnostics (screening) for this purpose**. In addition, other diseases/infectious agents *not* presently included on the Regional Quarterly Reporting List are included in the *Asia Diagnostic Guide*, since they are of interest to the region and infect commercially significant species.

Each chapter on specific diseases is presented with information on the following:

- **Causative Agent(s)**- an introductory paragraph on the causative agent(s) responsible for the disease.
- **Host Range** – the range of hosts that can be infected (both naturally and experimentally).
- **Geographic Distribution** - known/recorded geographic range of the disease (updated, where applicable, using the Asia-Pacific Quarterly Aquatic Animal Disease Reports for 1999-2000 (OIE, NACA/FAO quarterly reports).
- **Clinical Aspects** - the effects of the disease are described, ranging from gross observations and behavioural changes, lesions and other external clues, to gross and microscopic internal pathology.
- **Screening Methods** – are the examination methods applied to check healthy appearing aquatic animals to determine whether or not they are infected by a potentially significant infectious agent.
- **Diagnostic Methods** – are the examination procedures used to try and determine the cause of a disease or clinical infection.

  Screening and diagnostic methods are divided into two types:

  Presumptive Diagnosis – preliminary diagnosis based on gross observations and circumstantial evidence. Where more than one infectious agent may be responsible, confirmatory diagnosis (usually laboratory Level II and/or III) may be required; and

  Confirmatory Diagnosis – positive identification of the causative agent, with a high degree of diagnostic confidence.
- **Modes of Transmission** - deals with the known modes of transmission (spread) of the disease and the factors associated with its spread (environmental, handling, life-history stage, reservoirs of infection, *etc.*). This area of diagnostics is known as epidemiology (or epizootiology) and available observations from this field of study are also included.
- **Control Measures** – describes any control measures which are known to work, should the disease appear.
- **References** – recent relevant publications about the disease.

The chapters for each host group also include **three Annexes** providing information of the (a) list of OIE Reference Laboratories, (b) a list of regional disease experts who can provide information and valuable health advise, and (c) useful guides/manuals. A **Glossary** is also included.

I.4 Health and Aquatic Animals

Unlike other farm and harvesting situations, where the animals and plants are visible, aquatic animals require more attention in order to monitor their health. They are not readily visible, except under tank-holding conditions, and they live in a complex and dynamic environment. Likewise, feed consumption and mortalities may be equally well hidden under water. Unlike the livestock sector, aquaculture has a wide range of diversity in species cultured, farming environment, nature of containment, intensity of practice and culture system used. The range of diseases found in aquaculture is also varied, some with low or unknown host specificities and many with non-specific symptoms. Disease is now recognized as one of the most important challenges facing the aquaculture sector.

The complexity of the aquatic ecosystem makes the distinction between health, sub-optimal performance and disease obscure. Diseases in aquaculture are not caused by a single

event but are the end result of a series of linked events involving the interactions between the host (including physiological, reproductive and developmental stage conditions), the environment and the presence of a pathogen (Snieszko 1974). Under aquaculture conditions, three factors are particularly important affecting host's susceptibility: stocking density, innate susceptibility and immunity (natural/acquired). Environment includes not only the water and its components (such as oxygen, pH, temperature, toxins, wastes) but also the kind of management practices (*e.g.* handling, drug treatments, transport procedures, *etc.*). Pathogens may include viruses, bacteria, parasites and fungi; diseases may be caused by a single species or a mixture of different pathogens. The introduction of infectious diseases is another major concern in aquaculture. As in livestock, the aquaculture and fisheries sector will continue to face increasing global exposure to disease agents as it intensifies trade in live aquatic organisms and their products (Subasinghe *et al.* 2001).

The first and most important defenses against preventable disease losses under such complex situations are:

monitoring *as regularly as possible* and *appropriate* action at the *first* sign(s) of suspicious behaviour, lesions or mortalities.

These fundamental approaches – despite having been long instilled in human and agricultural production – still require reinforcement in many aquatic animal production sectors. Some farmers and harvesters still hesitate to act at the first sign of health problems, due to concern that it may reflect on their production capability, or that it will result in failure in the competitive market place. Hiding or denying health problems, however, can be as destructive to aquatic animals as it is elsewhere. It is important to recognise that disease is a challenge that everyone has to face, and having the resources that can effectively deal with it, are the primary weapons against misplaced ignorance and fear.

I.5 Role of Diagnostics in Aquatic Animal Health Management and Disease Control

Diagnostics play two significant roles in aquatic animal health management and disease control. As described above, some diagnostic techniques are used to screen healthy animals to ensure that they are not carrying infection at sub-clinical levels by specific pathogens. This is most commonly conducted on stocks or populations of aquatic animals destined for live transfer from one area or country to another. Such screening provides protection on two fronts: (a) it reduces the risk that animals are carrying few, if any, opportunistic agents which might proliferate during shipping, handling or change of environment; and (b) it reduces the risk of resistant or tolerant animals transferring a significant pathogen to a population which may be susceptible to infection. The second role of diagnostics is to ***determine the cause of unfavourable health or other abnormality*** (such as spawning failure, growth or behaviour) in order to recommend mitigating measures applicable to the particular condition. This is the most immediate, and clearly recognised, role of diagnostics in aquatic animal health.

Accurate diagnosis of a disease is often incorrectly described as complicated and costly. This may be the case for some of the more difficult to diagnose diseases or newly emerging diseases. Disease diagnosis is ***not solely a laboratory test***. A laboratory test may confirm the presence of a specific disease agent, or it may exclude its presence with a certain level of certainty. Incorrect diagnosis can lead to ineffective or inappropriate control measures (which may be even more costly). For example, a "new" disease agent may get introduced to a major aquaculture producing area, or the animals may all die in shipment/during handling. Disease diagnostics should be made as a continuum of observations starting on the farm and, in fact, commencing prior to the disease event. The different levels of disease diagnostics which can be undertaken when investigating a disease situation are discussed in the section below.

I.6 Levels of Diagnostics

The *Asia Diagnostic Guide* is built on a framework of ***"three levels" of diagnostics***, agreed upon during the Second Regional Workshop of the TCP held in Bangkok in February 1999 (see FAO/NACA 2000). Table I.6.1 below outlines the diagnostic activities at each level, who is responsible, and the equipment and training required. It should be noted that none of the levels function in isolation, but build on each other, each contributing valuable data and information for optimum diagnoses. Level 1 provides the foundation and is the basis of Levels II and III since findings using higher level(s) can only be meaningfully interpreted only in conjunction with observations and results obtained from lower levels.

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Level I (farm/production site observations, record-keeping and health management) is strongly emphasized throughout the *Asia Diagnostic Guide* as this forms the basis for triggering the other diagnostic levels (II and III). ***Level II*** includes the specialisations of parasitology, histopathology, bacteriology and mycology, which require moderate capital and training investment, and which, generally-speaking, cannot be conducted at the farm or culture site. ***Level III*** comprises the types of advanced diagnostic specialisation which requires significant capital and training investment. As the reader will note, immunology and biomolecular techniques are included in Level III, although field kits are now being developed for farm or pond-side use (Level I) as well as use in microbiology or histology laboratories (Level II). These efforts are good indication that technology transfer is now enhancing diagnostics and, with solid quality control and field validation, it is certain that more Level III technology will become field accessible in the near future (Walker and Subasinghe 2000).

One of the most important aspects of maximising the effectiveness of the three diagnostic levels is ensuring that Level I diagnosticians have access to, and know how to contact Levels II and III support (and at what cost), and *vice versa*. Level III diagnostic support is usually based on referrals, so has little contact with field growing conditions. They, therefore, need feedback to ensure any diagnosis (and actions recommended) are relevant to aquatic animal production situation being investigated.

Thus, the baseline aim for initiating diagnostic capability is Level I. Confirmatory diagnostics, or second opinion, *where required*, can be obtained by referral until such capabilities are developed locally. The period required to develop Level II and/or Level III diagnostic infrastructures, usually depends on the disease situations being faced and tackled by Level I diagnosticians in the area/country and the resources available. Where there are few problems, there is little incentive to build diagnostic capability. This is a vulnerable position, and strong links with Level II and/or III diagnostics are good precautionary measures and strongly promoted under the regional program – especially for introductions of live aquatic animals into a relatively disease-free area.

Table I.6.1 Diagnostic Levels, Associated Requirements and Responsibilities.

Level	Activity	Work requirements	Responsibility	Technical requirements to support activities
I	Observation of animal and environment Gross clinical examination	Knowledge of normal (feeding, behaviour, growth) of stock. Frequent / regular observation of stock. Regular, consistent record-keeping and assistance (Levels II, III). maintenance of records – including fundamental environmental information. Knowledge contacts for health diagnosis Ability to submit and/or preserve representative specimens for optimal diagnosis (Levels II, III).	Farm worker/manager. Fishery extension officers. On-site veterinary support. Local fishery biologists.	Field keys. Farm record keeping formats. Eqiopment lists Model clinical observation sheets. Pond/Site record sheets. Preservation/transportation guidelines for Levels II/IIIdiagnoses. Model job descriptions/skill requirements. *Asia Diagnostic Guide for Aquatic Animal Diseases*
II	Parasitology Bacteriology Mycology Histopathology	Laboratories with basic equipment and personnel trained/experienced in aquatic animal pathology. Keep and maintain accurate diagnostic and laboratory case records. Ability to preserve and storage specimens for optimal Level III diagnoses. Knowledge of/ contact with different areas of specialisation within Level II. Knowledge of who to contact for Level III diagnostic assistance.	Fish biologists/ technicians. Aquatic Veterinarians. Parasitologists/ technicians. Mycologists/ technicians Bacteriologists/ technicians. Histopathologists/ technicians.	Model laboratory record-keeping system Protocols for preservation/ transport of samples to Level III Model laboratory requirements/ equipment/ consumables lists Model job descriptions/ skill lists Access to Level II and Level III specialist expertise *Asia Diagnostic Guide for Aquatic Animal Diseases* *OIE Diagnostic Manual for Aquatic Animal Diseases* Regional General Diagnostics Manuals
III	Virology Electron microscopy Molecular biology Immunology	Highly equipped laboratory with highly specialised and trained personnel. Keep and maintain accurate diagnostic and laboratory case records. Preserve and store specimens. Maintenance of contact with people responsible for sample submission.	Virologist/ technician. Ultrastructural histopatholo-gist/ technicians. Molecular biology scientists/ technicians.	Model laboratory requirements/ equipment/ consumables lists Model job descriptions/ skill requirements Contact information for reference laboratories Protocols for preservation of samples for consultation/ validation *Asia Diagnostic Guide for Aquatic Animal Diseases* *OIE Diagnostic Manual for Aquatic Animal Diseases* General molecular and microbiology diagnostic references

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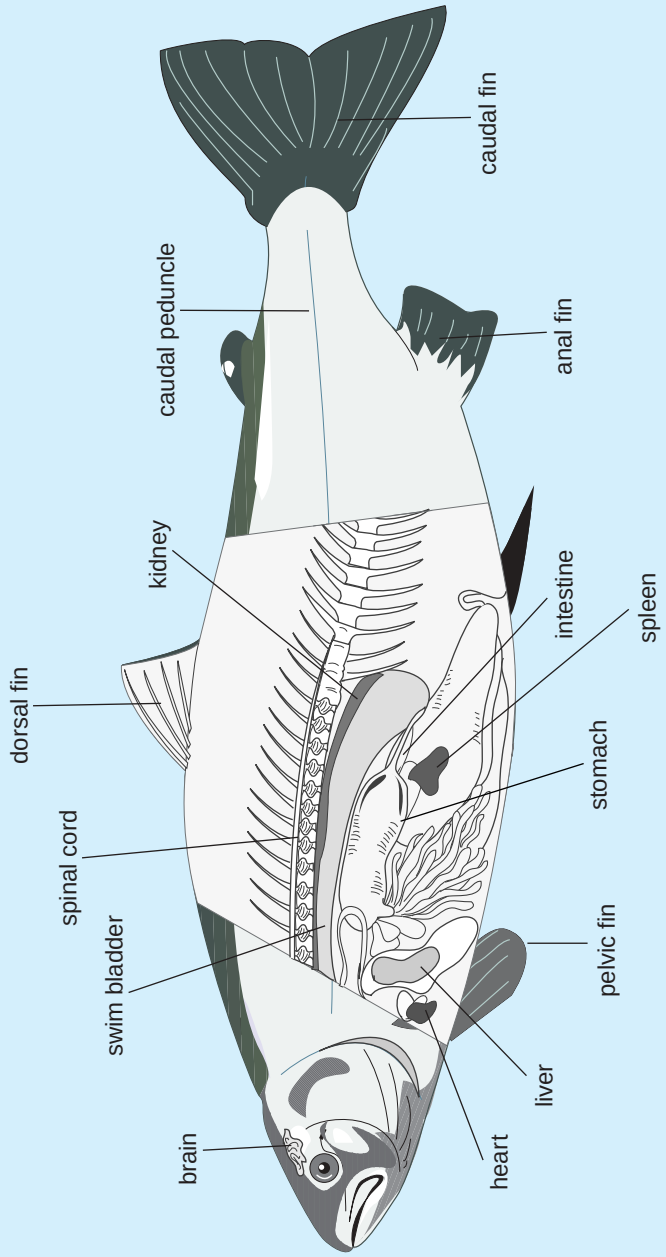
Basic Anatomy of a Typical Bony Fish



Basic anatomy of a typical bony fish.