

Procedures for the quarantine of live aquatic animals A manual





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Inset, top left: Examining fish samples. Courtesy of J. Yulin/China. Inset, bottom right: Trout farm in Bosnia and Herzegovina; FAO/R.P. Subasinghe. Centre collage, clockwise from top left: Typical severe mycotic granulomas from muscle section of fish with epizootic ulcerative syndrome; FAO/M.G. Bondad-Reantaso. Inner shell of Pinctada maxima showing Polydora-related shell damage or mud-filled blisters; FAO/M.G. Bondad-Reantaso. Necrotic gills of koi herpes virus-infected koi carp; FAO/R.P. Subasinghe. Carapace of shrimp showing distinctive white spots of white spot syndrome virus; FAO/R.P. Subasinghe.

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FAO FISHERIES TECHNICAL PAPER

502

A manual

by

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Preparation of this document

This manual was prepared as part of FAO's continuing effort to provide valuable information sources that can be used to improve aquatic animal biosecurity at the regional, national and local levels. The development of capacity to quarantine aquatic animals is in line with FAO Technical Guidelines for Responsible Fisheries No. 5, Suppl. 2. Aquaculture development. Health management for responsible movement of live aquatic animals, the second supplement of a series of technical guidelines that support the aquaculture development within the FAO's Code of Conduct for Responsible Fisheries.

This document is one of a number of publication outputs under the Aquatic Animal Biosecurity Project implemented by the Aquaculture Management and Conservation Service of the FAO Fisheries and Aquaculture Department and funded under the Strategic D1 Objective of FAO's Cooperation Agreement with Norway, one of the aims of which is to strengthen national capacities to promote an integrated biosecurity approach.

Abstract

Quarantine is an important risk management measure and a key activity that should be considered when developing national strategies for aquatic animal health management. It can also be used effectively to increase biosecurity at the farm production level.

This manual outlines the technical requirements for setting up quarantine facilities at three levels, based on the general level of risk (as determined by risk analysis) represented by the specific consignment of aquatic animals being moved: (i) the quarantine of "high risk" species (e.g. aquatic animals being moved either internationally through introductions and transfers or domestically between regions of different health status that are destined for use in aquaculture, capture fishery development or other applications where release or escape of animals or any pathogens they may be carrying into the natural environment is likely to occur; (ii) the quarantine of "lower risk" species (e.g. aquatic animals destined for the ornamental trade) to improve biosecurity for aquatic animals whose trade is an established practice; and (iii) the routine guarantine of aquatic animals at production facilities (e.g. new, domestically produced or locally captured broodstock or juveniles or animals whose movement has been contingent upon additional, more stringent, risk management measures, such as the use of Specific Pathogen Free stocks, international health certification, pre-border and/or border quarantine, etc.).

This manual should be useful to government policy-makers and responsible national and state agencies in assessing their need for quarantine capacity and in implementing aquatic animal quarantine in an effective and cost-efficient manner within the framework of national and state aquatic biosecurity programmes. It also provides useful guidance to responsible agencies, their technical staff and the private sector in setting up of effective quarantine facilities and their daily operation.

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Abbreviations and acronyms

AGND	Nutrition and Consumer Protection Division (FAO)
AGPP	Plant Protection Service (FAO)
ALOP	appropriate level of protection
ALOR	acceptable level of risk
APEC	Asia-Pacific Economic Cooperation
AQIS	Australian Quarantine and Inspection Service
ASEAN	Association of Southeast Asian Nations
ASEC	ASEAN Secretariat
BMPs	Better management practices
CA	Competent Authority
CCRF	Code of Conduct for Responsible Fisheries
CFHPR	Canadian Fish Health Protection Regulations
CuSO4	Copper sulfate
DAFF	Department of Agriculture, Fisheries and Forestry, Australia
DFO	Department of Fisheries and Oceans, Canada
EIFAC	European Inland Fisheries Advisory Commission
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FIES	Fisheries and Aquaculture Information and Statistics Service
	(FAO)
FIMA	Aquaculture Management and Conservation Service (FAO)
F1	Filial generation
GPS	Global positioning system
KMN04	Potassium permanganate
ICES	International Council for the Exploration of the Sea
LFHO	Local Fish Health Officer
MAF	Ministry of Agriculture and Fisheries, New Zealand
NACA	Network of Aquaculture Centres in Asia-Pacific
OIE	World Organisation for Animal Health
	(formerly the Office international des épizooties)
PCR	polymerase chain reaction
PEI	Prince Edward Island
pН	potential of hydrogen
PL	postlarvae
ppm	parts per million
SOPs	standard operating procedures
SPF	specific pathogen free
SPR	specific pathogen resistant

TAADstransboundary aquatic animal diseasesUVUltraviolet

1. Introduction

1.1 PURPOSE, APPROACH AND TARGET AUDIENCE

The purpose of this manual is to bring together in a concise and practical format, recommendations for the construction, security and operation of facilities for the quarantine of live aquatic animals.

Quarantine should be seen as one of a wide range of risk management options that can be applied, either alone or in combination, to reduce the risk posed by aquatic animal pathogens. The decision of whether or not to require quarantine or other biosecurity measures should be done on a case-by-case basis and determined by a risk analysis (Arthur *et al.*, 2004; Murray *et al.*, 2004; OIE, 2006a).

Government agencies responsible for establishing and implementing national strategies for aquatic animal health, particularly those in developing countries, have often had difficulty in establishing effective national biosecurity programmes

(Section 1.4). In some cases this has led to considerable investment in infrastructure, staffing and operational costs for quarantine facilities but little actual improvement in national biosecurity. To assist governments in establishing more effective biosecurity programmes, this document thus also attempts to place "quarantine", as one of a number of risk management options, within the context of national biosecurity programmes and the risk analysis process.

The technical requirements for setting up quarantine facilities are presented at three levels, based on the general level of risk (as determined by risk analysis) represented by the specific consignment of aquatic animals being moved. Thus, a country may need to adopt several sets of quarantine protocols, the standards to be applied to each proposed



Penaeid shrimp are considered as high risk species, requiring stringent quarantine procedure during trans-boundary movement.

movement of live aquatic animals being determined on a case-by-case basis using the risk analysis process and/or previous experience.¹ The three levels of

¹ Quarantine protocols have also been developed for groups of commodities having similar end uses such as ornamental finfishes (Kahn *et al.*, 1999) and live food finfishes destined for direct consumption in restaurants (ASEAN, in press); however such protocols need to incorporate sufficient stringency of risk management to address the maximum level of risk posed by an individual commodity within the group.



Routine quarantine of aquatic animals improves biosecurity of farming systems.

quarantine protocol discussed in this document are:

• quarantine of "high risk" species, e.g. aquatic animals being moved either internationally (introductions and transfers) or domestically between regions of different health status that are destined for use in aquaculture, capture fishery development or other applications where release or escape of animals or any pathogens they may be carrying into the natural environment is likely to occur;

• quarantine of "lower risk" species, e.g. aquatic animals destined for the ornamental

trade to improve biosecurity for aquatic animals whose trade is an established practice; and

 routine quarantine of aquatic animals at production facilities, e.g. new, domestically produced or locally-captured broodstock or juveniles or animals whose movement has been contingent upon additional, more stringent, risk management measures, such as the use of specific pathogen free (SPF) stocks, international health certification, pre-border and/or border quarantine, etc.).

This manual is aimed at helping government policy-makers and responsible national and state agencies in assessing their need for quarantine capacity and in implementing aquatic animal quarantine in an effective and cost efficient manner within the framework of national and state aquatic biosecurity programmes. It should also provide useful guidance to responsible agencies, their technical staff and the private sector in setting up effective quarantine facilities and their daily operation.

1.2 DEFINITIONS

Acceptable level of risk (ALOR) – a risk level judged by an importing country to be compatible with the protection of public health, aquatic animal health and terrestrial animal health within the country (modified from OIE, 2006a) (also "Appropriate Level of Protection").²

Aquatic animal import health standard - a legal document specifying the requirements to be met for the effective management of risks associated with the importation of a consignment of live aquatic animals before the consignment may be imported, moved from a biosecurity control area or quarantine facility or given a biosecurity clearance.

² Referred to simply as "acceptable risk" by OIE (2006a), for risk analysis purposes, the concept of ALOR is often more easily applied than that of "appropriate level of protection" (ALOP). ALOR is essentially the inverse of ALOP; a country having a very high ALOP would have a very low ALOR.

Aquatic animals – all life stages (including eggs and gametes) of fish, molluscs and crustaceans originating from aquaculture establishments or removed from the wild, for farming purposes, for release into the aquatic environment or for human consumption (OIE, 2006a).

Appropriate level of protection (ALOP) – the level of protection deemed appropriate by the country establishing a sanitary or phytosanitary measure to protect human, animal or plant life or health within its territory (also "Acceptable level of risk").

Better management practices (BMPs), also termed "best management practices" – management practices aimed at increasing both quantity and quality of products taking into consideration food safety, animal health, environmental and socio-economical sustainability. BMP implementation is generally voluntary. The term "better" is preferred rather than "best" because aquaculture practices are continuously improving (today's 'best' is tomorrow's 'norm'), *http://www.enaca. org/modules/tinyd11/index.php?id=*6.

Biosecurity (also termed "quarantine measures") – the sum total of a country's activities and measures taken to protect its natural aquatic resources, capture fisheries, aquaculture and biodiversity and the people who depend on them from the possible negative impacts resulting from the introduction and spread of serious transboundary aquatic animal diseases (TAADs).

Biosecurity – in general terms, is a strategic and integrated approach to analyzing and managing relevant risks to human, animal (including aquatic), plant life and health and associated risks to the environment (FAO, 2007).

Biosecurity clearance – official written notification issued by the Competent Authority stating that a consignment of aquatic animals has met any pre-border and border requirements (including any quarantine requirements) as specified in the aquatic animal import health standard and can now be released into the custody of the importer.

Chain of custody – documentation showing all persons/agencies who have had legal responsibility for assuring the conditions of importation (including quarantine) as specified by the Competent Authority in an aquatic animal import health standard for a consignment of live aquatic animals during its movement from the exporter or facility of origin until biosecurity clearance is granted by the Competent Authority and release to the importer.

Commodity – aquatic animals, aquatic animal products, biological products and pathological material (OIE, 2006a).

Competent Authority (CA) – the Veterinary Services, or other Authority of an OIE Member Country, having the responsibility and competence for ensuring or supervising the implementation of the aquatic animal health measures recommended in the World Organisation for Animal Health's (OIE) *Aquatic Animal Health Code* (modified from OIE, 2006a).

Consignment (also termed "shipment") – a group of live aquatic animals described in an aquatic animal import health standard, an international health certificate, a health certificate and/or in a permit to import or export.

Container – a transport appliance: a) of permanent type and sufficiently strong to enable repeated use; b) specially constructed to facilitate transport of live aquatic animals by one or several means of transport; c) provided with fittings that make it easy to manipulate, particularly for transshipment from one kind of transport vehicle to another; d) constructed in a watertight way, easy to load and unload and capable of being cleansed and disinfected; and e) ensuring safe and optimal transport of live aquatic animals.

Contingency plan – a documented work plan designed to ensure that all needed actions, requirements and resources are provided in order to eradicate or bring under control outbreaks of specified diseases of aquatic animals (OIE, 2006a).

Diagnosis – determination of the nature of a disease (OIE, 2006a).

Disease – clinical or non-clinical infection with an etiological agent (modified from OIE, 2006a).

Disinfectants – chemical compounds capable of destroying pathogenic microorganisms or inhibiting their growth or survival ability (OIE, 2006a).³

Disinfection – the application, after thorough cleansing, of procedures intended to destroy the infectious or parasitic agents of diseases of aquatic animals, including zoonoses; this applies to aquaculture establishments (i.e. hatcheries, fish farms, oyster farms, shrimp farms, nurseries, etc.), vehicles and different equipment/objects that may have been directly or indirectly contaminated (OIE, 2006a).

External audit – evaluation of a quarantine facility conducted by the Competent Authority or its agent to determine the degree of conformity with prescribed criteria and provide a basis for ongoing improvement.

Hazard – any pathogen that could produce adverse consequences on the importation of a commodity (OIE, 2006a).

Hazard identification - the process of identifying the pathogenic agents



Inspection of consignments by the competent authority is a crucial part of the quarantine process.

that could potentially be introduced in the commodity considered for importation (OIE, 2006a).

Health certificate – a certificate issued by an exporting country's Competent Authority attesting to the health status of a consignment of aquatic animals (also "International aquatic animal health certificate").

Inspection – the control carried out by the Competent Authority in order to ensure that an aquatic animal is/aquatic animals are free from the diseases/infections considered in the OIE Aquatic Code; the inspection may call for clinical examination, laboratory

³ Disinfectants mentioned in this manual are summarized in Annex 2.

tests and generally, the application of other procedures that could reveal an infection that may be present in an aquatic animal population (OIE, 2006a).

Inspector – a technically competent person authorized by the Competent Authority for purposes of inspecting and verifying compliance with the requirements of the Competent Authority concerning the import and export of live aquatic animals.

Internal audit – an audit carried out by the company or organization operating a quarantine facility to evaluate its own performance in relation to the prescribed criteria.

International aquatic animal health certificate – a certificate issued by a member of the personnel of the Competent Authority of the exporting country certifying the state of health of the aquatic animals and a declaration that the aquatic animals originate from a source subjected to official health surveillance according to the procedures described in the OIE *Manual of Diagnostics Tests for Aquatic Animals* (OIE, 2006b) (modified from OIE, 2006a).

Introduction – the human-assisted movement of an aquatic animal to an area outside its natural range.

Live food finfish – finfish that are of a size suitable and intended for immediate human consumption. Such animals will not be grown further but may be fed for short-term maintenance at the end users' premises. They shall not be diverted to other purposes (e.g. broodstock, research, recreation, restocking) or to other facilities holding other aquatic animals.

Monitoring – collection and analysis of information necessary to detect changes in a pathogen's prevalence or intensity of infection.

Movement – human-mediated movement of aquatic animals within or across political borders (international, state/provincial or regional boundaries) or between differing geographic areas (e.g. between drainage basins) or zones of differing disease status.

Operator – the person who has overall responsibility for the operation and maintenance of a quarantine facility.

Ornamental aquatic animal – any species of freshwater or marine finfish or invertebrate whose name appears on a national or state list of ornamental aquatic animals approved for importation. Ornamental aquatic animals are typically species that are maintained in indoor aquaria and have no use in foodfish aquaculture in the importing country.

Pathogen – an infectious agent capable of causing disease.

Permit – authorization issued by the supervisor for entry/exit of people, animals and goods onto or off the quarantine facility (also "Biosecurity clearance").

Precautionary approach – as pertaining to the safe movement of live aquatic animals, the obligation that, in instances where countries must make decisions in the face of incomplete knowledge, that both importing and exporting countries act responsibly and conservatively to avoid the spread of serious pathogens.

Protocol (also referred to as "procedure") – a document specifying, as applicable, the purpose and scope of an activity; what shall be done and by

whom; when, where and how it shall be done; what materials, equipment and documentation shall be used and how it shall be controlled.

Quarantine – maintaining a group of aquatic animals in isolation with no direct or indirect contact with other aquatic animals, in order to undergo observation for a specified length of time and, if appropriate, testing and treatment, including proper treatment of the effluent waters (OIE, 2006a).

Quarantine facility (also referred to as a "quarantine premise" or a "transitional facility") – any place approved for the quarantine of live aquatic animals.

Quarantine officer – a technically competent person authorized by the Competent Authority for purposes of inspecting and certifying compliance with the health requirements of the Competent Authority concerning the import and export of live aquatic animals.

Quarantine period – a minimum period of quarantine, typically as specified in an aquatic animal import health standard or other legally binding document (e.g. national or state regulations).

Risk – the likelihood of the occurrence and the likely magnitude of the consequences of an adverse event to public, aquatic animal or terrestrial animal health in the importing country during a specified time period (OIE, 2006a).

Risk analysis – the complete process composed of hazard identification, risk assessment, risk management and risk communication (OIE, 2006a).

Risk assessment – the evaluation of the likelihood and the biological and economic consequences of entry, establishment or spread of a hazard within the territory of an importing country (OIE, 2006a).

Risk communication – the interactive exchange of information on risk among risk assessors, risk managers and other interested parties (OIE, 2006a)

Risk management (also referred to as "Risk mitigation") – the process of identifying, selecting and implementing measures that can be applied to reduce the level of risk (OIE, 2006a).

Specific pathogen free (SPF) – aquatic animals that have been produced and are tested and held under rigorous conditions of biosecurity that provide assurances that they are free of certain specified pathogens. Once animals leave an SPF facility, they are no longer considered to have an SPF status.

Standard operating procedures (SOPs) – a set of instructions having the force of a directive, covering those features of operations that lend themselves to a definite or standardized procedure without loss of effectiveness - *http://en.wikipedia.org/wiki/Standing_operating_procedure.*

Surveillance – a systematic series of investigations of a given population of aquatic animals to detect the occurrence of disease for control purposes, which may involve testing samples of a population (OIE, 2006a).

Transboundary aquatic animal diseases (TAADs) – aquatic animal diseases that are highly contagious or transmissible, with the potential for very rapid spread irrespective of national borders, and that cause serious socio-economic consequences.

Transfer – the movement of an aquatic animal to an area within the established or historical range of the species.

Traceability – the capacity to identify facilities and other locations to and from which live aquatic animals have moved during the import/export process (also "chain of custody").

Zone – a portion of one or more countries comprising (a) an entire water catchment from the source of a waterway to the estuary or lake, or (b) more than one water catchment, or (c) part of a water catchment from the source of a waterway to a barrier that prevents the introduction of a specific disease or diseases, or (d) part of a coastal area with a precise geographical delimitation, or (e) an estuary with a precise geographical delimitation, that consists of a contiguous hydrological system with a distinct health status with respect to a specific disease or diseases for which required surveillance and control measures are applied and basic biosecurity conditions are met for the purpose of international trade. All areas of the zone much have the same health status. The zones must be clearly documented (e.g. by a map or other precise locators such as GPS co-ordinates by the Competent Authority (or Authorities) (OIE, 2006a).

Zoning – identifying zones for disease control purposes (OIE, 2006a).

1.3 WHAT IS QUARANTINE?

"Quarantine" has been defined in many ways, ranging from the very broad definition of "quarantine measures" as encompassing all activities related to the prevention of the international and domestic spread of serious animal diseases (what is now usually referred to as "biosecurity") (FAO/NACA, 2000), to, in its briefest form, the holding or rearing of animals under conditions which prevent their escape or the escape of organisms and potential disease agents infecting or associated with them into the natural environment (Arthur, 1996). The definition of quarantine adopted for this manual is that of the World Organisation for Animal Health (formerly the Office International des Épizooties, OIE), as given in the *Aquatic Animal Health Code* (OIE, 2006a):

"Quarantine means maintaining a group of aquatic animals in isolation with no direct or indirect contact with other aquatic animals, in order to undergo observation for a specified length of time and, if appropriate, testing and treatment, including proper treatment of the effluent waters."

At the international level, the primary purpose of quarantine is to minimize the risk of introducing pathogens into the territory of the importing country and their transmission to susceptible species. An additional purpose, unrelated to pathogen concerns and thus not discussed further in this manual, is to prevent the introduction of potentially harmful aquatic organisms (pests or "fellow travellers") that have not been approved for introduction (ICES, 2005). At the subnational level, quarantine can similarly be applied by states to prevent the spread of pathogens between different river systems, watersheds, islands, disease zones or political units. At the production facility level, quarantine can be successfully applied at individual hatcheries and grow-out facilities to prevent the

BOX 1 The components of a national aquatic animal health strategy (FAO/NACA, 2000)

- National pathogen list
- Disease diagnosis
- Health certification and quarantine measures
- Disease zoning
- Disease surveillance and reporting
- Contingency planning
- Import risk analysis
- National strategies and policy frameworks
- National and regional capacity building



Well trained staff is a basic requirement for effective quarantine.

entry of serious pathogens into the facility via infected broodstock or juvenile stages.

The basic requirements for effective quarantine include:

• adequate physical infrastructure appropriate to the level of containment required (e.g. secure facilities, secure intake water source, etc.);

• established operating protocols (including traceability and chain of custody) and

• well-trained staff.

1.4 QUARANTINE WITHIN AQUATIC ANIMAL BIOSECURITY AND THE RISK ANALYSIS PROCESS

In the past, quarantine was often seen as a separate activity, and as a procedure that should be applied to all imports of living aquatic animals, often with the unrealistic goal of "zero risk" of disease entry to the importing country. This thinking has changed considerably in the past 10 years, so that national governments are increasingly viewing quarantine as one aspect of a 'National Aquatic Animal Health Strategy'. In Southeast Asia, for example, the components of such a national programme have been defined through a regional Technical Cooperation Project (TCP) of the Food and Agriculture Organization of the United Nations (FAO) implemented by the Network of Aquaculture Centres in Asia-Pacific (NACA) that has the support of 21 countries in the Asia-Pacific and a number of international agencies. One of the major outputs of this programme was the Asia Regional Technical Guidelines on Health Management for the Responsible Movement of Live Aquatic Animals and the Beijing Consensus and Implementation Strategy (FAO/NACA, 2000). These guidelines, which outline an agreed-upon general approach and framework that countries in the Asia-Pacific should use in developing and implementing national programmes to reduce the

risk of pathogen spread via movements of live aquatic animals and their products, have been officially adopted as a policy document by the Association of South East Asian Nations (ASEAN).

The components of a National Strategy are shown in Box 1. It can be noted that health certification and quarantine measures are key components that countries should consider when developing a national aquatic animal health strategy.

In cases where a risk assessment has determined that the level of risk associated with trade in a commodity exceeds the acceptable risk indicated by the appropriate level of protection (ALOP) of the importing country, the importing country can then consider ways to reduce the risk to an acceptable level. The possible options for risk management will vary depending on the nature of the commodity and the individual hazard. Quarantine is one of the options that may be applied (Chapter 1.6).

Note that during the risk analysis, management options for each hazard (pathogen) must be carefully evaluated as to their likely effectiveness and the risk presented by the hazard reassessed based on the expected results.

1.5 EXAMPLES FROM ACTUAL EXPERIENCE

1.5.1 Importations of Atlantic salmon into western Canada

Atlantic salmon (Salmo salar) are nonnative to western Canada. They were first

imported into British Columbia from Scotland in 1985 (Arthur, 1995). Following the signing of a legal agreement between the importing company and the Government of Canada, the exporting facility was certified in accordance with the provisions of the Canadian Fish Health Protection Regulations (DFO, 1984) and an on-site visit was made by Canadian Fish Health Inspectors. After approval, live eggs were surface-disinfected using an iodophor prior to exportation. Upon arrival in British Columbia, they were held in government-approved quarantine facilities constructed at the expense of the importer. Juveniles were reared from the eggs and the young salmon held in quarantine for 12 months during which they were inspected at intervals for disease agents by the inspectors. At the end of this period, they were released into cages for rearing. This, and subsequent imports of Atlantic salmon from Ireland, the United States and eastern Canada were used to develop Canada's highly successful salmon culture industry.

A formal protocol for the importation of Atlantic salmon into British Columbia was subsequently developed by Canada's Department of Fisheries and Oceans (DFO) (Annex 1). While quarantine is an important component of the importation procedure, a number of other risk mitigation measures are also

Well designed aquaculture facilities generally include quarantine or observation areas.



required, which in combination, greatly reduce the likelihood of introducing serious pathogens (Chapter 1.6). To date no pathogens are known to have been introduced along with the highly successful introduction of this species.

1.5.2 Importation of bay scallop into eastern Canada

A second example, also from Canada, is the introduction of the bay scallop (*Argopecten irradians*) into eastern Canada. The bay scallop, which is native to the northeastern United States, was first imported into Atlantic Canada in 1979 for possible aquaculture development. Imported scallops where held in quarantine on Prince Edward Island (PEI) through four generations and rigorously inspected for diseases prior to release of progeny in selected grow-out sites in 1983. By 1989, an industry had become established, with commercial quantities of seed being produced in private hatcheries in Nova Scotia for grow-out in PEI. However, it was subsequently discovered that, despite the best efforts of the government, at least two protistan pathogens, a *Pseudoklossia* sp. and *Perkinsus karssoni*, were introduced along with this species (McGladdery, Bradford and Scarratt, 1993; McGladdery and Zurbrigg, 2006). This example highlights the difficulty posed in safely introducing species or stocks whose health history is poorly documented and whose natural pathogen fauna is insufficiently known, as well as the high risk associated with introducing or transferring live molluscs in general (Hine, 2004).



Quarantine facilities designed for molluscs are equipped with special shallow trays or tanks as seen here.

1.5.3 Implementation of quarantine in developing countries

Several developing countries, particularly those in Asia, have been attempting to implement quarantine of aquatic animals as part of their national aquatic animal health strategies (for example, Arthur, 1996; FAO, 1997; Lavilla-Pitogo and Nagasawa, 2004; Whittington and Chong, in press). Indonesia and Malaysia, in particular, have devoted considerable efforts, human and financial resources to establishing national quarantine services, including border quarantine and inspection facilities (Latiff, 2004; Sunarto *et al.*, 2004). Unfortunately,

these efforts have not been successful in preventing the entry and spread of exotic transboundary aquatic animal pathogens into national territory. This has been due to several reasons, the most important of which is probably the fact that quarantine has not yet been fully integrated into national biosecurity programmes as part of the pathogen risk analysis process. Thus, little attempt has been made to identify and prioritize risks and to look at ways to reduce them effectively by drawing upon the entire arsenal of risk management measures that are available (Chapter 1.6).

1.6 RISK MANAGEMENT MEASURES COMPLEMENTARY TO QUARANTINE

Quarantine should be seen as one of a wide range of risk management measures that can be applied, either alone or in combination, to reduce the risks posed by aquatic animal pathogens. The decision whether or not to require quarantine or other biosecurity measures should be done on a case- by-case basis and determined by a risk analysis (Murray *et al.*, 2004; Arthur *et al.*, 2004; OIE, 2006a). This Chapter briefly highlights some of the other complementary approaches that can be applied and provides some references that can be consulted for further information. Risk reduction measures are subdivided into pre-border and postborder measures (border measures are the quarantine protocols presented in this manual).

1.6.1 Pre-border measures

Pre-border measures are often critically dependent on the inspection, certification and compliance regime of the exporting country and are most effective when undertaken as a cooperative undertaking by the Competent Authorities (CA) of the importing and exporting countries.

- Certification of production source. The inspection, testing and certification of hatcheries and other aquaculture production facilities as free from specific pathogens is a highly effective method to assure freedom from many serious diseases (Daelman, 1996). An example of the procedures required by the Government of Canada for the certification of salmonid production facilities for movement of eggs and fish into Canada is given in DFO (1984).
- Use of specific pathogen free (SPF) *commercially available.* stocks. The development of SPF stocks



SPF Litopenaeus vannamei broodstock is now commercially available.

for some species of penaeid shrimp (SPF *Litopenaeus vannamei*, *L. stylirostris* and *Penaeus monodon* are currently available) is a similar private-sector initiative to provide shrimp growers and hatcheries with broodstock and postlarvae of known health status with regard to certain pathogens (FAO, 2006). Unlike the government-regulated programme for salmonids used in Canada, however, the pathogens for which freedom is certified varies between SPF production facilities and shrimp species. There is also no universally accepted standard (e.g. type, number and frequency of diagnostic testing that must be performed) as to the criteria that must be met for a production facility to achieve SPF production status.

• Zoning. Sourcing stock from production facilities located in disease-free zones is another highly effective method to assure that the aquatic animals being moved are free from certain serious pathogens. Such a system is

currently in place in the European Union (Daelman, 1996). Guidance on disease zoning can be found in FAO (2004) and OIE (2006a).

- **Restrictions on life cycle stages.** Juvenile stages and especially fertilized eggs generally carry fewer subclinical infections than do adult animals. Restricting importations to surface-disinfected fertilized eggs is often an effective way to prevent the movement of parasites, most bacteria and some viruses.
- Lists of approved species. Allowing importation only of certain preapproved "lower risk" species is an effective means to reduce the likelihood of pathogen introduction. Such lists should be country-specific as determined by risk analysis, taking into consideration the various national factors, including possible end uses (for example, Doyle, Beers and Wilson, 1996; Anon., 2002; AQIS, undated (b), undated (c)).
- Lists of approved exporting countries. Importing countries may wish to establish lists of exporting countries that have met preset risk management conditions and thus can be pre-approved as lower risk sources for certain types of aquatic animals. Such conditions might include, for example:
 - presence of disease surveillance, monitoring and reporting programmes
 - existence of zoning programmes
 - existence of production facility health certification programmes
 - evaluation of the CA
 - existence of standard operating procedures (SOPs) or better management practices (BMPs) for production facilities and exporters and
 - existence of contingency plans for serious disease outbreaks.
- On-site inspection of exporting facilities. For movements of "high risk" species, the CA of the importing country may wish to make on-site visits to proposed hatchery or other production facilities to verify the biosecurity measures that are in place to support claims of health status. Such visits have been used, for example, by Canada's DFO for the importation of Atlantic salmon (Annex 1) and more recently, by the Thai Department of Fisheries (DoF) in the approval of production facilities for SPF penaeid shrimp (Arthur *et al.*, in press).
- Evaluation of Competent Authorities. In cases where, as part of a risk assessment, the CA of an importing country has uncertainty regarding the zoosanitary measures used by a potential exporting country, an evaluation of the CA may help to relieve any specific concerns. The procedures for evaluation of the CAs are given in OIE (2006a).
- International and other health certificates. Requiring international health certificates (OIE, 2006a, 2006b) for specific OIE-listed diseases of concern to the importing country can provide a high level of assurance that consignments are free of the specified diseases. It should be noted that importing countries should not require certification for diseases that are not relevant to their country situation and/or the species of aquatic animal being moved. Other types of health certificates are of limited value and must be individually evaluated based on the reliability of the diagnostic test(s) performed, the

sampling regime, etc. Health certificates based on visual inspection for gross signs of pathology (Level I diagnostics, FAO/NACA, 2001b) and/or general "healthiness" have little value in preventing the international spread of transboundary aquatic animal diseases (TAADS).

- Pre-border quarantine and temporary holding. Risks to the importing country posed by "high risk" species can be reduced by conducting quarantine and disease testing of the stock/consignment or aquatic animals to be imported in the exporting country, or in a third country having appropriate quarantine capacity. Pre-border quarantine holding of "lower risk" aquatic animals in the exporting country can also allow time for any diseases or infections to become evident. Australia, for example, requires that freshwater ornamental fish be held for a period of 14 days in the approved exporting country (AQIS, undated(b)). In quarantine situations involving "high risk" species, the use of co-habitation experiments in which key native species are held in contact with the exotic species or effluent waters from the quarantine holding tank can be performed to investigate pathogen presence and the susceptibility of native species. Placing quarantined animals under increased stress may also assist in the overt expression of subclinical infections.
- **Preshipment treatment.** In some cases, the use of pre-shipment treatments can reduce the risk of pathogen transfer. The surface disinfection of eggs using iodophores, for example, is one such treatment. However, treatment of external parasites and bacterial infections may only reduce infection levels, removing the clinical signs of disease but not eradicating the pathogen(s).
- Inspection, certification and compliance audits. Establishing auditing procedures to verify that exporters, importers, CAs and private contracting agencies are strictly adhering to specified protocols and requirements should be considered.

1.6.2 Post-border measures

Complementary post-border risk management measures include:

- Restrictions on initial use. Placing restrictions on the initial use of introduced or transferred aquatic animals provides the opportunity to detect any introduced diseases prior to the animal's general release into the natural environment and increases the opportunity for control and eradication (ICES, 2005).
- Monitoring programmes. Inclusion of a disease surveillance component within monitoring programmes such as those outlined by ICES (2005) for introduced or transferred aquatic animal species can be used to confirm that serious diseases have not been spread to new environments and in the case where serious exotic pathogens have escaped detection in quarantine, will help minimize their impacts by allowing containment or eradication programmes to be initiated at an early stage.



Monitoring health is an important component of pre-boarder quarantine measures.

• **Contingency planning.** All proposals for introductions and transfers should include planning for actions to be taken in case escape of animals or pathogens from quarantine occur or a serious pathogen fail to be detected during quarantine and be released into aquaculture facilities or the natural environment. FAO (2005) and OIE (2006a) provide guidelines for contingency planning.

2. Standards and guidelines for quarantine

There are few international standards or guidelines for the setting up and operation of quarantine facilities for aquatic animals. While the use of quarantine is one of the cornerstones to preventing the international spread of the serious pathogens listed in the OIE's *Aquatic Animal Health Code* (OIE, 2006a) and *Manual of Diagnostic Tests for Aquatic Animals* (OIE, 2006b) (the Aquatic Code and Aquatic Manual, respectively), there is no available detailed guidance as to the minimum standards for construction, security or operation of quarantine facilities that should be applied. Appendix 3.2.1 of the Aquatic Code and Chapter 1.1.5 of the Aquatic Manual provide valuable information on the appropriate and safe use of chemicals and other treatments for the disinfection of aquaculture facilities that has direct applicability to the operation of quarantine facilities.

The International Council for the Exploration of the SEA (ICES) in its "ICES Code of Practice on the Introductions and Transfers of Marine Organisms" (ICES, 2005), provides a recommended protocol for the introduction or transfer of live marine organisms that includes a decision-making process incorporating consideration of risks due to possible ecological (pest), pathogen and genetic impacts of the species being moved to the receiving country. Once a decision has been made to introduce or transfer an aquatic organism, the ICES Code provides a general protocol on how the movement should occur, with long-term quarantine being a fundamental component. Appendix C of the ICES Code provides brief general guidelines for the operation of quarantine facilities for aquatic animals destined for introduction or transfer.

In 1996, FAO assisted Asian countries in initiating the development of a "Strategy for the Development and Implementation of Health Certification and Quarantine Guidelines for the Responsible Movement of Aquatic Animals in the Asia-Pacific Region" (FAO, 1997). This initiative leads the FAO, its partner organizations (NACA and OIE, among others) and 21 participating countries to develop the Asia Regional Technical Guidelines on Health Management for the Responsible Movement of Live Aquatic Animals (FAO/NACA, 2000, 2001a). Included in these documents is a general guidance to countries on establishing effective quarantine programmes for live aquatic animals. Most recently, in support of FAO's Code of Conduct for Responsible Fisheries (CCRF), FAO (2007) has provided general guidance for the safe transboundary movement of live aquatic animals, including recommendations on the use of biosecurity measures such as quarantine.

Recently the Association of Southeast Asian Nations (ASEAN), through a project coordinated by the ASEAN Secretariat (ASEC), NACA and AusVet, has drafted a set of standard operating procedures (SOPs) for health certification and



Code of Conduct for Responsible Fisheries provides general guidelines on safe transboundary movement of live aquatic animals.

quarantine measures for the safe movement of live food finfish between ASEAN Member Countries. Included with the SOPs is an annex briefly outlining standards for quarantine facilities (ASEAN, 2007).

European The Union (EU) has implemented a comprehensive programme to assure health standards for aquatic animals traded between EU Member Countries that is based on the definition of important pathogens and their hosts (disease lists), regionalization and zoning, controls on movements between zones of different status and disease testing that are spelled out in EU Directives, Decisions and Regulations (Daelman, 1996). Quarantine must be applied in the case of wild-caught animals from deepsea waters that are to be used for breeding in approved zones or farms. It may also be applied as a post-border health condition to consignments of aquatic animals originating from listed third countries (non-EU Member Countries).

At the national level, a number of countries

have established protocols for the operation of quarantine facilities, those developed by Australia (AQIS undated(a)) and New Zealand (MAF, 2001) being among the most comprehensive. Protocols for the quarantine of aquatic animals have also been enacted in the legislation of many developing countries such as Indonesia, Malaysia and Namibia.

At the production facility level, guidance on the quarantine of new broodstock in shrimp hatcheries can be found in FAO (2006).

Additional guidance on the setting up of quarantine facilities for aquatic animals can be found in Ledua and Adams (1988), Muir (1990), Porter (1992), Humphries (1995) and Maes and Carmichael (2002), among others.

3. General considerations

3.1 IMPORTANCE OF RISK ANALYSIS

A proposal to introduce or transfer an aquatic animal species into a nation's territory may be unique or may be identical or conform closely to a previous proposal. Applications must therefore be carefully evaluated on a case-by-case basis to determine if a risk analysis is required, whether an unacceptably high level of risk of pathogen introduction exists (based on a new or previously conducted risk analysis) and if so, what risk management measures, if any, can be applied to reduce the risk to an acceptable level. In cases where risk management is technically and financially possible, the specific measure or combination of measures to be applied (e.g. use of SPF stocks, sourcing from stocks whose health status is well documented, use of surface-disinfected eggs, availability and use of specific diagnostics tests for health certification of individual broodstock, etc.) will vary depending on species of aquatic animal, its life cycle stage, origin, etc.

3.2 GENERAL PRINCIPLES OF QUARANTINE

The following general principles for the use of quarantine for aquatic animals are taken mainly from FAO (2007):

- Where risk analysis has shown that the level of risk posed by the proposed importation of a consignment of live aquatic animals is unacceptable, quarantine is one of a number of potential risk mitigation measures that singly or in combination with other measures, can be considered to reduce the risk to within the national appropriate level of protection (ALOP).
- The stringency of quarantine applied should be commensurate with the estimated level of risk, which is a function of the source and destination of the aquatic animal movement. Importations of exotic species for aquaculture development and those originating from wild populations or other sources of unknown or poorly documented health status will often require stringent quarantine measures. For the first movement (introduction) of an exotic species, the use of protocols outlined by the International Council for the Exploration of the Sea (ICES, 2005) and the European Inland Fisheries Advisory Commission (EIFAC) (Turner, 1988) is strongly recommended.
- Because transmission of pathogens can occur across major taxonomic groups of aquatic animals, countries should avoid making scientifically unsound, arbitrary distinctions between marine and freshwater species, or between wild, cultured or ornamental species, with respect to the risk posed and the level of quarantine needed.

- Because it is extremely difficult and often impossible to eradicate aquatic animal diseases once established in the environment, particular care should be taken over introductions intended for release into natural waters.
- Quarantine procedures, including observation for clinical signs of disease and diagnostic testing, can be conducted in the country of origin, in a country of transit and/or in the receiving country.



Quarantine procedures such as diagnostic testing ca be conducted in the country of origin, country of transit, and/or in the receiving country.

• Quarantine facilities should meet minimum standards for location, design, infrastructure and equipment, physical security, treatment of intake and discharge waters, staff expertise and training and operating protocols to ensure their effective operation and that aquatic animals and any pathogens they may carry will not escape into the surrounding environment. Countries may need to develop such standards.

• Movements involving a high or unknown health risk (e.g. from areas where exotic diseases are known to occur) should only take place where full containment facilities and support services (diagnostics

capability, security, inspection) are in place. Where facilities do not currently meet these requirements, only low risk movements should be approved.

- Disinfectants, antibiotics and other chemicals and drugs for use in quarantine facilities should be chosen and applied in conformation with local laws and regulations and in a manner that ensures the protection of personnel, the aquatic animals being held and the external environment from their possible harmful effects.
- A wide range of products and procedures can be applied in the cleaning and disinfection of quarantine facilities. Competent Authorities should evaluate the effectiveness of such products and procedures against relevant pathogens under local conditions. Decisions on which products to use should take into account their legality, microbiocidal efficacy and their safety for personnel, aquatic animals and the environment. Approved procedures (SOPs) for the use of disinfectants should be established.⁴
- All effluent and wastes generated by a Quarantine Facility should be treated in a manner that effectively destroys all pathogens. To ensure continuous operation and complete containment, quarantine effluent systems should equipped with fail-safe backup systems. As treated effluent and waste may contain substances deleterious to the environment (e.g. active disinfectants), they should only be disposed of in a manner that minimizes environmental impact.

⁴ The use of disinfectants poses risks to the health of quarantine staff, the stocks being quarantined and the wider environment. For detailed guidance in their selection and safe usage, DAFF (2006), Danner and Merrill (2006) and OIE (2006a, 2006b).

- Where possible, countries should reduce the level of risk involved by importing eggs, embryonic or juvenile stages, as these generally carry fewer subclinical infections than do adult animals and are often easier than adults to maintain under quarantine conditions.
- When possible, candidate stocks for introduction or transfer should be moved on a batch-by-batch basis, a batch being a group of animals of the same age, from the same population and maintained as a discrete group on the same water source. Mixing of animals, water or equipment between batches should not occur.
- Co-habitation experiments in which key native species are held in contact with the exotic species or effluent waters from the quarantine holding tank can be performed to investigate pathogen presence in imported aquatic animals and the susceptibility of native species (however, note that negative results do not demonstrate an absence of pathogens). Placing quarantined animals under increased stress may also assist in the overt expression of subclinical infections.
- Many diseases, especially those caused by external parasites, can be treated. However, because chemical therapy can cause additional health complications, such as the development of antibiotic-resistant strains of bacteria, it should be used responsibly, with due caution and expert advice.
- As many treatments, such as those for monogenean gill parasites, are often only partially effective, it is critical to ensure that the pathogen is eliminated and that subclinical infections do not occur.
- Should a serious untreatable disease or pathogen be encountered in aquatic animals held in quarantine, the entire stock should be destroyed and the facility appropriately disinfected.
- Introductions from sources that have passed a quarantine containment process may receive "approval" status if conditions do not change at the export site, further reducing quarantine requirements/duration.

3.3 SOME FACTORS LIMITING THE APPLICATION OF QUARANTINE

The inability to prevent the entry and spread of exotic diseases into new areas has been due to a number of reasons, including:

- most importantly, the lag time between when a new disease emerges, when it is first recognized as a serious pathogen of international importance, and when accurate and reliable diagnostics tests are developed and become generally available;
- the diversity of forms in which trade occurs;
- the sheer volume of aquatic animals traded;
- the lack of simple and accurate diagnostics tests for some pathogens;
- the ability of pathogens to take advantage of novel host species and new environments; and
- the limited capital and human resources that governments are able to commit to this effort (Arthur, 2004).

At the national level, the following necessary supporting services must also be in place for effective quarantine to occur:

- adequate legislation;
- effective enforcement (e.g., border customs and inspection, post-border follow up);
- knowledgeable and supportive aquaculture industry;
- sufficient political will;
- competent and readily available diagnostics support;
- existence of reliable diagnostics tests for major pathogens;
- good working relationships between importing and exporting country CAs;

• good knowledge base of pathogens present in the exporting and importing countries (surveillance and monitoring, disease surveys); and

• good information base on pathogen biology, prevention, treatment, etc.



Sheer volume of aquatic animal traded sometimes makes it difficult to implement effective quarantine.

Quarantine, particularly of exotic species, can be an expensive activity. Responsibility for the costs of operating a Quarantine Facility and other related financial issues (e.g. costs of permits, supervision, diagnostic and other services, treatments, loss of stock due to mortality or their ordered destruction, etc.) should be clearly spelled out to all parties via legal documentation. Generally, the importer of a consignment of live aquatic animals should bear all costs associated with complying with the aquatic animal import standards.

Box 2 shows an example semiquantitative scoring system that could be

used to determine the level of quarantine required for an importation of live aquatic animals. Readers are also referred to the "risk-assessment worksheets" developed by the United States Fish and Wildlife Service (USFWS) to assist resource managers in minimizing health risks associated with the movement of special-case aquatic species into and out of quarantine facilities (Bell *et al.*, 2006).

3.4 USE OF DISINFECTANTS, ANTIBIOTICS AND OTHER CHEMICALS AND DRUGS IN QUARANTINE FACILITIES

Disinfectants and other chemicals used in quarantine facilities can be highly toxic to fish and humans (e.g. hypochlorites, iodine, etc.) and thus must be handled and applied with due care using the methods and safety equipment appropriate to the individual chemical being applied and the specific application. The recommendations and safety advice on the use of individual disinfectants supplied by the manufacturer and on appropriate material safety data sheets should be strictly followed. Readers are referred to the detailed information on the safe use of disinfectants in aquaculture found in DAFF (2006) and Danner and Merrill (2006).

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The effectiveness of a given disinfectant depends on many factors. For hypochlorites, for example, these include such variables as the concentration of available chlorine, the treatment time, the nature of the material being treated (e.g. water, hard surfaces, porous surfaces, absorbent material, etc.), the presence and amount of organic matter occurring in or on the treated material (e.g. the effectiveness of pretreatment filtration of water), the pH of the material to be treated and the nature of any pathogens present



Disinfection using wheel baths for vehicles and people entering aquaculture facilities reduce risk of contamination.

BOX 2

An example of a semi-quantitative system for assessing the quarantine stringency that should be applied to an importation of live aquatic animals (for illustrative purposes only; modified from Humphries, 1995)

		Risk category (se Lower	core) Higher
Age at transfer	Egg Larvae or juveniles Adult	+(1) +(1)	+(100)
Source	Farm or hatchery bred Wild-caught	+(1)	+(100)
Geographical origin	Within native range Outside native range	+(1)	+(100)
Country or regional disease status	Free of specified diseases Status uncertain Specified diseases present	+(1)	+(100) +(100)
Major diseases in candidate species	None reported Recognized host	+(1)	+(100)
Interpretation	Score <105 200–400 >400	Quarantine strate Minimum quaran Higher stringency Prolonged quaran testing of parent s transfer of progen	gy tine tine & tock with

(DAFF, 2006). There exists in the literature, therefore, a wide range of treatment regimes for the application of a given disinfectant that may be equally effective. Some useful equations for the calculation of the quantities of hypochlorite required to obtain final concentrations (mg/litre) and for determination of the quantity of thiosulphate needed to neutralize the residual chlorine in a given volume of water are given in Box 3.

The treatment protocols outlined in this manual for "high risk" and "lower risk" species have been modified mainly from AQIS [undated(a)], MAF (2001) and ICES (2005), while those for use in the "routine quarantine" of aquatic animals at aquaculture production facilities are modified mainly from FAO (2003, 2006). They are given as general guidelines and thus procedures and concentrations of disinfectants and other chemicals mentioned in this manual may need to be adjusted based on local experience and existing local conditions. As a general rule, quarantine personnel should apply disinfectants and other chemicals in ways to minimize the quantities used while still achieving effective treatment.

Although protocols for some production-level quarantine facilities include the use of antibiotics (e.g. FAO, 2006), in general, their routine use in aquaculture, including quarantine facilities, should be strongly discouraged due the possibility of bacteria developing antibiotic resistance (Inglis, 2000). Antibiotics should only

BOX 3 Some useful equations for working with hypochlorites					
Calculation of working volumes for disinfection					
As liquid	_ % conc. stock soln. x 1 000 000 mg/litre x No. litres stock soln. added				
(mg/litre)	No. litres water to be treated				
As powder Conc. active chlorine (mg/litre)	= $rac{\% ext{ conc. stock powder x 1 000 mg/g x No. grams powder added}}{ ext{ No. litres water to be treated}}$				
Calculation of concentration thiosuphate required to neutralize residual chlorine					
Conc. thiosulphate =- (mg/litre)	% conc. stock soln. x No. litres stock soln. added No. litres water to be treated				

Note: for hypochlorite, 1 ppm = 1 mg/litre
be used where a disease is clearly known to be of bacterial etiology and following sensitivity testing to select an appropriate antibiotic and to determine the dose that is effective against the particular bacterium for which treatment is being applied. Similarly, although some protocols for shrimp broodstock (e.g. FAO, 2006) suggest for the use of probiotics or immune enhancers, their value still need to be clearly demonstrated and thus whether or not to use them is a decision that we leave to individual production facility managers.

4. Recommended standards of construction, security and operation for quarantine facilities for "high-risk" movements (introductions and transfers)

4.1 GENERAL

Chapter 4.0 outlines the recommended minimum standards for the construction, security and operation of quarantine facilities used to hold aquatic animals considered to pose a "high risk" of introducing serious aquatic animal disease. These include imported live aquatic animals that are destined for use in aquaculture development, fisheries enhancement or other activities that involve intended release or probable escape into natural waters (i.e. introduced or transferred species) and whose individual health status and/or the health status of the population from which they originate is partially or completely unknown and which are considered to be potential carriers of serious aquatic animal diseases of concern to the importing country. The following procedures are adapted primarily from the protocols developed by AQIS (undated(a)), MAF (2001) and ICES (2005).

The protocols outlined in ICES Code of Practice on the Introductions and Transfers of Marine Organisms (ICES, 2005) recommend that such "high-risk" aquatic animals should normally be held in strict quarantine throughout their lives, during which repeated observation, sampling and testing for pathogens should be conducted. Progeny reared from the imported parent stock (F1 generation), following additional observation and testing, may be approved for limited release under controlled conditions, during which further monitoring of their health status should be conducted. If no pathogens are detected during this initial period (which can be expected to last several years), subsequent generations may be approved for wider use in aquaculture or for release into the wild. In all cases, the original parent stock should not be released from quarantine and should be destroyed, preferably by concurrent lethal sampling and testing for pathogens.

The standards recommended in this Chapter should be applied only to the quarantine of those aquatic species that have received written approval by the regulating agency or CA (typically the national Quarantine Service, Ministry of Fisheries, Department of Agriculture, Veterinary Service, etc.) to be introduced or transferred into the national territory. Such approval should be based on a risk analysis (Arthur *et al.*, 2004; ICES, 2005; OIE, 2006a; FAO, 2007) and an application for a Permit to Introduce or Transfer Live Aquatic Animals, as specified in the appropriate national regulations.



Appropriate provision created through national acts and regulatory procedures are important to implement effective quarantine.

The approval of an application to introduce or transfer an aquatic animal should, among others, be contingent upon the use (construction, establishment or lease) of an approved Quarantine Facility meeting the minimum standards outlined in this Section. Each application should be considered on its individual merits with consideration being given to the quarantine risk and serviceability associated with each establishment's location and construction and on the capability of the applicant to successfully operate such a facility. Examples of the approval processes and application forms used for quarantine facilities for

ornamental aquatic animals and their operators are given in MAF (2001). The *Import Health Standard for the Importation into New Zealand of Ornamental Fish and Marine Invertebrates from All Countries* (Anon., 2002) provides a useful example of a set of procedures and legal requirements (i.e. an aquatic animal import health standard) to import aquatic animals. Similar requirements for Australia are given in AQIS (undated (b), undated(c)). Approval of a Quarantine Facility should be contingent upon the operator's formal agreement to undertake any training or other courses or briefings as mandated by the CA.

It is the responsibility of the operator of an approved Quarantine Facility to ensure that the premises and all operations comply with all local, state and federal regulations. Documented evidence of compliance with these requirements must be produced to the supervising Quarantine Officer on request.

The operator should ensure that all staff entering the Quarantine Facility are adequately trained in the husbandry of the species being held in quarantine and are familiar with the applicable standards of performance as outlined in the aquatic animal import standard and any SOPs.

The CA should require that notification in writing be given at least 30 days prior to any intended change in ownership, senior management, quarantine operating procedures/arrangements or contemplated modifications to the Quarantine Facility.

Non-compliance with the criteria outlined in these recommendations, once they have been modified to fit specific national needs and circumstances and formally adopted into national legislation, may be justification for withdrawal or suspension of approval of the Quarantine Facility, the possible destruction of stock and the instigation of legal action. The importation of "high risk" aquatic animals destined for introduction or transfer into aquaculture facilities or natural waters often entails a significant risk that serious pathogens accompanying them may escape and become established in natural or cultured populations. Thus, an extremely high level of biosecurity must be maintained at all times. The Quarantine Facility should be constructed and operated in a manner that will assure a high level of security, guaranteeing the isolation of the imported aquatic animals, such that the animals, any pathogens they may carry and any pests or other living organisms contained in their transport waters will not be released from the facility. The possible entry of pathogens of domestic origin that might infect the stock held under quarantine, thorough contaminated influent water, or their entry along with personnel, feeds and fomites must also be prevented.

During the quarantine period, the operator should ensure that no living aquatic animals, equipment or materials are removed from the Quarantine Facility.

4.2 PERIOD OF QUARANTINE

No set period of quarantine should be established. The period of holding in the Quarantine Facility will depend on the results of observation and testing of the imported stock and the resulting F1 generation. In all cases, once the CA is satisfied that the F1 or a subsequent generation is safe for limited release, the parent stock should be destroyed and the Quarantine Facility thoroughly disinfected.

An application to introduce or transfer an aquatic animal entails a commitment to maintain the animals under conditions of strict quarantine for a number of years. The quarantine period will need to take into account the life history of the aquatic animal being introduced or transferred.

If a pathogen or infectious disease is detected at any point while the imported aquatic animals and their progeny are under quarantine, the supervising Quarantine Officer may require treatment and further testing. If the disease is of a serious and/or untreatable nature, destruction of all aquatic animals held in the facility should be ordered and complete disinfection of the building, water and all equipment should be required before permission to restock is granted.

4.3 STANDARDS OF CONSTRUCTION

4.3.1 Location of quarantine facilities

The location of a Quarantine Facility should be determined on a case-by-case basis. Premises should not be approved in the vicinity of private or government fish hatcheries, aquaculture facilities, watercourses or areas subject to frequent flooding.

4.3.2 General requirements

Access to the Quarantine Facility should be through property owned or leased on a long-term basis by the operator and should be available to Quarantine Officers during normal business hours and at such time that aquatic animals are entering or leaving the facility. The operator should notify the supervising Quarantine Officer of the times when the premises will be attended and any alterations to the regular hours.

The Quarantine Facility should be located within a single operational entity that is structurally separated from all other operations and is dedicated solely to the holding of the shipment. It should not share a building having areas that are used for different purposes and should not serve as an access way to other buildings or activities. The Quarantine Facility should not to be used for any purpose, what-so-ever, other than as a place for the performance of quarantine.

The Quarantine Facility should be weatherproof and maintained in a state of good repair.

The Quarantine Facility should be a secure, lockable building that is surrounded by a lockable person-proof security fence.

The holding capacity of the Quarantine Facility should be commensurate with the proposed quantities of the species of aquatic animal for which a permit is granted. Provision must be made for the growth and maturation of the original parent stock and the holding of all F1 and subsequent generations.

The Quarantine Facility should be equipped for the sterilization of all equipment that comes in contact with aquatic animals or tank water during the quarantine period.

The Quarantine Facility should be equipped with back-up systems for essential components (e.g. electricity, water circulation, aeration, temperature control, filtration, etc.) to maintain biosecurity and the health of stocks in the case of electrical or mechanical failures.

4.3.3 Specific construction and equipment requirements

The Quarantine Facility should comply with the following specific construction and equipment requirements:

- (a) Windows should be screened to prevent the entry of insects.
- (b) Floor and walls should be constructed of concrete, tiles or other impervious material to enable hose down and disinfection with retention of all wastewater. The floor should be sufficiently smooth and with sufficient grade to drain to an enclosed holding tank.
- (c) Floor to wall junctions and all gaps and cracks in the walls, floor and ceiling should be effectively sealed such that the quarantine area is capable of containing all leaks and floods that might occur.
- (d) Lighting should be of sufficient intensity to allow proper inspection of all aquatic animals.
- (e) Floor drainage with an insertable plug or other mechanism to prevent the accidental escape of aquatic animals or uncontrolled release of water should be installed. Drainage should be to an approved holding tank. The holding tank should be of suitable size to contain the total volume of all tanks used for the holding of aquatic animals.

- (f) Doors should be equipped with self-closing mechanisms to ensure that they remain shut after entry, or there should be a self-closing insect-proof screen door installed.
- (g) Access to the Quarantine Facility should only be through a personnel entrance leading to a separate outer change room provided with facilities for staff and Quarantine Officers to wash their hands and change outer clothing prior to entering or leaving the quarantine area.
- (h) A footbath containing disinfectant should be placed at the entrance door to the quarantine facility.
- (i) All holding tanks for aquatic animals should:
- be identified with permanent numbers so that individual tank records can be correlated with them;
- be fitted with lids or other approved coverings so as to prevent transmission of pathogens between adjacent tanks due to splash from the aeration/filter system, and to prevent the escape of aquatic animals;
- have water intake lines equipped with automatic shut-off valves;
- be arranged in a manner that permits ready access for inspection purposes, including a minimum width of 75 cm for corridors between rows of tanks or tanks and walls;



Foot baths containing disinfectants should be placed at the entrance door to the facility.

- other than the aquatic animals, contain only sterilizable materials (e.g. plastic) that do not interfere with inspection;
- have at least the front transparent to provide good visibility of their contents, and be stacked for adequate viewing; and
- have its own set of nets, buckets, beakers and other items associated with the tank use, to ensure that none are shared between tanks (also Chapter 4.6.1).
- (j) As all aquatic animals within the facility should be considered to have the same quarantine status, the use of a shared water recirculation system is permissible but not advisable, as it may facilitate the spread of pathogens between tanks.
- (k) All entry and exit points to the Quarantine Facility should prominently display a permanently affixed, professionally made, quarantine sign that states "Quarantine Area-Authorized Persons Only". Such signs should be highly visible (e.g. black lettering of about 5 cm in height on a yellow background).
- (l) A suitable wash-up trough should be located in the quarantine area for the cleaning and disinfecting of equipment. An approved disinfectant should be

available at the wash-up trough. A suitable draining rack should be provided for air drying of equipment.

- (m) A designated refrigerator or freezer should be provided solely for the storage and preservation of dead aquatic animals. The refrigerator or freezer should be clearly identified as being for quarantine use only, be lockable, and located within the quarantine area.
- (n) Equipment necessary to carry out the disinfection all wastewater (both the overseas transport water and all domestic waters used in the Quarantine Facility) should be supplied.
- (o) Secure storage facilities for food used for aquatic animals should be provided such that contamination or infestation by pests is prevented.
- (p) A fully stocked first aid cabinet should be provided and maintained.
- (q) Amenities that should be provided for use by Quarantine Officers include access to a desk and chair, a telephone with a direct outside line, toilet facilities, hand washing facilities (within the quarantine area) and a hygienic means of drying hands, and suitable arrangements for daily cleaning of amenities.

4.4 STANDARDS OF OPERATION

4.4.1 Influent water

All influent water entering the Quarantine Facility should be from an approved groundwater source certified to be free from biological material, including any possible infective agents. Alternatively, water from other sources may be used, however, it should be filtered to remove suspended matter and then sterilized using a method approved by the CA before being used in the Quarantine Facility.

4.4.2 Wastewater sterilization and disposal

All wastewater to be discharged from the Quarantine Facility should be appropriately sterilized. Sterilized wastewater should not be discharged directly into natural waterways. Disposal of wastewater should also conform to any state and local government requirements.

Wastewater should be sterilized in accordance with one of the following methods:

(a) Chlorination⁵

- (i) All water should pass through an approved filter capable of removing suspended organic material prior to hypochlorite treatment.
- (ii) All water should pass to a retention vessel where sufficient hypochlorite is be added to achieve a minimum concentration of 200 parts per million (ppm) (200 mg/litre) at 1 hr post-treatment. Sodium hypochlorite (bleach) should

⁵ Chlorine compounds are corrosive, relatively unstable and are inactivated by the presence of organic matter. They can be highly toxic to fish and humans and thus must be handled and applied with due care. For detailed reviews of their use as disinfectants in aquaculture, DAFF (2006) and Danner and Merrill (2006).

be used at 1.6 ml of hypochlorite solution (12.5 percent available chlorine) per litre of water, while calcium hypochlorite powder (e.g. Pool Chlor[®], 65–70 percent available chlorine) should be used at 0.3 g of powder per litre of water.

- (iii) Before the treatment period commences, the chlorinated effluent should be brought to a pH between 5.0 and 7.0.
- (iv) Following addition of hypochlorite, wastewater should be agitated for at least 10 min to ensure thorough mixing of hypochlorite.
- (v) After a retention period of not less than 1 h, the chlorine concentration is measured using an approved method (e.g. commercially available chlorine test kit). Tanks not achieving a minimum chlorine concentration of 200 ppm (200 mg/litre) at the allotted time should be re-treated until the requirement is met.
- (vi) The chlorine in the wastewater should be neutralized by adding sodium thiosulphate at a rate of 1.25 g (2.5 ml of 50 percent sodium thiosulphate solution) per l of treated wastewater, then agitated for not less than 10 min before discharge.6
- (vii) Chlorination records should be maintained noting: the amount of compound added, the volume of effluent, the time that treatment period commenced, the pH at commencement of the treatment period, the 1 hr posttreatment concentration, the amount of sodium thiosulphate added to achieve neutralization and the of residual chlorine concentration at discharge.
- (viii) Chlorinated water should not be discharged directly into adjacent Effective water treatment facilities are essential for waterways.



removing pathogens prior to discharge of effluent waters.

(b) Heat treatment

Prior to discharge, wastewater shall be

heated to at least 85 °C for a minimum of 30 minutes. Water heating units should be approved by the CA and be fitted with temperature and flow recorders.

(c) Ultraviolet (UV) light radiation

As particles in the water may shade pathogens from the effects of UV light, all water to be treated should pass through an approved filter capable of removing suspended organic material prior to irradiation.

⁶ Theoretically, 1 mg thiosulphate is required to reduce 1 mg chlorine dioxide (Siemens, 2006). Thus the specified treatment would potentially reduce chlorine at levels of 1 250 mg/litre and below. To reduce the quantity of chemical required, residual chlorine level at the end of treatment may be measured and the amount of thiosulphate required for neutralization calculated.

Commercial UV water treatment units operating in the spectral range of 190-280 nm (254 nm recommended) delivering doses of at least 130 mWs/cm² are required. As UV bulbs will burn long after their effectiveness has waned, the burning time of the UV lamp should be monitored, and the lamp replaced



Combination of carbon, cartridge and UV filtration is an effective way of treating effluent waters.

according to manufacturer's specifications.

4.3 DISINFECTION OF EQUIPMENT

Before removal from the quarantine area, and before any restocking, all tanks and tank equipment must be thoroughly cleaned and disinfected with (i) hypochlorite solution at 200 ppm concentration for 5 min or with (ii) an approved iodophore solution containing iodine at 0.5 percent available iodine for 5 min or (iii) by another disinfection procedure approved by the supervising Quarantine Officer.

Filter material should be disposed of by autoclaving followed by incineration or deep burial.

4.4 DISPOSAL OF DEAD AQUATIC ANIMALS

Dead aquatic animals should only be disposed of as directed by the CA. Aquatic animals that have died while under quarantine should held in an approved freezer, an approved refrigerator, or preserved using another method as specified by the CA until removed for laboratory examination or released for disposal by the supervising Quarantine Officer. Upon approval, dead aquatic animals should be disposed of by sterilization using of an approved autoclave, followed by incineration or deep burial.

4.5 DISPOSAL OF PACKING MATERIALS

All containers (bags, boxes and cartons) used to hold aquatic animals during transit should be disinfected using the methods of disinfection specified under "Disinfection of Equipment" (Section 4.3) and then disposed of by incineration, deep burial or another method approved by the supervising Quarantine Officer.

4.6 WORK PRACTICES

4.6.1 Cleanliness and sanitation

The Quarantine Facility and holding tanks should be kept clean at all times. Adequate cleaning facilities (e.g., pressurized water supplies, brooms, shovels, etc.) should be provided to enable maintenance of appropriate standards of hygiene.

No animals other than aquatic animals and live food for aquatic animals should be permitted in the quarantine area. All feeds used within the Quarantine Facility should have prior approval of the supervising Quarantine Officer and be of assured sanitary condition. Live food should not be used unless no other alternative food is acceptable to the animals under quarantine. Live food should be certified to the specifications set by the Competent Authority to ensure their freedom from potential disease agents.

Equipment used in the handling of aquatic animals and tank cleaning and maintenance should not be shared between tanks. A separate set of equipment (nets, cleaning equipment, etc.) should be kept for each tank or series of tanks operated on an individual water filtration system. In the case where several tanks are linked by a shared water recirculation system, a single set of equipment can be used for all tanks within the shared system.

All nets and other equipment should be regularly disinfected by an approved

Cleanliness and sanitation is an important concept in quarantine.

method of disinfection. Equipment or other material should not be removed from the quarantine area during the period that the shipment is under quarantine conditions. In exceptional circumstances, and with the written approval of the supervising Quarantine Officer and his verification that proper disinfection has been accomplished, a request to remove specific items of equipment may be granted.

All footwear and protective clothing used in the quarantine area should be restricted to this site.

The operator should provide protective clothing (jumpsuits, waterproof apron or outer-wear and rubberized footwear) to staff and visitors to use in the facility. Protective clothing should be kept inside the quarantine area (street footwear should left outside the quarantine area and within the changing area). Cloth protective clothing that should be routinely washed may be removed from the quarantine area after washing for the purpose of drying. During the period in which aquatic animals are under quarantine, protective clothing (with the exception of washed clothes removed for drying) should be removed only for destruction. Should removal of unusable protective clothing become necessary, it should first be sterilized by autoclaving or use of an approved disinfectant such as Betadine[®] (5 percent solution) and then removed and destroyed by incineration under the supervision of the Quarantine Officer.

A footbath containing hypochlorite, Betadine® or another approved disinfectant should be maintained at the entrance of the quarantine area proper. The bath should be routinely replenished for adequate disinfection and a record of bath maintenance maintained.⁷ A sign stating "Footwear must be Immersed in Footbath on Exit/Entry from Quarantine Area" should be appropriately displayed.



⁷ Once iodophor solutions have lost their brown colour to become colourless, they are no longer active and should be replaced (DAFF, 2006).

All wastewater disposals should meet any state and local government requirements, be by an approved method (Section 4.4.2), and should not flow directly into natural waterways.

All filter material should be disinfected by autoclaving on another method approved by the supervising Quarantine Officer prior to removal from the Quarantine Facility and then disposed of by incineration or deep burial.

Staff and visitors who have had contact with water or aquatic animals should wash their hands and forearms with soap and water prior to exiting the Quarantine Facility.

Staff and visitors should plan their daily activities such that when they leave the Quarantine Facility they do not visit any other aquatic animal facility that day.

4.6.2 Handling of aquatic animals

Upon arrival of a shipment of aquatic animals at the approved port of entry, and following verification of the accuracy of details of the shipment and its preliminary inspection and clearance by customs officers, the shipment should be resealed by the supervising Quarantine Officer with an approved tamperproof seal (e.g. Tyden seal, lead seal or padlock) and then transferred to the custody of the operator, who should guarantee the secure transport of the aquatic animals, under quarantine conditions, to the Quarantine Facility.

Upon their arrival at the Quarantine Facility, the integrity of the seal should be verified by the supervising Quarantine Officer, the seal removed and the animals transferred to new water. The overseas water should be subjected to an approved disinfection treatment (Section 4.4.2).

In the event that a shipment of imported aquatic animals is incorrectly represented in any manner, the shipment may be destroyed under supervision of the Quarantine Officer.

The progeny of aquatic animals that breed during the quarantine period may be removed to another tank or room in the facility but are subject to all quarantine conditions.

A standard Tank Record Sheet should be maintained for each tank (Section 4.8.2).

Periodically throughout the day, the operator should observe all aquatic animals for signs of illness and abnormal behavior.

All dead aquatic animals should be held for inspection by a Quarantine Officer. All animals found dead on arrival or that die during the quarantine period should be placed in a labeled plastic bag as soon as possible and kept under refrigeration or preserved as specified by the Quarantine Officer until diagnostic examination can be completed. Information on labels should identify the shipment, species, tank number, number of mortalities, date of death and name of collector.

Any equipment that has been in contact with dead aquatic animals should be disinfected before re-use.

Any unusual levels of mortality, changes in behavior or unusual signs of disease, parasites or pests should be immediately reported to the supervising Quarantine Officer.

The use of any drug or chemical to treat aquatic animals should have the prior approval of the CA and be recorded on tank record sheets.

The operator should ensure that no aquatic animals leave the quarantine area under any circumstances without the approval of the supervising Quarantine Officer (i.e. the granting of biosecurity clearance).

On approval by the CA F1 or subsequent generation aquatic animals may be released from the Quarantine Facility for limited trials in aquaculture facilities or for stocking in enclosed water bodies. The CA may specify the precise conditions, period and any further risk management measures under which the aquatic animals are to be maintained. Prior to removal from the Quarantine Facility, aquatic animals should be transferred into clean water.

All original stock and any F1 or subsequent generation aquatic animals not approved for release from quarantine should remain under quarantine conditions. When determined by the CA or at the request of the operator, the operation of the Quarantine Facility may be terminated under the direct supervision of the supervising Quarantine Officer. In which case, all remaining aquatic animals, including all original parent stock, should be humanely killed by a method approved by the supervising Quarantine Officer, tested for pathogens if required, appropriately sterilized (.e.g. heat sterilization through autoclave, etc.) and then disposed of by incineration or deep burial. The facility and all tanks and equipment should be thoroughly cleaned and disinfected using approved disinfectants, and all filters, clothing and other similar materials autoclaved or disinfected and then destroyed by incineration or deep burial. Upon written sanitary certification by the supervising Quarantine Officer, the premises may then be disposed of as seen fit by the operator, or may be used as the basis for a new application for an approved Quarantine Facility.

4.7 OCCURRENCE OF AN OUTBREAK OF A SERIOUS EXOTIC DISEASE

If a serious exotic disease is diagnosed, the operator should be immediately notified. In such cases, the supervising Quarantine Officer or other representative of the CA may direct the management of disease control. Disease control measures may include the extension of quarantine, treatment and/or the destruction of stock.

Measures to be taken are likely to include:

- treatment and/or destruction of stock from infected tanks or of all aquatic animals present in the facility at the time of the outbreak, and their sanitary treatment, removal and incineration;
- decontamination of the interior of the facility, all tanks and equipment, and all waters present in the facility at the time of the outbreak; and
- approval of the CA prior to the reuse of the facility.

4.8 RECORD KEEPING REQUIREMENTS

4.8.1 Summary records

A complete history of the stock of aquatic animals being contained in the Quarantine Facility should be maintained. The operator should, for auditing purposes, maintain all documentation (shipping bills, health certificates, biosecurity clearance, etc.) and records for a minimum period of 36 months after closure of the Quarantine Facility, during which time they will, upon request, be readily made available to a Quarantine Officer. The following summary information concerning the quarantined stock(s) should be recorded:

- overseas supplier, country of origin and waybill;
- date of arrival of parent stock;
- date(s) of release of F1 or subsequent generation from quarantine;
- total number of animals in original shipment(s) and total mortalities in each shipment upon arrival;
- original number of animals stocked in each tank;
- details of any clinical signs of disease and number of affected individuals, by tank;
- details of any mortalities, by tank;
- details of any health certificates;



Standard tank record sheets must be maintained for each quarantine tank.

• details of any diagnostic tests and examinations;

• details of any F1 progeny produced (date and number) and their corresponding transfer tank number;

• for parent stock, and for any F1 or subsequent generation aquatic animals that for any reason have not been approved for release from quarantine upon termination of the quarantine license: number and size of aquatic animals destroyed, date and method of destruction and disposal and signature of the supervising Quarantine Office; and

• for F1 or subsequent generation aquatic animals, if approved for limited release from quarantine: number and size of aquatic animals released, date of release, destination, summary of any risk management measures or restrictions to be employed and signature of the supervising Quarantine Officer.

4.8.2 Tank record sheets

A corresponding approved Tank Record Sheet should be maintained for each holding tank and must be kept up to date at all times. Tank Record Sheets should be retained for a minimum of 36 months following release from quarantine of the portion of the shipment held in the specific tank, or their destruction. This sheet should display the following information:

- tank number;
- number of aquatic animals in tank;
- exporter identification details, including country of export;
- importer's name;
- date of arrival;
- shipment or airway bill number;
- number of aquatic animals dead on arrival;
- details of any observed disease conditions and number of sick aquatic animals;
- daily record of number of aquatic animal deaths in tank;
- details of any prophylactic or therapeutic treatments given;
- disposal details;
- disinfection details; and
- details of any F1 progeny produced (date and number) and their corresponding transfer tank number.

4.8.3 Operations and entry logbooks

Details of wastewater treatment (including chlorination records, if applicable),; filter cleaning, replacement or disposal; internal audit; and general maintenance should be recorded in an operations logbook.

A separate entry logbook should be used to record details of the entry and exit of authorized personnel into the Quarantine Facility.

4.9 AUDITING

The operator should undertake systematic periodic internal audits at least on a quarterly basis, to ensure that the standards for the operation of the Quarantine Facility as in the relevant legislation are maintained and to identify and correct any deficiencies. The operator should record in the logbook, any variations from the prescribed criteria encountered and the corrective measures taken.

Periodic external audits of the Quarantine Facility should be conducted by the supervising Quarantine Officer or other approved personnel to verify the security and proper functioning of the facility.

4.10 SECURITY

Control and security of the Quarantine Facility is of the utmost importance and is the responsibility of the operator. The Quarantine Facility and its perimeter fencing should be securely locked when the facility is not in active use or when unattended. Increased after working hour's security should be considered to prevent unauthorized entry and theft, particularly where valuable broodstock or foodfish are being held.

Procedures should be adopted to ensure that access to the premises is limited to authorized persons only. Signs should be prominently displayed on all sides of the external perimeter fencing and on all entrances to the facility to show that it is a Quarantine Facility and that unauthorized entry is prohibited. The entry of staff into the Quarantine Facility should be restricted to the minimum required to perform necessary maintenance and observation of the quarantined animals. A list of authorized staff should be provided to the supervising Quarantine Officer by the operator. Except in an emergency situation, no other persons should enter the Quarantine Facility unless written prior approval has been granted from the supervising Quarantine Officer.

A logbook of all entry and exit into and out of the Quarantine Facility should be maintained. All personnel entering the facility should be required to enter the following information:

- name of authorized person;
- date of entry/exit;
- time of entry;
- reason for entry;
- time of exit;
- signature at exit; and
- notation of any irregularities.

Signature at exit indicates that the exiting staff has confirmed that the Quarantine Facility was in proper order at the time of his/her exit and that the premises have been left in a secure manner. The operator should ensure that all staff conform to these requirements and should verify the accuracy of record keeping on a weekly basis. The logbook should be made available for examination by the supervising Quarantine Officer upon request.

4.11 CONTINGENCY PLANS

The operator should develop a contingency plan addressing actions to be taken in the event of a vehicle breakdown during transport of aquatic animals from customs arrival to the Quarantine Facility, and due to on-site emergencies that may arise, such as fire, flood, electrical failure or breakdown of essential equipment (aerators pumps, etc.). In the case of emergency, the supervising Quarantine Officer should be notified as soon as possible.

5. Recommended standards of construction, security and operation for quarantine facilities for "lower-risk" movements: standards for ornamental aquatic animals

5.1 GENERAL

The standards recommended in this chapter apply primarily to the quarantine of those freshwater and marine ornamental aquatic animals whose importation is part of established trade and whose names are included on a national list of ornamental aquatic animal species approved for importation (see, for example, Anon., 2002; AQIS, undated(b), undated(c)). Such lists should be derived from risk analyses and thus will differ from country to country based on the individual country situation. Information presented in this Chapter is based primarily on the quarantine protocols developed by MAF (2001) and AQIS (undated(a)).

It should be noted that some commonly traded ornamental species are also cultured as foodfish in the tropics (e.g. Osphronemus goramy, Barbonymus gonionotus, Scatophagus spp.), while others, such as goldfish (Carassius auratus auratus), dwarf gourami (Colisa lalia) and koi carp (Cyprinus carpio carpio) are known to carry serious diseases affecting aquaculture and capture fisheries in both tropical and temperate latitudes (see, for example, Bondad-Reantaso et al., 2005; Whittington and Chong, in press). Importation of species with potential use other than in aquaria, or which can potentially live in the environment or with related native species should be subjected to more intensive scrutiny and the use of more stringent quarantine procedures, such as those outlined in Chapter 4. Importational health certificates stating freedom from the diseases of concern. More stringent quarantine or other risk management measures may also be required for such species as determined by risk analysis.

Examples of the approval processes and application forms used for quarantine facilities for ornamental aquatic animals and their operators are given in MAF (2001). The *Import Health Standard for the Importation into New Zealand of*

Ornamental Fish and Marine Invertebrates from All Countries (Anon., 2002) provides a useful example of a set of procedures and legal requirements (i.e. an aquatic animal import health standard) to import aquatic animals. Similar requirements for Australia are given in AQIS (undated(b), undated(c)). An application to operate an approved Quarantine Facility for ornamental aquatic animals is subject to approval by the CA. The decision to approve or reject an application to establish a Quarantine Facility should be made based on the proposal's individual merits, taking into account such factors as the quarantine risk and serviceability associated with the proposed establishment's location and construction and on the capability of the applicant to successfully operate such a facility. Approval of a Quarantine Facility should be contingent upon the operator's formal agreement to undertake any training or other courses or briefings as mandated by the CA.

It is the responsibility of the operator (i.e. the holder of a licence to operate an approved Quarantine Facility) to ensure that the premises and all operations comply with all local, state and federal regulations. Documented evidence of compliance with these requirements must be produced to the supervising Quarantine Officer on request.

The CA should be notified in writing at least 30 days prior to any intended change in ownership, senior management, quarantine operating procedures/ arrangements or contemplated modifications to the Quarantine Facility.

Non-compliance with the recommended criteria, once incorporated into national legislation, may result in approval of the Quarantine Facility being withdrawn or suspended and legal action instigated.

The Quarantine Facility should be constructed and operated in a manner that assures the isolation the imported ornamental aquatic animals, such that the animals, any pathogens they may carry and any pests or other living animals contained in their transport waters will not be released from the facility.

During the quarantine period, the operator should ensure that no living aquatic animals, equipment or materials are removed from the Quarantine Facility without the approval of the supervising Quarantine Officer or without adequate disinfection using approved methods.

5.2 PERIOD OF QUARANTINE

The normal minimum period of quarantine should be determined by the CA based on national circumstances, including consideration of the national Appropriate Level of Protection (ALOP, Arthur *et al.*, 2004, 2007), recognizing that the risk of pathogen introduction decreases as the period of quarantine is increased. Examples include the requirement of a minimum quarantine period of six weeks for shipments of freshwater ornamental aquatic animals and three weeks for marine ornamental aquatic animals as used by New Zealand (Anon., 2002) and 7–21 days for freshwater ornamental finfish and 7 days for marine ornamental finfish as specified by Australia (AQIS undated(b), undated(c)). However, given

the increasing evidence that ornamental fish play a significant role in the spread of TAADs (e.g. Bondad-Reantaso *et al.*, 2005; Whittington and Chong, in press), the stringency of risk management measures applied to the routine trade in these species will probably need to be increased, including increased length of quarantine and more extensive diagnostics testing.

If, at the end of the specified quarantine period, the supervising Quarantine Officer believes that a consignment of aquatic animals still presents an unacceptable risk of disease introduction, the consignment may be held in quarantine for further investigation, observation, treatment, testing or for any other purpose appropriate to the circumstances. If the risk cannot be effectively managed, destruction of the consignment should be ordered.

5.3 STANDARDS OF CONSTRUCTION

5.3.1 Location of quarantine facilities

Quarantine facilities should be located within the metropolitan area of an approved port of entry that has a permanently based Quarantine Officer.

Premises should not be approved in the vicinity of private or government aquatic animal hatcheries, aquaculture facilities, watercourses or areas subject to frequent flooding.

5.3.2 General requirements

Access to the Quarantine Facility should be through property owned, rented or leased by the operator and should be available to Quarantine Officers during normal business hours and at other times when consignments are entering or leaving the facility. The operator should notify the supervising Quarantine Officer of the times when the facility will be attended and any alterations to the regular hours.

The Quarantine Facility should be located within a single operational entity, and as such be structurally separated from any other operations. It may share a building with other areas that are used for different purposes (including wholesale or retail activities involving live aquatic animals or their products); however, the Quarantine Facility should not used as an access way to other parts of the building.

The Quarantine Facility should be weatherproof and maintained in a state of good repair.

The Quarantine Facility should be located within a secure, lockable building or within a building that is located in an area surrounded by a lockable person-proof security fence.

The Quarantine Facility should not to be used for any purpose, what-so-ever, other than as a place for the performance of quarantine.

The holding capacity of the Quarantine Facility should be commensurate with the proposed quantities and number of species of aquatic animal to be handled. The Quarantine Facility should be equipped with back-up systems for essential components (e.g. electricity, water circulation, aeration, temperature control, filtration etc.) to maintain biosecurity and the health of stocks in the case of electrical or mechanical failures.

The Quarantine Facility should have facilities for the sterilization of all equipment that comes in contact with aquatic animals or tank water during the quarantine period.

5.3.3 Specific construction and equipment requirements

The Quarantine Facility should comply with the following specific construction and equipment requirements:

- (a) Windows should be screened to prevent the entry of insects.
- (b) The floor and walls should be constructed of concrete, tiles or other impervious material to enable hose down and disinfection with retention of all wastewater. The floor should be sufficiently smooth and with sufficient grade to drain to an approved septic tank, municipal sewerage or enclosed holding tank.
- (c) Floor to wall junctions and all gaps and cracks in the walls, floor and ceiling should be effectively sealed such that the quarantine area is capable of containing all leaks and floods that might occur.
- (d) Lighting should be of sufficient intensity to allow proper inspection of all aquatic animals.
- (e) A floor drainage with an insertable plug or other mechanism to prevent the accidental escape of aquatic animals or uncontrolled release of water should be installed. Drainage should be to an approved septic tank, municipal sewer or an enclosed holding tank.
- (f) Doors should be equipped with locks and with self-closing mechanisms to ensure that they remain shut after entry, or there should be a self-closing insect-proof screen door installed.
- (g) Facilities should be provided for staff and Quarantine Officers to wash their hands prior to leaving the Quarantine Facility. A footbath containing disinfectant should be placed at the entrance door to the quarantine facility.
- (h) All tanks used for the holding of aquatic animals should:
 - Be identified with permanent numbers so that records for shipments can be correlated with them.
 - Be fitted with lids or other approved coverings so as to prevent transmission of pathogens between adjacent tanks due to splash from the aeration/filter system, and to prevent the escape of aquatic animals.
 - Be arranged in a manner that permits ready access for inspection purposes, including a minimum width of 75 cm for corridors between rows of tanks or tanks and walls.
 - Other than the aquatic animals, contain only sterilizable materials (e.g. plastic) that do not interfere with inspection.

- Have at least the front transparent to provide good visibility of their contents, and be stacked for adequate viewing.
- (i) The use of shared water recirculation systems should be avoided, as it may facilitate the spread of pathogens between tanks.
- (j) All entry and exit points to the Quarantine Facility should prominently display a permanently affixed, professionally made, quarantine sign that states "Quarantine Area Authorized Persons Only". The sign should be highly visible (e.g. black lettering of 5 cm in height on yellow background).
- (k) A suitable wash-up trough should be located in the quarantine area for the cleaning and disinfecting of equipment. An approved disinfectant should be available at the wash-up trough. A suitable draining rack should be provided for air drying of equipment.
- (l) A designated refrigerator or freezer should be provided solely for the storage and preservation of dead aquatic animals. The refrigerator or freezer should be clearly identified as being for quarantine use only and located within or close to the quarantine area. If outside the quarantine area, it should be lockable.
- (m) Equipment necessary to carry out the disinfection all wastewater (both overseas water and domestic water) used in the Quarantine Facility should be supplied.
- (n) Secure storage facilities for food for aquatic animals should be provided such that contamination or infestation by pests is prevented.
- (o) A fully stocked first aid cabinet should be provided and maintained.
- (p) Amenities to be provided for use by Quarantine Officers should include access to a desk and chair, a telephone with a direct outside line, toilet facilities, hand washing facilities (within the quarantine area) and a hygienic means of drying hands, and suitable arrangements for daily cleaning of amenities.

5.4 STANDARDS OF OPERATION

5.4.1 Wastewater disposal

• Freshwater – All wastewater of domestic origin to be discharged from the Quarantine Facility that has not been used for the transport or holding of aquatic animals should directly enter an approved septic tank or municipal sewerage system, or be sterilized as described below (Chapter 5.4.2.) Sterilized wastewater may be discharged elsewhere provided that it does not flow directly into natural waterways. All wastewater, whether of overseas or domestic origin, that has been used for the transport or holding of live freshwater animals or for the cleaning of tanks and associated equipment should be disinfected using an approved method prior to disposal. Disposal of wastewater must also conform to any state and local government requirements.

• Marine – All marine wastewater (including overseas water), discharged from the Quarantine Facility should directly enter an approved septic tank or municipal sewerage system or be sterilized as described in Chapter 5.4.2. Sterilized wastewater should not be discharged directly into natural waterways.

5.4.2 Sterilization of wastewater

Where sterilization of wastewater is required before disposal, it should be sterilized using one of the following methods:

(a) Chlorination⁸

- (i) All water should pass through an approved filter capable of removing suspended organic material prior to hypochlorite treatment.
- (ii) All water should pass to a retention vessel where sufficient hypochlorite must be added to achieve a minimum concentration of 200 parts per million (ppm) (200 mg /litre) at 1 hr post-treatment. Sodium hypochlorite (bleach) should be used at 1.6 ml of hypochlorite solution (12.5 percent available chlorine) per litre of water, while calcium hypochlorite powder (e.g. Pool Chlor®, 65-70 percent available chlorine) should be used at 0.3 g of powder per litre of water.
- (iii) Before the treatment period commences, the chlorinated effluent should be brought to a pH between 5.0 and 7.0.
- (iv) Following addition of hypochlorite, wastewater should be agitated for at least 10 min to ensure thorough mixing of hypochlorite.
- (v) After a retention period of not less than 1 hr, the chlorine concentration is measured using an approved method. Tanks not achieving a minimum chlorine concentration of 200 ppm (200 mg/litre) at the allotted time should be re-treated until the requirement is met.
- (vi) The chlorine in the wastewater may be neutralized by adding sodium thiosulphate at a rate of 1.25 g (2.5 ml of 50 percent sodium thiosulphate solution) per litre of treated wastewater, then agitated for not less than 10 min before discharge.⁹
- (vii) Chlorination records should be maintained noting: the amount of compound added, the volume of effluent, the time that treatment period commenced, the pH at commencement of the treatment period, the 1 hr post-treatment concentration, the amount of sodium thiosulphate added to achieve neutralization and the residual chlorine concentration at discharge.

⁸ Chlorine compounds are corrosive, relatively unstable and are inactivated by the presence of organic matter. They can be highly toxic to fish and humans and thus must be handled and applied with due care. For a detailed review of their use as disinfectants in aquaculture, DAFF (2006) and Danner and Merrill (2006).

⁹ Theoretically, 1 mg/litre thiosulphate is required to reduce 1 mg/litre chlorine dioxide (Siemens, 2006). Thus the specified treatment would potentially reduce chlorine at levels of 1 250 mg/litre and below. To reduce the quantity of chemical required, residual chlorine level at the end of treatment may be measured and the amount of thiosulphate required for neutralization calculated.

(viii) Chlorinated water should not be discharged directly into adjacent waterways.

(b) Heat treatment

Prior to discharge, wastewater shall be heated to at least 85 °C for a minimum of 30 min. Water heating units must be approved by the CA and be fitted with temperature and flow recorders.

(c) Ultraviolet (UV) light radiation

All water to be treated should pass through an approved filter capable of removing suspended organic material prior to irradiation.

Commercial UV water treatment units operating in the spectral range of 190–280 nm (254 nm recommended) delivering doses of at least 130 mWs/cm² are required. As UV bulbs will burn long after their effectiveness has waned, the burning time of the UV lamp should be monitored, and the lamp replaced according to manufacturer's specifications

5.5 DISINFECTION OF EQUIPMENT

Before removal from the quarantine area, and before restocking with a new shipment of aquatic animals, all tanks and tank equipment should be thoroughly cleaned and disinfected with (i) hypochlorite solution at 200 ppm concentration for 5 min or with (ii) an approved iodophore solution containing iodine at 0.5 percent available iodine for 5 min or (iii) by another disinfection procedure approved by the supervising Quarantine Officer.

Filter material should be disposed of by incineration, by autoclaving and deep burial, or by another approved method.

5.6 DISPOSAL OF DEAD AQUATIC ANIMALS

Dead aquatic animals should only be disposed of as directed by the CA. Aquatic animals that have died while under quarantine should held in an approved freezer, an approved refrigerator, or preserved using another method as specified by the CA until removed for laboratory examination or released for disposal by the supervising qQuarantine Officer. Upon approval, dead aquatic animals should be disposed of by incineration or by autoclaving and deep burial.



Disinfection of equipment before and after their use in quarantine is a must.

5.7 DISPOSAL OF PACKING MATERIALS

Wet bags, boxes and cartons should be either disinfected using the methods of disinfection specified under "Disinfection of Equipment" (Chapter 5.5) or disposed of by incineration or another method approved by the supervising Quarantine Officer.

5.8 WORK PRACTICES

5.8.1 Cleanliness and sanitation

The Quarantine Facility and holding tanks should be kept clean at all times. Adequate cleaning facilities and equipment (e.g. pressurized water supplies, brooms, shovels, etc.) should be provided to enable staff to maintain appropriate standards of hygiene.

No animals or plants other than the aquatic animals being quarantined and their live food should be permitted in the quarantine area.

Handling of all packaging material used to transport live aquatic animals should comply with the following procedures:

- Damaged bags, damaged polystyrene boxes and cartons that are wet or contaminated with overseas water should be either incinerated or disinfected by an approved method prior to disposal (Section 5.2).
- Imported bags and polystyrene boxes containing leaked oversea water that are in good condition may be reused provided they are first disinfected by an approved method.
- Boxes and cartons that are free of overseas water may be reused without disinfection.

The use of dedicated equipment (nets, cleaning equipment, etc.) for each individual tank (or tanks connected by a shared water recirculation system) is recommended. At minimum, all nets and other equipment should be disinfected by an approved method of disinfection before being moved between tanks housing different consignments and before removal from the quarantine area.

All equipment, footwear and protective clothing used in the quarantine area should be restricted to this site. Equipment should only be removed from the quarantine area after cleaning and disinfection in an approved manner.

The operator should provide protective clothing (waterproof apron and footwear) to staff and visitors to use in the facility. Protective footwear (e.g. gumboots) and aprons should be kept inside the quarantine area (street footwear left outside the quarantine area). Prior to protective footwear or clothing being removed from the quarantine area, they should be cleaned using an approved disinfectant such as Betadine[®] (5 percent solution). Disposable overshoes may



All packing material should be properly disposed.

used provided they are destroyed after use by incineration or by autoclaving followed by deep burial.

All wastewater disposal should meet any state and local government requirements, followed the specifications for disposal given in Chapter 5.4.1, and should not flow directly into natural waterways.

All filter material should be disinfected prior to removal from the quarantine area or disposed of by incineration or by autoclaving and deep burial. Staff and visitors who have had contact with water or aquatic animals should wash their hands and forearms with soap and water prior to exiting the Quarantine Facility.

5.9 HANDLING OF AQUATIC ANIMALS

Upon arrival of a shipment of aquatic animals at an approved port of entry, and following verification of the accuracy of details of the consignment and its preliminary inspection by customs officers, the consignment should be transferred to the custody of the operator, who should guarantee its secure transport, under quarantine conditions, to the Quarantine Facility.

Upon their arrival at the Quarantine Facility, freshwater animals should be transferred to new water and the overseas water should be subjected to an approved disinfection treatment (Section 5.4.2). Each tank used to contain freshwater aquatic animals should only contain a single species of animal, and must be kept separate and isolated from animals from other shipments.

Each tank used to contain marine animals may contain different species but only from the same shipment.

The use of shared water recirculation systems is not recommended. If separate shipments of aquatic animals share a common water recirculation system, then no aquatic animals should be approved for release from quarantine until the last shipment to enter the system has satisfactorily completed its quarantine requirements. In the event that diseases or pests of quarantine concern are known or suspected, all aquatic animals sharing the same water recirculation system should be subject to additional quarantine risk management measures, including their destruction, treatment or detention beyond the normal quarantine period.

If consignments of aquatic animals having different quarantine periods are held in the same room, the operator should ensure that:

- each consignment is held in a discrete group of tanks that are physically separated from tanks holding animals from another consignment and at a distance sufficient to prevent splashing of water between tanks of different groups;
- each group of tanks should have its own set of dedicated equipment (nets, feeding equipment, thermometers etc.); and
- each group of tanks should be clearly marked to show the date of arrival of the aquatic animals.

In the event that all or part of a consignment of imported aquatic animals is incorrectly identified or listed by the exporter and includes species not on the list of ornamental aquatic animals approved for importation, the operator should notify the supervising Quarantine Officer within seven days of importation. Unapproved species should either be re-exported by the operator or destroyed under supervision of the Quarantine Officer.

The progeny of imported aquatic animals that breed during the quarantine period may be removed to another tank in the facility but should be subject to all quarantine conditions that applied to the parents.

A standard Tank Record Sheet should be maintained for each tank (Chapter 5.11.2).

Periodically throughout the day, the operator should observe all aquatic animals held in the Quarantine Facility for signs of illness and abnormal behavior.

Any sudden or unusually high levels of mortality or changes in behavior (levels of mortality or illness above 20 percent of a tank over a five-day period) or unusual signs of disease, parasites or pests should be immediately reported to the supervising Quarantine Officer.

All dead aquatic animals should be held for inspection by a Quarantine Officer. All animals from a given consignment that are found dead on arrival or that die during the quarantine period should be placed in a labeled plastic bag as soon as possible and kept under refrigeration or preserved as specified by the Quarantine Officer until diagnostic examination can be completed.

Information on labels should identify the shipment, species, tank number and day of death.

Any equipment that has been in contact with dead aquatic animals should be disinfected before re-use.

The use of any drug or chemical to treat aquatic animals should have the prior approval of the CA and be recorded on tank record sheets. The use of any treatments may result in extension of the quarantine period or other measures considered necessary by the supervising Quarantine Officer.

The operator should ensure that no aquatic animals leave the quarantine area under any circumstances without the approval of the supervising Quarantine Officer (i.e. the granting of a biosecurity clearance), excepting dead animals moved to a nearby lockable refrigerator or freezer.

On completion of quarantine, freshwater aquatic animals should be transferred into clean water prior to release from the Quarantine Facility.

Aquatic animals should be removed from the quarantine area following their satisfactory completion of the quarantine period and the issuance of a biosecurity clearance by the Competent Authority stating that all requirements specified by the aquatic animal import health standard have been met.

5.10 OCCURRENCE OF AN OUTBREAK OF A SERIOUS EXOTIC DISEASE

If a serious exotic disease is diagnosed, the operator should be immediately notified. In such cases, the supervising Quarantine Officer or other representative

Busy quarantine facilities receive many samples regularly and adequate number of trained staff is essential to implement effective quarantine.



of the Competent Authority may direct the management of disease control. Disease control measures may include the extension of quarantine and/or the destruction of stock.

Measures to be taken are likely to include:

- destruction of infected consignments or of all aquatic animals present in the facility at the time of the outbreak, and their sanitary removal and incineration;
- decontamination of the interior of the facility, all tanks and equipment, and all waters present in the facility at the time of the outbreak; and
- approval of the CA prior to the reuse of the facility.

5.11 RECORD KEEPING REQUIREMENTS

5.11.1 Documentation and summary records

Summary records (electronic or manual) and associated original documents (shipping bills, health certificates, biosecurity clearance, etc.) for all shipments of aquatic animals entering the Quarantine Facility should be maintained. The operator should, for auditing purposes, maintain such records and documents for a minimum period of 36 months after release of the aquatic animals from quarantine, during which time they will, upon request, be readily made available to a Quarantine Officer. The following summary information should be recorded for each consignment:

- overseas supplier and country of origin;
- dates of arrival;
- number of each aquatic species, in total and by tank;
- details of any accompanying health certificates;
- details of any clinical signs of disease, and number of affected aquatic animals, by species and tank;
- details of any mortalities, by species and tank;
- details of any treatments approved/ applied;
- biosecurity clearance and date of release from quarantine; and
- records of internal and external audits and corrective actions.



If a serious exotic disease is diagnosed during official quarantine, the operator should be immediately notified.

5.11.2 Tank record sheets

A corresponding approved Tank Record Sheet should be maintained for each holding tank and should be kept up to date at all times. Tank Record Sheets should be retained for a minimum of 36 months following release of shipments from quarantine. This sheet should display the following information:

- quarantine entry number;
- tank number;
- number and species of aquatic animals in tank;
- exporter identification details including country of export;
- importer's name;
- date of arrival;
- shipment or airway bill number;
- number/species of aquatic animals dead on arrival;
- details of any observed disease conditions and number of sick aquatic animals;
- daily record of number of aquatic animal deaths in tank;
- details of any prophylactic or therapeutic treatments given;
- disposal details;
- disinfection details;
- signature of authorizing Quarantine Officer and date released; and
- number of aquatic animals released.

5.12 LOGBOOKS

Details of wastewater treatment (including chlorination records, if applicable); filter cleaning, replacement or disposal; internal audit; and general maintenance should be recorded in an operations logbook.

A visitor's logbook that is used by the operator to record the name and address of any visitors and the visit date should be placed near the entrance to the facility.

5.13 AUDITING

The operator should undertake systematic periodic internal audits at least on a semi-annual basis, to ensure that the standards for the operation of the Quarantine Facility are maintained and to identify and correct any deficiencies. The operator should record in the logbook, any variations from the prescribed criteria encountered and the corrective measures taken.

Periodic external audits of the Quarantine Facility should be conducted by the supervising Quarantine Officer or other approved personnel to verify the security and proper functioning of the facility.

5.14 SECURITY

Control and security of the Quarantine Facility are the responsibility of the operator. The Quarantine Facility should be securely locked when not in active use or when unattended.

Procedures should be adopted to ensure that access to the premises is limited to authorized persons only. A prominent sign should be displayed at the entrance to the facility to show that it is a Quarantine Facility and that unauthorized entry is prohibited. Unnecessary entry of staff and visitors into the Quarantine Facility should be avoided. The operator should ensure that the visitor's logbook is properly maintained.

5.15 CONTINGENCY PLANS

The operator should develop a contingency plan addressing actions to be taken in the event of a vehicle breakdown during transport of aquatic animals from customs arrival to the Quarantine Facility, and due to on-site emergencies that may arise, such as fire, flood, electrical failure or breakdown of essential equipment (aerators, pumps, etc.). In the case of emergency, the supervising Quarantine Officer should be notified as soon as possible.

6. Recommended procedures for quarantine of aquatic animals in aquaculture production facilities

6.1 GENERAL

This Chapter presents recommended procedures for the application of quarantine in aquaculture production facilities using as an example, requirements for the quarantine of penaeid shrimp broodstock in a commercial hatchery production system modified from FAO (2003, 2006). Operators of production facilities for finfish, molluscs and other crustaceans should be able to adapt this guidance to their particular needs and available resources.

Quarantine is one component of a production-level biosecurity programme, which includes a set of standard operating procedures (SOPs) and is an essential part of good hatchery or farm management. A biosecurity programme for a shrimp hatchery should include the following elements:

- use of disease-free and healthy shrimp stocks;
- use of quarantine areas for all incoming stock;
- analysis of all incoming stock for disease (i.e. through polymerase chain reaction (PCR) or other immunodiagnostic technology);
- treatment of all incoming water sources to eliminate pathogens;
- sterilization and maintenance of clean equipment and materials;
- use of personal hygiene measures including washing of hands, feet and clothing;
- knowledge of potential pathogens, the sources of risk and methods for their control and/or eradication;
- development and use of stocks that are resistant to specific pathogens (SPR); and
- maintenance of optimal environmental conditions within all phases of the facility.

The quarantine of all broodstock to be introduced into the hatchery is an essential biosecurity measure. Before being introduced into the production system, the broodstock should be held in quarantine and screened for subclinical viral infections (i.e. by PCR). Broodstock infected with serious



Biosecure Litopenaeus vannamei *broodstock maturation facility.*

untreatable diseases such as those listed by the World Organisation for Animal Health (OIE, 2006a) should be immediately destroyed and only animals testing negative for important pathogens should be transferred to the maturation unit.

A well-run hatchery should ensure that all water discharged from the facility is free from pathogens.

6.2 PERIOD OF QUARANTINE

The quarantine period will vary depending on the time required to complete the health screening procedure. In all cases animals should be kept under observation in the Quarantine Facility until all tests are completed and each shrimp's health status is known.

6.3 STANDARDS OF CONSTRUCTION

6.3.1 General

A well-designed shrimp hatchery will consist of separate facilities for quarantine, maturation, spawning, hatching, larval and postlarval (PL) rearing, indoor and outdoor algal culture (where applicable), hatching of *Artemia* and feed preparation. Additionally there should be supporting infrastructure for the handling of water (facilities for abstraction, filtration, storage, disinfection, aeration, conditioning and distribution), laboratories for disease diagnosis/bacteriology, as well as areas for maintenance, packing of nauplii and PL, offices, storerooms and staff living quarters and facilities.



Use of personal hygiene measures is important in maintaining biosecurity.

6.3.2 Location of quarantine facilities

The area designated for broodstock quarantine should be completely isolated from the rest of the maturation and hatchery facilities, since it is an area having a high risk for disease transmission. Isolation includes the spatial separation of the buildings as well as the separation of water and air lines. If this is not possible, the hatchery design should be altered so that there is no possibility of contamination from the quarantine or holding area into the other production areas.

6.3.3 Specific construction and equipment requirements

The quarantine facility should be in an enclosed and covered building with no direct access to the outside. It should be adequately isolated from all rearing and production areas to avoid any possibility of cross-contamination.

Facilities for disinfection of feet (footbaths containing hypochlorite solution at >50 ppm active ingredient) and hands (bottles containing povidone iodine

(20 ppm and/or 70 percent alcohol) should be provided for use upon entering and exiting the unit.¹⁰

Entry into the quarantine facility should be via a staff dressing room equipped with lockers and shower facilities. This room should lead into a second dressing room where working clothes and boots are stored, which in turn, leads into the quarantine area proper.

Typically, individual holding tanks of >100 litres per individual broodstock should be provided.

Plastic buckets and other similar containers should be available in adequate numbers in the quarantine room to facilitate effective daily routine movement of shrimp in and out of the facility.

The Quarantine Facility should have an independent supply of water and air, with separate treatment and disinfection systems and a system for the treatment of effluents to prevent the potential escape of pathogens into the environment (Section 6.4.).

All implements used in the Quarantine Facility should must be clearly marked and should remain in the quarantine area. Facilities for disinfection of all equipment at the end of each day should be available.

6.4 STANDARDS OF OPERATION

Entrance to the quarantine area should be restricted to the personnel assigned to work exclusively in this area.

Quarantine staff should enter through a dressing room where they remove their street clothes and take a shower before entering a second dressing room to put on working clothes and boots. At the end of the working shift, the sequence is reversed.

The individual sections of the quarantine area should be designated "dirty" or "clean"



Closed biosecure L. vannamei hatchery.

depending on whether they contain shrimp that are not yet screened for infection (pre-testing) or that have been passed (post-testing). Shrimp should only move one way, from the "dirty" to the "clean" sections of the Quarantine Facility and all movements should be controlled to ensure no mixing between the two areas.

To avoid having to discard entire batches due to individual infection, potential broodstock should be held in isolation (unless they are SPF), at least until their disease status is ascertained, and preferably at all times in the Quarantine Facility.

On introduction into the quarantine area, the broodstock must be well acclimatized, the duration of acclimatization depending upon the temperature and salinity of the transport water.

¹⁰ Once iodophor solutions have lost their brown colour to become colourless, they are no longer active and should be replaced (DAFF, 2006).

The receiving quarantine tanks should be prepared at least one day ahead of arrival to match the expected conditions in the water of the arriving shrimp. Upon arrival the water quality in the tank receiving the broodstock should be checked to ensure that it is acceptable and that the salinity, temperature and pH are the same as that in the transportation bags. The still-closed bags are then floated in the receiving tank until the temperature inside and outside the bags is the same.¹¹ Then the bags are opened and an airstone connected to a low flow of air (or preferably oxygen) inserted. The bags are gradually filled with water from the tank over a 20–60 min period. After this time, the broodstock should be taken from the bag and passed through a dip of povidone iodine solution (20 ppm), potassium permanganate (100 ppm) or formalin (50–100 ppm) for 30–60 s and then released into the receiving tank.

Fresh (sterilized) or pelleted feeds are fed as for the maturation systems, feeding little and often to demand so as to maintain water quality. High quality feeds to demand should be immediately offered, as the shrimp may be hungry. Over the next few hours, the temperature of the receiving tanks is gradually allowed to increase to ambient (which should be 27–29 °C) at a rate of <2 °C/hr and (if required) the salinity to normalize to ambient (which should be 30–35 ppt) at <2 ppt/hr.

Handling of broodstock shrimp should be reduced to a minimum at all times and dissolved oxygen concentrations maintained at saturation. If shrimp arrive healthy but begin dying after a few days, they usually have high levels of bacteria in the haemolymph. After confirmation this can sometimes be reduced using 5–7 daily antibiotic baths (10 ppm oxytetracycline) or by feeding diets containing 1–2 ppm oxytetracycline. The health of the gills should be monitored regularly and if excessive fouling by algae or filamentous bacteria is found, treatment in an aerated bath with 0.1 ppm of copper control (based on copper sulphate, CuSO₄), or if epicommensal protozoans are found, application of a one-hour aerated bath treatment with 30–50 ppm formalin is indicated. Any shrimp having serious melanized (black) lesions on the body, large areas of white muscle or bright red coloration should be discarded immediately before they infect the others.

Prophylactic treatment of broodstock with formalin at 50–100 ppm for 30–60 min under strong aeration should be conducted before introducing the stock into the maturation/hatchery systems. Only spawners free from pathogens such as those viruses listed by the World Organisation for Animal Health (OIE, 2006a) should be transferred into the maturation/hatchery systems.

Staff working in the quarantine area should not be permitted to enter other production sections and should follow sanitary protocols at all times.

Used plastic containers and hoses should be washed and disinfected with hypochlorite solution (20 ppm) before reuse.

¹¹ A decision based on operator experience may be required. In cases where water quality within the bag may be poor due to e.g. a lengthy transport period, it may be better to open the bag immediately.

6.4.1 Treatment of intake water

Seawater to be used in the facility should enter a storage tank where it should be treated with hypochlorite solution (30 ppm active ingredient for not less than 30 min) before inactivating with sodium thiosulphate (1 ppm for every 1 ppm of residual chlorine) and strong aeration.

Water quality requirements in the quarantine system are a temperature of 27-29 °C, salinity of 29-34 ppt and a pH of 7.8-8.5, maintained by 200-300 percent of water (filtered to <20 µm) exchange daily (preferably on

a flow-through rather than rapid-change basis), permitting adequate feeding of the broodstock while maintaining optimal and stable water quality.

6.4.2 Treatment of effluent water

Wastewater from the quarantine facility should be released into a special concrete or lined sedimentation tank. From the sedimentation tank, it should overflow into a treatment tank where it should be chlorinated (>20 ppm active chlorine for >60 min or 50 ppm for >30 min) and then dechlorinated through aeration or neutralization using sodium thiosulphate to achieve a residual chlorine concentration < 5 ppm prior to discharge.

6.4.3 Disposal of dead animals

All mortalities or infected animals should *farms*. be incinerated or disposed of in another approved manner.



Inlet water filters are now commonly used in shrimp farms.
7. Conclusions

Quarantine is an important risk management measure that can be applied to reduce the risk posed by serious aquatic animal diseases when aquatic animals are moved internationally or domestically between different regions or zones, or when new broodstock or other life cycle stages are introduced into hatcheries and other aquaculture production facilities.

For international movements, the decision to require pre-border, border and/ or post-border quarantine of live aquatic animals should be made based on risk analysis, and stringency of quarantine to be applied should be commensurate with the estimated risk. The first movement (introduction) of a new species (an exotic) is likely to require use of highly stringent protocols, such as those outlined by ICES (2005).

The quarantine of broodstock and other life cycle stages entering aquaculture production facilities can be routinely applied to reduce the likelihood of introducing serious diseases to the facility that will cause morbidity, mortality and associated production and financial losses.

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ANNEX 1 Policy for the importation of Atlantic salmon into British Columbia

INTRODUCTION

The introduction of new disease agents or new strains of enzootic disease agents has caused significant economic losses to countries around the world with industries that depend upon the harvest of wild and cultured fish.

The Protocol described below has been developed in recognition of the importance of protecting the commercial fisheries, recreational fisheries, and cultured fish stocks of British Columbia from the importation of non-indigenous disease agents and parasites such as, but not limited to:

- Infectious Pancreatic Necrosis Virus,
- Viral Hemorrhagic Septicemia Virus,
- Exotic strains of the Infectious Hematopoietic Necrosis Virus,
- The causative parasite of whirling disease,
- Erythrocytic Inclusion Body Syndrome,
- Atypical Aeromonas salmonicida_(furunculosis).

Proposals to import Atlantic salmon into British Columbia will be assessed and authorized by the Department of Fisheries and Oceans (DFO) on the basis of their compliance with this protocol. DFO will consult with provincial fisheries agencies (Ministry of Agriculture, Fisheries and Food; Ministry of Environment, Lands and Parks) on all decisions made in regard to this Policy.

PROTOCOL

All importations must comply with the Canadian Fish Health Protection Regulations (CFHPR). All applications to import eggs or milt must be received at least 45 days prior to the "taking of the eggs or milt" destined for B.C.

Only surface-disinfected, fertilized eggs will be imported. No live fish or unfertilized eggs will be allowed. Milt may be allowed if:

- a) the broodstock from which the milt is collected complies with the CFRPR; and
- b)100 % of the ripe males from which milt is collected are lethally sampled for the viral disease agents of concern; and
- c)eggs fertilized with imported milt must be held under quarantine and isolation as outlined below.

- 1. Egg imports will be allowed only from broodstock that has been held in captivity by the source company for one full generation. The eyed eggs must arrive in B.C. a minimum of 15 days before hatch.
- 2. Importations will only be allowed from:
 - a) facilities inspected and approved by a Local Fish Health Officer (LFHO)¹ at least 15 days prior to receipt of eggs or milt in B.C.;
 - b)facilities operated within a program of regular fish health monitoring and documentation by an CFHPR approved fish health official;
 - c) freshwater facilities that use a fish-free ground water supply and isolation areas for egg incubation; and
 - d)facilities that provide access to complete fish health, mortality, pedigree and production records for the facility and stock from which the sex products destined for B.C. have been collected.
- 3. The acceptability of a facility as a source of Atlantic salmon eggs and milt will be determined by a LFHO and will be based upon the extent and reliability of the available fish health data and disease histories for all species held in the facility. The acceptability will also depend upon the extent and reliability of the fish health data and disease history of the watershed in which the facility or fish holding units are located.
- No importations will be allowed from facilities or sites:
 - a) in which a salmonid pathogen not known to occur in B.C. exists;
 - b)in which a fish pathogen exists that has been designated as a problematic strain by a LFHO because of drug resistance or enhanced pathogenicity;
 - c) that do not take measures designed to prevent the movement, importation, control, and eradication of fish disease of concern to B.C.
 - 4. All imported Atlantic salmon eggs must be held under strict quarantine immediately upon their arrival in B.C. in an approved facility. The quarantine procedures will consist of:
 - a) disinfection of the facility's effluent until all stocks of fish under quarantine have reached an average size of 3 grams. The minimum length of time that the fish are quarantined with effluent disinfection shall not be less than 120 days. The quarantine period must be followed by total isolation of the fish from all other fish in the facility until they are introduced to sea water. The isolation must be carried out in the quarantine facility or an approved alternate land based facility.
 - b) discharge of all effluent from the facility "to ground" in an approved manner for the entire quarantine period. The maximum volume of effluent that can be "discharged to ground" on the lands of the quarantine facility must be determined by a qualified hydrologist approved by DFO.
 - c) testing by a DFO approved laboratory of 30 moribund and dead fish every
 4 weeks during the quarantine period in addition to the examination of 120

¹ as defined in Chapter 1 of the CFHPR; appointed by the Minister of Fisheries and Oceans for Canada.

fish per stock 2 weeks prior to the introduction of each stock to sea water. Additional spot checks may be carried out on moribund and dead fish by a LFHO. As well, additional fish health testing may be required at anytime by the LFHO.

- d) no live fish may be removed from the quarantine facility until all fish and eggs in the facility have met the minimum quarantine requirements and the written approval of a LFHO has been obtained.
- e) all dead fish or fish eggs must be placed in a solution of 10% formalin for a minimum of 5 days before removal from the quarantine facility.
- 5. The importers of the fertilized Atlantic salmon eggs or milt shall bear all costs of the inspectors described in Chapter 4, all costs of the testing of fish described in Chapter 6(c), and all costs of the hydrological testing and surveys required under Chapter 6(b).
- 6. All companies operating a quarantine/isolation facility must agree to enter into a legal contract with DFO regarding the design, operation, and monitoring of the facility.
- 7. If a disease agent of concern to DFO is detected in the imported stocks during the quarantine and isolation period, all stocks within the quarantine/ isolation facility must be destroyed and a full program of facility disinfection carried out.
- 8. All mortality records and the results of all routine or clinical testing carried out on the quarantined or isolated fish stocks must be submitted every 4 weeks to DFO. The results of tests carried out under 6(c) by independent laboratories must be reported to DFO within 48 hours of their completion.
- 9. All requests for permission to import live Atlantic salmon eggs and/or milt into B.C. must be addressed to the Transplant Committee DFO, Pacific Biological Station, Nanaimo, B.C. V9R 5K6.

ANNEX 2

Disinfectants and disinfecting procedures mentioned in this manual

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Chemical or treatment (generic/trade names)	Use	Effective concentration/ level	Application	Notes	Manual reference (section)
Chemicals					3.4
Alcohol (ethanol)	disinfecting hands	70%	apply directly to hands	replenish bottles as needed	6.3.3
lodine (iodophore, povidone iodine, Betadine®, Wescodine®)	a) disinfecting equipment, aprons, footwear	a) 0.5% available iodine	a) minimum treatment time of 5 min		4.3, 4.5, 4.6.1, 5.5, 5.8.1
	b) footbaths	b) 200 mg iodine/ litre	 b) treatment time of a few seconds 	b) replenish footbaths regularly & record in logbook. Discard colorless iodophor solutions as they are no longer active	4.6.1
	c) disinfecting hands	c) 20 ppm povidone iodine	c) a few seconds	c) replenish bottles as needed	6.3.3
	d) surface disinfection of shrimp broodstock upon entry to quarantine unit	d) 20 ppm povidone iodine	d) 30–60 s dip	d) immediately transfer broodstock to clean water in receiving tanks following treatment	6.4

DISINFECTANTS AP	ND DISINFECTING PROCE	DURES MENTION	IED IN THIS MANUAL (c	ontinued)	
Chemical or treatment (generic/trade names)	Use	Effective concentration/ level	Application	Notes	Manual reference (section)
Sodium hypochlorite (hypochlorite, Chlorox®, bleach)	a) sterilizing wastewater & transport water	a) Final chlorine concentration > 200 ppm (200 mg/litre)	a) 1.6 ml hypochlorite solution (12.5% available chlorine)/ litre of water followed by agitation for at least 10 min & retention for at least 1 h	a) filter wastewater to remove organic material test pH of water to be treated and adjust to between 5.0–7.0, if necessary test level of residual chlorine to ensure conc. of 5 mg/litre or less before discharge. Retreat if necessary.	3.4,4,2, 5.4.2
	b) disinfecting equipment, boxes, cartons & other potentially contaminated material intended for reuse	b) As above	b) treat for a minimum of 5 min	b) thoroughly clean items before disinfection	4.3, 4.5, 5.5
	c) footbaths			 c) replenish footbaths regularly & record in logbook 	4.6.1, 6.3.3
	d) disinfecting hands	d) > 50 ppm active ingredient			6.3.3
	e) disinfecting plastic containers & hoses in shrimp broodstock quarantine units	e) 20 ppm			6.4
	f) disinfecting intake seawater for use in shrimp broodstock quarantine unit	f) 20 ppm active ingredient	f) not less than 30 min	f) treatment conducted in intake water storage tank; neutralize with sodium thiosuphate at 1 ppm for every 1 ppm of residual chlorine at strong aeration	6.4.1
	g) treating effluent waters from shrimp broodstock quarantine units	g) >20 ppm or > 50 ppm active chlorine	g) >60 min or >30 min	g) neutralize as above	6.4.2

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Chemical or treatment (generic/trade names)	Use	Effective concentration/ level	Application	Notes	Manual reference (section)
Calcium hypochlorite (hypochlorite powder, Pool Chlor® (65–70% available chlorine))	Sterilizing discharge water	a) final chlorine concentration > 200 ppm (200 mg/litre)	a) 0.3 g/litre water followed by agitation for at least 10 min & retention for at least 1 hr		3.4,4,4,2, 5.4.2
		b) as above		b) replenish footbaths regularly & record in logbook also uses e, f & g under sodium hypochlorite.	4.6.1, 6.3.3
Copper sulphate (CuSO _{4,} copper control)	disposing of dead aquatic animals, filter material, wet bags, boxes, cartons & other burnable potentially contaminated materials	0.1 ppm	aerated bath		6.4
Formalin	disposing of dead aquatic animals, filter material, wet bags, boxes, cartons & other potentially contaminated materials	a) 50–100 ppm	a) 30–60 s, as dip	 a) immediately transfer broodstock to clean water in receiving tanks following treatment 	6.4
		b) 30–50 ppm	b) 1 h aerated bath		6.4
		c) 50–100 ppm	c) 30–60 min, with strong aeration		6.4
Oxytetracycline	a) Decontaminating small equipment, filters etc.	a) 10 ppm	a) as bath, 5–7 times per day	identification of the bacteria involved & antibiotic sensitivity testing strongly	3.4, 6.4
	b) Disposing of dead aquatic animals	b) 1–2 ppm	b) in feed	recommended.	
Potassium permanganate (KMnO4)	external disinfection of shrimp broodstock upon arrival at quarantine	100 ppm	30–60 sec, as dip	immediately transfer broodstock to clean water in receiving tanks following treatment	6.4
Sodium thiosulphate (photographic hypo)	neutralizing chlorine in wastewater or transport water prior to discharge		1.25 g (2.5 ml of 50 % sodium thiosulphate solution/litre followed by agitation for not less than 10 min before discharge		3.4, 4.4.2, 5.4.2, 6.4.1, 6.4.2

DISINFECTANTS AND DISINFECTING PROCEDURES MENTIONED IN THIS MANUAL (continued)

DISINFECTANTS A	ND DISINFECTING PROC	EDURES MENTION	ED IN THIS MANUAL	(continued)	
Chemical or treatment (generic/trade names)	Use	Effective concentration/ level	Application	Notes	Manual reference (section)
Irradiation					
Ultra-violet light	disinfecting waste water & transport water	>130 mWs/cm ²	commercial UV water treatment units operating in the spectral range of 190–280 nm (254 nm recommended) are required.	filter all water to be treated to remove all suspended organic material prior to irradiation. monitor burning time of UV lamp & replace as per manufacturer's specifications	4.4.2, 5.4.2
Physical					
Filtration	removing suspended organic matter prior to wastewater or transport water sterilization			filter to be approved by Competent Authority. Record filter changes in logbook.	4.4.1,4,4,2, 4.3, 4.6.1, 4.8.3, 5.4.2, 5.8.1, 5.12
Heat treatment	Sterilizing discharge water		85°C for not less than 30 min	heating units must be fitted with temperature & flow recorders & approved by Competent Authority	4.4.2
Incineration	disposing of dead aquatic animals, filter material, wet ba boxes, cartons & other burnable potentially contaminated materials	e s,		incineration facility to be approved by Competent Authority.	4.4, 4.5, 4.6.1, 4.6.2, 4,7, 5.6, 5.7, 5.8.1, 5.10, 6.4.3
Deep burial	disposing of dead aquatic animals, filter material, wet bags, boxes, cartons & other potentially contaminated materials			burial site to be approved by Competent Authority.	4.4, 4.5, 4.6.2, 5.6, 5.8.1
Autoclaving	 a) Decontaminating small equipment, filters etc. b) Disposing of dead aquatic 				4.3, 4.6.1, 4.6.2, 5.5, 5.6, 5.8.1
	animals				4.4, 4.6.2

Quarantine is an important risk management measure and a key activity that should be considered when developing national strategies on aquatic animal health management. This manual outlines the technical requirements for setting up quarantine facilities at three levels, based on the general level of risk (as determined by risk analysis) represented by the specific consignment of aquatic animals being moved: (i) the quarantine of "high risk" species (e.g. aquatic animals being moved either internationally [introductions and transfers] or domestically between regions of different health status) that are destined for use in aquaculture, capture fishery development or other applications where release or escape of animals or any pathogens they may be carrying into the natural environment is likely to occur; (ii) the quarantine of "lower risk" species (e.g. aquatic animals destined for the ornamental trade) to improve biosecurity for aquatic animals whose trade is an established practice; and (iii) the routine quarantine of aquatic animals at production facilities (e.g. new, domestically produced or locally captured broodstock or juveniles or animals whose movement has been contingent upon additional, more stringent, risk management measures, such as the use of specific pathogen free stocks, international health certification and pre-border and/or border quarantine).

