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# Health management and biosecurity maintenance in white shrimp *(Penaeus vannamei)* hatcheries in Latin America

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by
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# PREPARATION OF THIS DOCUMENT

This document, Health management and biosecurity maintenance in white shrimp (*Penaeus vannamei*) hatcheries in Latin America, presents technical guidance for the effective and responsible operation of shrimp hatcheries in Latin America. This document was compiled through an extensive consultative process undertaken from 2001 to 2003 that involved inputs from government-designated National Coordinators, regional and international experts, representatives from several intergovernmental organizations, private sector representatives and the Food and Agriculture Organization of the United Nations. This process was made possible through the FAO Regional Technical Cooperation Programme project - Assistance to health management of shrimp culture in Latin America: TCP/RLA/0071 (A), which involved the participation of 14 countries of the region, several intergovernmental organizations, shrimp hatchery operators and farmers, and individual experts. It is envisaged that this document will provide a firm basis for the improvement of the health and quality of hatchery-produced *Penaeus vannamei* postlarvae in Latin America.

## Distribution

Shrimp hatchery operators and managers
Ministries and Directorates of Fisheries
FAO Fishery Regional and Subregional Officers
FAO Fisheries Department

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## ABSTRACT

Aquaculture is an important food-producing sector, and it provides much needed protein, employment, income and livelihoods support to many people in the world. Shrimp, in particular, is a high value commodity that is mainly produced in Asia and Latin America, especially for export purposes, and brings a wealth of revenue to many developing countries in those regions. Over the past decade, there have been considerable problems in shrimp aquaculture, mainly due to viral diseases. Latin America, in particular, where *Penaeus vannamei* is the main species produced, has been suffering from severe viral disease problems since the early 1990s. During the efforts to find lasting solutions to the disease problems affecting *P. vannamei* culture in Latin America, it was perceived that stocking with healthy postlarvae is a key factor for achieving better survival during production. However, to successfully produce healthy postlarvae requires a clear understanding of the basic principles of sound health management and hatchery biosecurity.

This document provides technical guidance on how to improve the health and quality of postlarvae produced in hatcheries through improved facility maintenance and husbandry, broodstock maturation, larval rearing, feeding, water quality management, biosecurity and health management, using interventions at different points of the hatchery production process. The document also provides valuable information on how Standardized Operating Procedures (SOPs) and Hazard Analysis Critical Control Point (HACCP) type interventions can be applied during hatchery production of *P. vannamei* postlarvae. This document is expected to facilitate the efforts of hatchery operators and managers to produce quality, disease-free, healthy *P. vannamei* postlarvae, thus improving overall production and the sustainability of white shrimp aquaculture.

# Preface

The Food and Agriculture Organization of the United Nations (FAO) is pleased to present this document entitled "Health management and biosecurity maintenance in white shrimp (*Penaeus vannamei*) hatcheries in Latin America" which was developed by representatives from 14 Latin American countries, and scientists and experts on shrimp hatchery production and health management, as well as by representatives from several regional and international agencies and organizations[1].

This document, a product of the FAO Regional Technical Cooperation Programme (TCP) project - Assistance to health management of shrimp culture in Latin America, provides valuable guidance for efforts in reducing the risks of disease in hatchery production of *P. vannamei* and subsequent increase in production. It will also provide opportunities for improving overall biosecurity in the hatchery systems, which is pivotal in ensuring a healthy production process. Improved hatchery practices and processes contributing to increased production of white shrimp in Latin America will address the overall objectives of improving rural livelihoods, generating income, providing employment and increasing food security of countries in Latin America.

The countries that participated in the development of this document are: Belize, Brazil, Costa Rica, Colombia, Cuba, Ecuador, El Salvador, Guatemala, Honduras, Mexico, Nicaragua, Panama, Peru and Venezuela.

This document refers to various disinfection protocols and practices used during the hatchery postlarval production process in Latin America. These procedures and protocols include the use of various chemicals and disinfectants. The chemical concentrations and exposure times given in this document are based on the existing practices in Latin America. FAO promotes the safe and responsible use of chemicals and disinfectants in aquaculture as part of an effort to reduce negative environmental impacts and improved human health safety. Persons who are using this document are encouraged to be considerate and responsible in the use of chemicals and disinfectants and are also encouraged to refer to OIE Guidelines on disinfection in shrimp aquaculture (OIE 2003).

FAO extends special thanks to all the governments, agencies and organizations that took part in this endeavour, as well as to all the individuals who generously contributed their time, effort and expertise to the compilation of this document and other information produced during the process.

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[1]See Annex I for the list of persons, agencies and organizations that participated in the development of this document.

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# Abbreviations and acronyms

| | |
|---|---|
| APEC | Asia-Pacific Economic Cooperation |
| BP | Baculovirus Penaei |
| CCP | Critical Control Point |
| CV | Coefficient of variation |
| EDTA | Ethylene diamine tetra acetic acid |
| FAO | Food and Agriculture Organization of the United Nations |
| HACCP | Hazard Analysis Critical Control Point (HACCP) |
| IHHN | Infectious Hypodermal and Haematopoietic Necrosis |
| LAN | Local Area Network |
| NACA | Network of Aquaculture Centres in Asia-Pacific |
| NC | National Coordinator |
| OIE | Office international des épizooties |
| PCR | Polymerase Chain Reaction |
| PL | Postlarvae, Postlarval |
| PVC | Poly Vinyl Chloride |
| SEMERNAP | Secretariá de Medio Ambiente, Recursos Naturales y Pesca (Environment, Natural Resources and Fishery Ministry) |
| SOPs | Standard Operating Procedures |
| SPF | Specific Pathogen Free |
| SPR | Specific Pathogen Resistant |
| SPT | Specific Pathogen Tolerant |
| TSV | Taura Syndrome Virus |
| UV | Ultra Violet |
| WSSV | White Spot Syndrome Virus |
| YHV | Yellow Head Virus |

# 1 Introduction

Disease has become a major constraint to shrimp aquaculture in Latin America. Especially since the outbreak of white spot disease (caused by the white spot syndrome virus, WSSV), shrimp production has decreased significantly in many countries and farmers are facing serious difficulties in continuing production. The resulting economic losses and their impacts are now significantly affecting national economies and the livelihoods of poorer sectors. For example, the shrimp exports from Ecuador in December 1999 fell to below 1985 levels. Provision of assistance for combating this situation is considered highly appropriate and timely. Such assistance will help secure shrimp aquaculture development, national income through trade (both local and international), and livelihoods of farmers and other service providers.

When the patterns of spread of diseases and pathogens of shrimp are examined, especially those for viral pathogens, there is convincing evidence that most major disease outbreaks are associated with the movement of live shrimp (broodstock, nauplii and postlarvae (PL)). It is important to remain very cautious over the international or regional movement of live shrimp stocks bound for aquaculture. This precaution applies even to domesticated stocks and to a single shrimp species cultivated in different places. However, movements should be permitted when proper quarantine and screening procedures have been applied.

Our understanding of the avenues and options for controlling shrimp diseases, especially WSSV, has improved over the past few years, mainly through the experiences gained in Asia and in Latin America. The ultimate solution for combating shrimp disease problems is to culture certified, domesticated stocks that are free of specific pathogens on nutritious, dry feeds in biosecure ponds under conditions that are nonstressful to the shrimp. This should be the ultimate goal for the shrimp industry.

With respect to stress, while it is impossible to control weather, we do have the ability to control many important variables, such as pond carrying capacity, feed inputs and water exchange. At present, dry feeds appear to be adequate, although there is obviously still room for improvement in their quality. The biggest potentially controllable problems that farmers currently face are uncertainty regarding the quality of postlarvae used in culture, and the lack of biosecurity of the pond environment from the entry of pathogens and their carriers.

The simplest way to solve the postlarval quality problem is to change from the use of postlarvae derived from captured broodstock to those derived from domesticated stocks. However, this practice requires considerable research effort and field-testing, and is still in its infancy. At least we can try to ensure biosecurity in ponds through appropriate screening of postlarvae for important pathogens prior to stocking. The procedures for screening postlarvae for important pathogens (predominantly WSSV) are known; however, additional training, capacity building, and upgrading of hatcheries and diagnostic centres are necessary.

Currently, harmonized technical standards for the hatchery production of postlarvae are lacking. It is imperative that such technical standards be developed, standardized, validated, and agreed upon by the hatchery producers, both nationally and internationally.

In November 1999, an FAO Expert Workshop was held in Cebu, Philippines, where representatives from 14 shrimp-producing countries, including five Latin American countries, attended. The workshop discussed and agreed upon a number of strategies for controlling shrimp disease problems and made recommendations for future activities. These ideas were further discussed at the recent APEC/NACA/FAO/SEMARNAP Expert Workshop on Transboundary

aquatic animal pathogen transfer and the development of harmonized standards on aquaculture health management, held in Puerto Vallarta, Jalisco, Mexico, 24–28 July 2000. A consensus was achieved that the strategies should be incorporated into a regional technical cooperation project aimed at assistance, and the member countries of FAO in which the proposed project would be implemented gave their consent for formulation of the project proposal.

Developing regional technical guidelines and standards on quarantine and health certification for safe transboundary movement of live aquatic animals (broodstock, nauplii and postlarvae of shrimp), and harmonizing them within the region was considered timely and appropriate. However, this will take some time to realize, and compliance will remain an issue until appropriate national capacities are developed. Nevertheless, FAO's experience in developing technical guidelines on health management for safe transboundary movement of aquatic animals in Asia can be duly utilized for the benefit of Latin America (see FAO/NACA 2000, 2001a). Capacity building among national institutions, involved staff and shrimp farmers is important. Farmers should be made aware of the options and opportunities available for controlling diseases, especially WSSV. Developing good farm and hatchery management practices and documenting them with adequate scientific evidence and field data are also appropriate and timely.

Considering the above points, it became clear that the most timely and effective means to assist the Americas to deal with the existing shrimp disease situation would be: i) developing interventions for improving postlarval quality, ii) building capacity among farmers and appropriate state agencies and iii) developing a comprehensive information network within the region. The Government of Ecuador made a formal request to FAO for technical assistance to combat serious shrimp disease problems in Ecuador. FAO, in consultation and agreement with the shrimp-producing countries in the Americas, decided to prepare a Regional Technical Cooperation Programme Project addressing the above issues.

The Project, which began in 2001, involved the participation of 14 countries: Belize, Brazil, Costa Rica, Colombia, Cuba, Ecuador, El Salvador, Guatemala, Honduras, Mexico, Nicaragua, Panama, Peru and Venezuela. Representatives of each country responded to a questionnaire on shrimp maturation and hatchery practices in their country. The questionnaire covered a number of aspects of production, concentrating on maturation and hatchery types, sizes, species, management, physical and chemical treatments and disinfection procedures used; health management; production and quality assessment methods; transportation methods; and problems encountered.

The technical guidance provided in this document was developed by the National Coordinators (NCs) and experts who participated in the project and is based on the information provided by the participating governments.

# 2 The contribution of marine shrimp to global aquaculture production

In the year 2000, total global aquaculture production was reported as 45.71 million metric tonnes (mmt) valued at US$56.47 thousand million. Over half of this was in the form of finfish (23.07 mmt or 50.4% of total production), followed by molluscs (10.73 mmt or 23.5%), aquatic plants (10.13 mmt or 22.2%), crustaceans (1.65 mmt or 3.6%), amphibians and reptiles (100 271 metric tonnes (mt) or 0.22%) and miscellaneous aquatic invertebrates (36 965 mt or 0.08%). Although crustaceans (a category comprised mainly of penaeid shrimps) represented only 3.6% of total production by weight, they comprised 16.6% of total global aquaculture by value in 2000.

Over half (54.9%) of global aquaculture production originated from marine or brackish coastal waters in 2000, as compared with 45.1% for freshwater aquaculture production. Although brackishwater production represented only 4.6% of total global aquaculture production by weight in 2000, it contributed 15.7% of total production by value. The main species groups reared in brackish water are high-value crustaceans and finfish (50.5% and 42.7%, respectively), while molluscs and aquatic plants dominate in marine waters (46.1% and 44.0%, respectively).

As in previous years, marine shrimp continued to dominate crustacean aquaculture, with shrimp production in 2000 reaching 1 087 111 mt (66.0% of global crustacean aquaculture production) and valued at US$6 880 068 900 (73.4% of total value). Aquaculture currently provides just over a quarter (26.1%) of total global shrimp landings. The main cultivated species are the giant tiger prawn (*Penaeus monodon*), the fleshy prawn (*P. chinensis*) and the whiteleg shrimp (*P. (Litopenaeus) vannamei*), these three species accounting for over 86% of total shrimp aquaculture production in 2000.

The growth in production of crustaceans has continued to be strong, increasing by 6.8% by weight from 1999, a rate slightly exceeding that for finfish (6.7%), molluscs (5.8%) and aquatic plants (6.1%). The growth of shrimp production, while still significant, has decreased to more modest levels over the last decade (averaging 5%) as compared to the double-digit growth rates observed during the seventies (23%) and eighties (25%).

## 2.1 Marine shrimp aquaculture production trends in Latin America

The countries of Latin America, although still relatively minor contributors to total world aquaculture production (1.9% of global production by weight, and 5.3% by value), have raised their output dramatically over the past 30 years, total aquaculture production increasing by over 714-fold by weight, from 1 221 mt in 1970 (0.03% of total global production) to 871 874 mt in 2000. Aquaculture continues to grow strongly in the region, increasing by a healthy 14.2% per year for the period 1990–2000, although this rate is considerably lower than the rapid increases seen in earlier decades (34.4% per year during the period 1970–1980 and 23.3% per year during 1980–1990). Overall growth during the period 1970–2000 averaged 24.5% per year.

The top ten cultured species by weight within the region in 2000 included Atlantic salmon (166 897 mt or 19.1%), whiteleg shrimp (139 264 mt or 16.0%), rainbow trout (97 479 mt or 11.2%), coho salmon (93 419 mt or 10.7%), tilapia (85 246 mt or 9.8%), common carp

(62 241 mt or 7.1%), *Gracilaria* seaweed (33 642 mt or 3.8%), silver carp (30 000 mt or 3.4%), Chilean mussel (*Mytilus chilensis*) (23 477 mt or 2.7%) and the Peruvian calico scallop (*Argopectin purpuratus*) (21 295 mt or 2.4%) (FAO 2003).

The top country producers within the region in 2000 included Chile (425 058 mt or 48.7%), Brazil (153 558 mt or 17.6%), Ecuador (62 011 mt or 7.1%), Colombia (61 786 mt or 7.1%), Mexico (53 802 mt or 6.2%), Cuba (52 700 mt or 6.0%), Venezuela (12 830 mt or 1.5%), Costa Rica (9 708 mt or 1.1%), Honduras (8 542 mt or 1.0%) and Peru (6 812 mt or 0.8%).

By value, aquaculture production within the region has increased over eight-fold, from US$337 million in 1984 to US$2.98 thousand million in 2000 (representing 5.3% of the total global aquaculture production by value). The main species groups by value in 2000 were finfish (US$1.89 billion or 63.4%), crustaceans (US$0.94 billion or 31.5%) and molluscs (US$128 million or 4.3%), with the top cultured species being the whiteleg shrimp (US$848 million or 28.4%), Atlantic salmon (US$567 million or 19.0%), coho salmon (US$346 million or 11.6%), rainbow trout (US$291 million or 9.7%), tilapia (US$221 million or 7.4%), common carp (US$176 million or 5.9%), Peruvian calico scallop (US$93 million or 3.1%), penaeid shrimp (species not given) (US$77 million or 2.6%), cachama (*Colossoma*) (US$75 million or 2.5%) and silver carp (US$21 million or 0.7%).

## 2.2 Shrimp aquaculture in Latin America: the health issues

The shrimp farming industry in Latin America has developed and emerged as one of the major foreign exchange earners in the region. Initially, shrimp producers relied almost entirely on the capture of wild postlarvae (PL) in the estuaries and coastal areas where these are found naturally. Seasonal and annual variations in the catch of PL, however, led to the development of shrimp hatcheries where postlarval production could be undertaken in a more controlled manner. These hatcheries used wild broodstock caught by fishermen and supplied to the hatcheries.

The fluctuations in catches of both wild postlarvae and broodstock as a result of the El Niño phenomenon had a major impact on the development of hatcheries. In years when wild seed was abundant, low postlarval prices and a general perception that wild seed were stronger meant that many hatcheries encountered financial difficulties. In years when wild seed was scarce, on the other hand, hatchery-produced seed could be sold at a premium. Despite this, many hatcheries experienced problems due to the unpredictability of the market situation.

In recent years, disease, or more specifically, shrimp health concerns, has led to a revival of interest in hatchery-produced PL. Shrimp from some countries were widely believed to be less sensitive to Taura Syndrome Virus (TSV) than those from other areas, and this led to a lucrative cross-border trade in broodstock, nauplii and postlarvae in the region. Unfortunately, the arrival of the White Spot Syndrome Virus (WSSV) in the region in the late 1990s exposed the local hatchery operators to the possibility that the disease might be spread by such transfers if they were not conducted under appropriate controls and regulation.

At the same time, several producers had been experimenting with the breeding of survivors of TSV outbreaks in an attempt to develop lines of shrimp with greater resistance to the virus. The WSSV epidemic and the risk of vertical transmission accelerated this and led to a greater interest in genetics and breeding and a recognition that the dependence on wild sources of shrimp represented a significant disease risk. Hatchery operators reviewed their operations and focused on improving the biosecurity and health management of their production systems.

Now, most countries in Latin America have begun domestication and genetic selection programmes using pond-reared broodstock in maturation systems. This has been done in an attempt to stabilize predictability and improve the disease resistance and growth rates of their shrimp stocks. Initial efforts used broodstock from a variety of countries around the region in order to ensure a wide genetic variability in the stocks, but subsequent closure of most borders to import of live shrimp has curtailed this activity.

Most countries in the region are concentrating on the production of Specific Pathogen Resistant (SPR) or Specific Pathogen Tolerant (SPT) shrimp, selecting the best surviving (but not necessarily disease-free) animals from pond on-growing facilities and on-growing them further in various facilities before transfer to maturation systems. Specific Pathogen Free (SPF) shrimp (i.e. those certified free of one or more specific disease agents, and held throughout their lives in closed systems) have also been used, but with less frequency and when used, these animals have generally been brought in from isolated breeding centres in the United States.

# 3 Requirements for effective hatchery production

In order to provide practical and effective technical guidance for shrimp hatchery management, it is first necessary to review the basic requirements for an effective hatchery production system. These include the presence of essential infrastructure, the development of Standard Operating Procedures (SOPs) (including Hazard Analysis Critical Control Point (HACCP) analysis), the maintenance of biosecurity, the provision of adequate amounts of clean water, the responsible use of chemicals, and the assurance of health status of stocks through laboratory testing. Many of these components are discussed in more detail in later sections of this document.

## 3.1 Infrastructure

**Hatcheries must be well designed and have adequate infrastructure, as these have an important impact on the quantity and quality of postlarvae produced**

Hatcheries should be designed (or modified, in the case of existing hatcheries) to ensure good biosecurity, efficiency, cost-effectiveness and the implementation of the hatchery Standard Operating Procedures (SOPs). The infrastructure requirements for successful biosecurity in the hatchery operation will be discussed under the relevant headings throughout this section.

**Shrimp hatcheries should consist of several units, each having appropriate infrastructure**

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

**Good hatchery design should include the physical separation or isolation of the different production facilities and effective perimeter security**

The physical separation or isolation of the different production facilities is a feature of good hatchery design and should be incorporated into the construction of new hatcheries. In existing hatcheries with no physical separation, effective isolation may also be achieved through the construction of barriers and implementation of process and product flow controls. The hatchery facility should have a wall or fence around the periphery of the property, with enough height to stop the entrance of animals and unauthorized persons. This will help to reduce the risk of pathogen introduction by this route, as well as increase overall security.

**To minimize the possibility of infecting existing broodstock via the introduction of new animals, there should be a quarantine unit for new broodstock**

The quarantine of all new animals to be introduced into the hatchery is an essential biosecurity measure. Before passing to the production system, the broodstock must be screened for subclinical levels of pathogens (i.e. via dot blot, polymerase chain reaction (PCR), immunoblot etc.). Broodstock infected with serious untreatable diseases should be destroyed immediately and only animals negative for pathogens introduced to the maturation unit.

## 3.2 Water quality and treatment

**Water treatment systems should be designed to provide high quality oceanic seawater**

Water for the hatchery should be filtered and treated to prevent entry of vectors and any pathogens that may be present in the source water. This may be achieved by initial filtering through subsand well points, sand filters (gravity or pressure), or mesh bag filters into the first reservoir or settling tank. Following primary disinfection by chlorination, and after settlement, the water should be filtered again with a finer filter and then disinfected using ultraviolet light (UV) and/or ozone. The use of activated carbon filters, the addition of ethylene diamine tetra acetic acid (EDTA) and temperature and salinity regulation may also be features of the water supply system.

**The design of the water distribution system should take into account the level of biosecurity required by the individual areas to which the water is distributed**

Each functional unit of the hatchery system should have the appropriate water treatment and, where necessary, should be isolated from the water supply for other areas (for example, quarantine areas). Separate recirculation systems may be used for part or the entire hatchery to reduce water usage and further enhance biosecurity, especially in high-risk areas.

**All water discharged from the facility should be free of pathogens**

All water discharged from the hatchery, particularly that known or suspected to be contaminated (for example, water originating from the quarantine areas) should be held temporarily and treated with hypochlorite solution (>20 ppm active chlorine for not less than 60 min) or another effective disinfectant prior to discharge. This is particularly crucial where the water is to be discharged to the same location as the abstraction point.

More specific water treatment procedures to be used for each phase of maturation and larval rearing are detailed in the appropriate sections.

## 3.3 Biosecurity

**Good biosecurity must be achieved, as it is paramount to the successful production of healthy PL**

Biosecurity has been defined as "…*sets of practices that will reduce the probability of a pathogen introduction and its subsequent spread from one place to another*…" (Lotz 1997). The basic elements of a biosecurity programme include the physical, chemical and biological methods necessary to protect the hatchery from the consequences of all diseases that represent a high risk. Effective biosecurity requires attention to a range of factors, some disease specific, some not, ranging from purely technical factors to aspects of management and economics. Various levels and strategies for biosecurity may be employed depending on the hatchery facility, the diseases of concern and the level of perceived risk. The appropriate level of biosecurity to be applied will generally be a function of ease of implementation and cost, relative to the impact of the disease on the production operations (Fegan and Clifford 2001). Responsible hatchery operation must also consider the potential risk of disease introduction into the natural environment, and its effects on neighbouring aquaculture operations and the natural fauna.

## 3.4 Standard operating procedures (SOPs)

**Each hatchery should develop its own set of Standard Operating Procedures (SOPs)**

Standard Operating Procedures (SOPs) outlining the control protocol for the hatchery should be described in a comprehensive document that covers each stage or process of the production cycle. The document should include details of all of the critical control points (CCP) and describe how to perform each task to control the associated risk. Once the protocol for hatchery operation is documented, the SOPs should be given to all personnel, and a copy should be available for all workers in an accessible place (dining room, meeting room etc.). A meeting should be held to introduce the protocol and explain the need for, and contents of the SOPs. This is a good opportunity to clearly identify and explain any points that generate doubts or that may be misinterpreted and to get practical input from the hatchery staff.

As new information becomes available, it will be necessary to update or modify the SOPs, and any changes must be communicated to all personnel. Any updated version of the SOPs should have the date of the modification and a clear statement that the new version supersedes all previous versions.

**All workers should sign a document indicating that they have read and understood the SOPs, and that they will comply with all requirements**

All job descriptions of hatchery management and staff should include a clause related to following the SOPs and the disciplinary consequences of failure to comply.

**Training in biosecurity maintenance should be an important component of the hatchery process**

It is advisable to have a group of people with higher technical training or experience who can supervise and train workers in the execution of each step of the SOPs. This point is of fundamental importance, as the workers may not understand either the standards required or the risks of non-compliance to the success of the hatchery. These technical personnel must organize meetings with the workers for each department to explain and discuss the importance of the execution of the SOPs.

**The biosecurity risk posed by each area of the hatchery should be determined**

Different areas of the hatchery may be classified according to the level of risk of disease introduction or transfer. Weirich *et al.* (in press) used this system to describe four classifications:

- Quarantine areas where a pathogen of concern is potentially present or suspected,
- High sensitivity areas requiring minimum exposure to avoid potential pathogen introduction or transfer,
- Medium sensitivity areas with lower risk of pathogen introduction or transfer, and
- Low sensitivity areas in which risks of pathogen introduction or transfer are unlikely.

These classifications can be modified if required and the changes reflected in an updated version of the SOPs. Specific protocols and restrictions may be adopted for each of these biosecurity levels to prevent pathogen entry or transfer.

## 3.5 Hazard analysis critical control point (HACCP) approach

**Development and implementation of biosecurity protocols can be made easier by a Hazard Analysis Critical Control Point (HACCP) approach**

The HACCP approach is a preventive risk management system based upon a hazard analysis and has been widely used to identify and control risks to human health in food-processing systems. Critical limits are set at critical control points (CCPs) in the system where controls must be applied to prevent, eliminate or reduce a hazard. Monitoring and corrective actions are then implemented (Weirich *et al.* in press). HACCP principles have been applied as a risk management tool to control viral pathogens at shrimp research and production facilities (Jahncke *et al.* 2001).

**HACCP analysis should also be applied to shrimp production, with particular emphasis on reducing or preventing disease risks**

Maximum biosecurity in shrimp production facilities can be achieved through the isolation of breeding, hatchery and production phases (Jahncke *et al.* 2001, 2002). Good facility design with a high degree of isolation can help to reduce the risk of transfer of pathogens from broodstock to their offspring. The critical control points (CCP) identified for the maturation and hatchery stages of shrimp production are the shrimp, the feeds and the water. Other potential risks to be

covered by the implementation of SOPs and HACCP are disease vectors (human and animal), facilities and equipment.

**A flow diagram should be created for the hatchery facility detailing all operations and the movement of shrimp and larvae through the production system**

For each operation, from broodstock receipt through maturation, larval rearing and, where applicable, nursery, all potential hazards, impacts on larval health and quality, and points of entry of pathogens should be identified. Following this systematic hazard analysis, CCPs should be identified. For each CCP, critical limits must be established and, where these limits are exceeded, appropriate corrective actions determined. A system to monitor the CCPs must be established along with a good system of documentation and recording.

**Critical Control Points (CCPs) must be identified for each area**

For different areas such as quarantine, maturation, hatchery, algal culture, *Artemia* production etc., it is necessary to identify critical control points. The following stages can be considered as CCPs, although these may not be the only ones and they can vary from one location to another:

- Facility entrance: Control at entrance for operational workers, administrative employees, vehicles and other disease vectors to prevent transfer of infections from other hatcheries and the environment at large.
- Water treatment: All the water used in production units must be appropriately (stage dependant) treated (chlorine, ozone, filtration etc.) to kill pathogens and their hosts.
- Maturation: Quarantine of incoming broodstock; checking and disinfection of fresh feed; cleaning of tanks and water and air lines; and disinfection of broodstock, eggs, nauplii and equipment.
- Hatchery: Regular dry-out periods; cleaning and disinfection of buildings, tanks, filters, water and air lines and equipment; quality control and disinfection of fresh feeds; separation of working materials for each room and each tank.
- Algae: Restricted entrance of personnel to algal laboratory and tank facilities; equipment, water and air disinfection; sanitation and quality control of algae and chemicals used.
- *Artemia*: Cyst disinfection, nauplii disinfection, tank and equipment cleaning and sanitation.
- Restriction of entrance to the hatchery in general and each area in particular to authorized personnel: All staff and administrative personnel entering the production areas must comply with the procedures in the SOPs.

**Hatchery workers must be restricted to their specific area of work**

The hatchery workers must be restricted to their specific area of work and should not be able to move freely to other areas not assigned to them. One practical way to manage this is to provide different colour uniforms for each area. This will allow quick identification of people in areas where they are not allowed.

**The SOPs should address risks due to staff whose duties require them to pass through areas of the hatchery with different biosecurity classifications**

For example, communication between staff working in different areas can be maintained while limiting movement between different areas of the hatchery by providing a central area where staff can meet to discuss and plan work schedules, and by communicating by intercom system, radios, text messaging, mobile phones, or a local area network (LAN) for the computer systems.

**All staff must take adequate sanitary precautions when entering and leaving a production unit**

Rubber boots must be worn by staff when in the production areas. The production units (hatchery, maturation, algal culture, *Artemia* etc.) must have one entrance/exit to avoid unnecessary through-traffic. The entrance must have a footbath with a solution of calcium (or sodium) hypochlorite with a final concentration not less than 50 ppm active ingredient. This disinfectant solution must be replaced when necessary. Next to the entrance door, each room must have a bowl with a solution of iodine-PVP (povidone iodine) at 20 ppm and/or 70% alcohol, and personnel must wash their hands in the solution(s) when entering or leaving the room.

**Special care must be taken with vehicles (personal or shrimp transport vehicles), because they may have visited other hatcheries or shrimp farms before arrival**

All vehicles must pass through a wheel bath with dimensions such as to assure complete washing of the wheels. The wheel bath must be regularly filled with an effective disinfectant solution (such as sodium (calcium) hypochlorite at >100 ppm active ingredient).

**The entry of potential disease vectors into the hatchery facility must be controlled**

Some shrimp viruses are found in a range of terrestrial animals, such as insects and birds (Lightner 1996, Lightner *et al.* 1997, Garza *et al.* 1997). While it is not possible to control all potential animal vectors, their entry can be minimized by the use of physical barriers such as fencing, while nets or mesh can be used to exclude birds and insects. Aquatic animals can be excluded by ensuring that there are no direct means of entry from open-water sources, especially via inlet pipes and drainage channels. All water entering the facility should filtered and disinfected, and all drainage channels should be screened and/or covered, where possible, to prevent the entry and establishment of wild aquatic animals.

## 3.6 Chemical use during the hatchery production process

**Chemicals must be used responsibly during the hatchery production process**

Chemicals (e.g. disinfectants, drugs, antibiotics, hormones etc.) have many uses in the hatchery production process, where they increase production efficiency and reduce the waste of other resources. They are often essential components in such routine activities as tank construction;

water quality management; transportation of broodstock, nauplii and PL; feed formulation; manipulation and enhancement of reproduction; growth promotion; disease treatment, and general health management.

However, chemicals must be used in a responsible manner, as they pose a number of potential risks to human health, other aquatic and terrestrial production systems and the natural environment. These include:

- Risks to the environment, such as the potential effects of aquaculture chemicals on water and sediment quality (nutrient enrichment, loading with organic matter etc.), natural aquatic communities (toxicity, disturbance of community structure and resultant impacts on biodiversity), and effects on microorganisms (alteration of microbial communities).
- Risks to human health, such as the dangers to aquaculture workers posed by the handling of feed additives, therapeutants, hormones, disinfectants and vaccines; the risk of developing strains of pathogens that are resistant to antibiotics used in human medicine; and the dangers to consumers posed by ingestion of aquaculture products containing unacceptably high levels of chemical residue.
- Risks to production systems for other domesticated species, such as through the development of drug-resistant bacteria that may cause disease in livestock or poultry.

It is thus essential that only qualified and adequately trained hatchery personnel be permitted to handle chemicals, that the chemical to be used for a particular situation is the most appropriate for the job, and that it is used in the correct manner (e.g. amount, duration and treatment conditions).

Before chemicals are used, management should always consider if other, more environmentally friendly interventions might be equally effective. Effective and safe use and storage of chemicals should be an integral component of the hatchery's Standard Operating Procedures (SOPs). A detailed review of the use of chemicals in shrimp culture, and in other aquaculture systems, can be found in Arthur *et al.* (2000).

The Office international des épizooties (World Organisation for Animal Health - http://www.oie.int), in its Manual of diagnostics tests and vaccines for aquatic animals provides acceptable and recommended dosages of various chemicals and disinfectants to be used in shrimp aquaculture (http://www.oie.int/eng/normes/fmanual/A_summry.htm) (OIE 2003).

Table 1 provides a summary of chemical names mentioned in this document and how they are used in hatchery production of *P. vannamei* in Latin America. Some of the dosages (concentrations and exposure times) provided in this table are slightly different from those given in OIE, 2003. The dosages given in Table 1 have been found more effective in *P. vannamei* hatchery production in Latin America and were agreed by the experts participated in producing this document.

**Table 1. Summary of chemicals and their uses mentioned in this document.**

| Use in Hatchery | Chemical | Recommended Concentration (Parts Active Ingredient) |
|---|---|---|
| Disinfection of inflow seawater | Sodium hypochlorite[2] | 20 ppm for not less than 30 min (or 10 ppm for not less than 30 min) |
| Chelation of heavy metals in inflow seawater | EDTA | Depends on concentrations of heavy metals in water |
| Disinfection of discharge water | Sodium hypochlorite | >20 ppm for not less than 60 min |
| Determination of presence of chlorine in water | Ortho-toluidine | 3 drops in 5 mL water sample[3] |
| Neutralization of chlorine in treated water | Sodium thiosulfate | 1 ppm for every 1 ppm residual chlorine |
| Chelation of heavy metals in: broodstock tank water and hatching tank water | EDTA | Must be determined based on heavy metal loading at location up to 20 ppm or both at 20–40 ppm |
| Disinfection of broodstock upon entry to quarantine | Iodine-PVP<br>Formalin | 20 ppm<br>50–100 ppm |
| Disinfection of broodstock following spawning | Iodine-PVP | 20 ppm for 15 sec (dip) |
| Washing and disinfecting eggs | Iodine-PVP or<br><br>Formalin,<br>and<br>Treflan | 50–100 ppm for 1–3 min, (or for 10–60 sec)<br>100 ppm for 30 sec<br><br>0.05–0.1 ppm (to reduce fungal infections) |
| Disposal of discarded larvae | Sodium hypochlorite | 20 ppm |
| Removal of epibiont fouling from postlarvae | Formalin | up to 20–30 ppm for 1 hr with full aeration |
| Stress testing of postlarvae | Formalin[4] | 30 min |
| Decapsulation of *Artemia* cysts | Caustic soda (NAOH) and Chlorine liquid[5] | 40 g in 4 mL (8–10% active ingredient) |
| Disinfection of *Artemia* nauplii | Sodium hypochlorite solution<br>or<br>Chloramine-T<br>or both | 20 ppm<br><br><br>60 ppm for 3 min |
| Treatment of water in spawning and hatching tanks | Treflan | 0.05–0.1 ppm |
| Footbath | Sodium (calcium) hypochlorite solution | >50 ppm (or >100 ppm) |
| Disinfection of equipment (containers, hoses, nets, etc.) | Sodium hypochlorite<br>or<br>Muriatic acid | 20 ppm (or 30 ppm)<br><br>10% solution |
| Disinfection of hands | Iodine-PVP<br>or<br>Alcohol | 20 ppm<br><br>70% |

[2]or calcium hypochlorite
[3] Presence of chlorine is indicated by a yellow colour
[4] Salinity change can also be used.
[5] See page 41 for details

**Table 1. Continued.**

| Use in Hatchery | Chemical | Recommended Concentration (Parts Active Ingredient) |
|---|---|---|
| Cleaning and disinfection of tanks used for broodstock spawning, egg hatching holding for nauplii and postlarvae, hatching of *Artemia* | Sodium hypochlorite<br>and/or<br>Muriatic acid[6] | 30 ppm ( or 20–30 ppm)<br><br>10% solution (pH 2–3) |
| Disinfection of previously cleaned and disinfected tanks prior to starting a new cycle | Muriatic acid | 10% solution |
| Disinfection of algal culture tanks | Sodium hypochlorite<br>followed by<br>Muriatic acid | 10 ppm<br><br>10% solution |
| Disinfection of sand filters | Sodium hypochlorite<br>or<br>Muriatic acid | 20 ppm<br><br>10% solution (pH 2–3) |
| Disinfection of cartridge filters | Sodium hypochlorite<br>or<br>Muriatic acid | 10 ppm<br><br>10% solution (pH 2–3) for 1 hr |
| Washing of feed preparation equipment (knives, tables, mixers, pelletisers, etc.) | Iodine-PVP | 20 ppm |

## 3.7 Health assessment

**Routine health assessments should be a component of good hatchery management**

The health assessment techniques described below for use in shrimp hatcheries are divided into three categories (levels) based on past experience gained from aquatic animal health management activities in Asia. The system was developed to measure the diagnostic capability required to diagnose diseases of aquatic animals, and thus the techniques commonly employed in shrimp hatcheries can be divided into the same three basic categories. The details of the different levels of assessment techniques are given in FAO/NACA (2000, 2001a, 2001b). They provide a simple and convenient separation based on the complexity of the techniques used (Table 2).

**Table 2. Diagnostic level descriptions adapted for use in shrimp hatchery systems.**

| | |
|---|---|
| **Level 1** | Observation of animal and environment. Examination based on gross features. |
| **Level 2** | More detailed examination using light microscopy and squash mounts, with and without staining, and basic bacteriology. |
| **Level 3** | Use of more complex methods such as molecular techniques and immunodiagnostics (e.g. PCR, dot blots etc.). |

[6] In the past, muriatic acid was referred to 3:1 HCl and $HNO_3$, but currently it is referred to as 34-37% HCl.

**Level 1 Health assessment techniques**

Level 1 techniques are commonly employed in most hatcheries. Detailed examination of large numbers of larvae is not practical and hatchery operators and technicians frequently use Level 1 techniques to get a preliminary feel for the health status of larvae and to prioritize more detailed examination. Level 1 observations are also frequently sufficient to make a decision about the fate of a hatchery tank or batch of larvae.

Selection of nauplii, for example, generally includes a decision based on phototactic response without the need for a more detailed microscopic examination. If a batch of nauplii shows poor phototaxis and weak swimming behaviour, it will be rejected without further examination.

**Level 2 Health assessment techniques**

Level 2 techniques are also frequently used in the decision-making process in shrimp hatchery management. Most, if not all hatcheries will have a microscope that is used to make more detailed examinations of the condition of the shrimp larvae and to observe directly various health-related features (cleanliness, feeding behaviour, digestion etc.).

Many hatcheries also routinely employ basic bacteriology to gain an understanding of the bacterial flora of the tanks and to identify possible pathogens when the larvae become weak or sick. This information may then be used to make a decision on whether the tank should be discarded or treated.

**Level 3 Health assessment techniques**

Level 3 techniques are becoming more commonly employed in shrimp hatcheries. Polymerase chain reaction (PCR) methods are used for the screening of postlarvae and broodstock for viral diseases, as are dot blot and other immunodiagnostic tests.

The various applications of the different diagnostic techniques in a shrimp hatchery are given in Table 3.

**Table 3. Use of Level 1, 2 and 3 diagnostics in shrimp hatcheries.**

| | |
|---|---|
| **Level 1** | Examination of broodstock for general health condition, sex determination, staging of ovarian development, moult staging, removal of sick/moribund individuals. |
| | Selection of nauplii by phototactic response, zoea/mysis stage feeding by observation of faecal strands, larval activity, postlarval activity and behaviour, stress tests. |
| **Level 2** | Examination of egg quality by microscope. Checking bacterial flora of normal or moribund animals. |
| | Microscopic examination of naupliar quality. Routine microscopic examination of larval condition and postlarval quality. Checking bacterial flora of rearing water and larvae. |
| **Level 3** | Screening of broodstock by dot blot or PCR. |
| | Screening of nauplii and postlarvae by dot blot or PCR. |

# 4 The pre-spawning process

For ease of reference, technical guidance on how to manage health and maintain biosecurity in shrimp hatcheries is arranged according to the basic hatchery production process, starting from broodstock selection through to transportation of postlarvae out of the facility. The process has been divided into two broad categories: the pre-spawning process and the post-spawning process. The pre-spawning process includes procedures for broodstock selection, maintenance, maturation, acclimatization, spawning and hatching. As these procedures require different facilities, the facility maintenance guidelines are described under the different specific facilities used in the hatchery production process. Broodstock handling, nutrition and feeding are also discussed.

## 4.1 Broodstock selection

**Healthy broodstock that are not carriers of serious pathogens must be selected in order to achieve successful hatchery production**

Some viral diseases such as Infectious Hypodermal and Haematopoietic Necrosis (IHHN) are believed to be transmitted vertically from parent to offspring (Motte *et al.* 2003). Such vertically transmitted diseases may be eliminated from the hatchery production system by the use of domesticated shrimp that are free from these pathogens through an appropriate Specific Pathogen Free (SPF) programme (see below).

If SPF (or "high health") shrimp free from known viruses are not available, broodstock should be tested for infection by an appropriate diagnostic test and any infected individuals destroyed. Shrimp testing negative for the disease or pathogen should still be considered a risk and placed in a quarantine facility until their health status is fully known.

Even after broodstock have been transferred from the quarantine unit, some hatcheries maintain a routine health check by monthly monitoring of the postlarvae produced. A proportion (e.g. 0.1%) of the population is sampled by PCR and haemolymph tests, and based on the results of these tests, appropriate action is taken. The number of animals to be sampled should be determined according to a sampling table that takes into consideration the size of the host population and the presumed prevalence of the pathogen (see, for example, OIE 2003).

Where possible, the animals selected as broodstock should come from a closed cycle operation, as this allows their performance history and health status to be known. Ideally, they should originate from shrimp farms located in areas with physico-chemical characteristics (salinity, temperature etc.) similar to those where the postlarvae will be stocked. Criteria used in the selection of broodstock depend on the source of the broodstock (wild or domesticated).

***Wild broodstock:*** Because performances and growth records are not available for wild broodstock, and because there is no chance for stock improvement, there has therefore been a trend away from their acquisition and use. Wild-source broodstock were formerly preferred by hatcheries due to the belief that they produced more and stronger nauplii. In recent years, however, the high risk of introducing viral pathogens with wild broodstock has changed this preference. Additionally, it is increasingly recognised that domesticated shrimp stocks need to be developed to enable enhancement of maturation, hatchery and pond performance, which has led

to a trend towards the use of broodstock reared in captivity. In the case of *Penaeus vannamei*, wild broodstock captured using nets from small boats are preferred, because those captured by trawlers suffer more damage. Wild females for use in a maturation facility should be 60 g body weight and with developed ovaries, and males should be approximately 40 to 50 g body weight.

***Domesticated broodstock:*** In the past ten years, sources of domesticated shrimp stocks have become more common, with domesticated stocks of both *P. vannamei* and *P. stylirostris* now being commercially available. Closed-cycle stocks are generally supplied at a smaller size than wild animals, males being approximately 30 g and females not less than 30–35 g and usually >40 g. Females are usually supplied in a nongravid condition. Domesticated stocks may come from one of several sources. Some countries have well-established domestication programmes, whereas others rely on imported stocks. The domesticated stocks may be either genetically improved through a specific genetic improvement programme to select for desirable traits or simply selected from stocks that are free from, or suspected to be resistant or tolerant to, specific pathogens.

Several specialized types of domesticated broodstock have been developed to reduce disease risks. Specific Pathogen Free (SPF) stocks are generally maintained in highly biosecure facilities and their offspring (designated "high health" rather than SPF) are supplied to the industry. Specific Pathogen Resistant (SPR) shrimp are those that are not susceptible to infection by one or several specific pathogens, and Specific Pathogen Tolerant (SPT) shrimp are those that are intentionally bred to develop resistance to the disease caused by one, or several, specific pathogens. Lines of *Penaeus stylirostris* that are resistant to IHHNV, for example, are available.

**When using domesticated shrimp, it essential to obtain adequate background information on the origin of the stocks and their past performance**

To avoid potential genetic problems and associated poor growth and survival due to inbreeding, details of the different families or origins of the domestic stocks, whether of foreign or native origin, must be obtained.

It is also useful to have performance and development data for the candidate families or lines under a range of environmental conditions. The selection protocol used is also important, i.e. whether the stocks were selected from ponds with better performance or for survival following a disease outbreak, and the exact timing of the selection procedures. Some criteria that are used for phenotypic selection (usually done first at harvest size and later, when the females are >30 g and males are >25 g) are: relative size and general physical appearance, absence of necrosis or other (clinical or subclinical) signs of disease or ill health in muscle and exoskeleton, clean pleopods, no rostrum deformities and a translucent body.

## 4.2 Procedures for broodstock quarantine

**Upon arrival at the hatchery, potential broodstock should be held in isolation until their disease status is ascertained**

The quarantine facilities are essentially a closed holding area where shrimp are kept in individual tanks until the results of screening for viruses (and for bacteria, where applicable) are known.

The broodstock quarantine unit should be physically isolated from the rest of the hatchery facilities. 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. Particular care should be taken with waste disposal and effluent treatment. Staff working in this area should not be permitted to enter other production sections and should follow sanitary protocols at all times.

**The quarantine unit should have the following characteristics:**

- It should be adequately isolated from all of the rearing and production areas to avoid any possible cross contamination.
- It should be in an enclosed and covered building with no direct access to the outside.
- There should be means provided for disinfection of feet (footbaths containing hypochlorite solution at >50 ppm active ingredient) and hands (bottles containing iodine-PVP (20 ppm and/or 70% alcohol) to be used upon entering and exiting the unit.
- Entrance to the quarantine area should be restricted to the personnel assigned to work exclusively in this area.
- Quarantine unit staff should enter through a dressing room, where they remove their street clothes and take a shower before going to another dressing room to put on working clothes and boots. At the end of the working shift, the sequence is reversed.
- An adequate number of plastic buckets and/or similar containers should be available 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.
- The seawater to be used in the facility must enter a storage tank where it will be treated with hypochlorite solution (20 ppm active ingredient for not less than 30 minutes) before inactivating with sodium thiosulfate (1 ppm for every ppm of residual chlorine) and strong aeration.
- All wastewater must be collected into another tank for chlorination (20 ppm for not less than 60 minutes) and dechlorination before release to the environment.
- All mortalities or infected animals must be incinerated or disposed of in another approved manner.
- Used plastic containers and hoses must be washed and disinfected with hypochlorite solution (20 ppm) before reuse.
- All the implements used in the quarantine unit 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.

**The quarantine unit should be structured so that shrimp move from "dirty" to "clean" areas as their health status becomes clear**

The individual sections of the quarantine area should be designated "dirty" or "clean" depending on whether they contain shrimp that are not yet screened for infection (pretesting) or that have been passed (posttesting). 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.

On entering the quarantine area the broodstock are passed through a dip of iodine-PVP solution (20 ppm) or formalin[7] (50–100 ppm). On the third day of quarantine, a pleopod is removed from each shrimp (if held individually) or from a sample of the population (if held as a group) for analyses. If shrimp are held collectively, random samples should be taken from each container to evaluate the general condition of the population held in that container. Groups of ten pleopods can be analysed as one sample. Any groups that give a positive result can be discarded or, in the case of a pooled sample from animals held individually, the shrimp can then be tested on an individual basis to identify and discard only the positive individuals. Infected animals should be disposed of by incineration or some other method (e.g. autoclaving and deep burial) that will prevent the potential spread of virus.

Further details on the construction and operation of a quarantine facility can be found in MAF (2001), Anon. (2002) and AQIS (2003).

**Broodstock must not be released from quarantine until their health status is clearly known**

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 for at least a minimum of 20 days prior to transferring them to the acclimatization area. Depending on the design of the facility and the location of the quarantine unit relative to the acclimatization facility, this may involve repacking the broodstock for shipment to a distant location or their movement to a separate section of the same facility using disinfected buckets with water from the acclimatization facility.

In either case, the equipment used for the transfer should be kept separate from that used in the quarantine room and disinfected before and after transport. All equipment used in the quarantine area should remain in the quarantine area and be disinfected at the end of each day in tanks specially designated for that purpose.

**Laboratory facilities and associated expertise must be determined based on the specific needs of the hatchery**

Basic laboratory facilities (e.g. a microscope, some microbiological capability etc.) will be required to carry out routine inspections of shrimp health. The addition of more complex facilities to carry out PCR tests, for example, will require the construction of dedicated facilities to avoid the possibility of contamination. The design and operation of these facilities is outside the scope of this document.

[7] When formalin is used, avoid using the whitish sediment at the bottom of the container (formaldehyde), as it is highly toxic.

## 4.3 Acclimatization

**Shrimp that pass the initial quarantine inspection must be acclimatized to the new conditions in the maturation facility**

During acclimatization, which lasts from seven days to a few weeks, the broodstock will be adjusted to the environmental conditions of the maturation facility and the types of feed that will be given. This is especially important where formulated diets will be used to supplement the natural feeds.

**The acclimatisation facility must have sufficient tank space to hold the shrimp that will be introduced into the maturation facility**

Such a facility also allows optimization of production of the maturation system. This is because well-acclimatized shrimp should be ready to begin producing nauplii soon after introduction to the maturation system, without excessively long periods of "down-time" (the number of days between introduction of a female into the maturation system and the first spawning) being lost.

**The broodstock should spend a minimum period of seven days (and up to several weeks) in acclimatisation before being stocked in the maturation tanks**

During this period any difference in temperature and/or salinity between the quarantine area and the maturation facility is gradually reduced. Feeding protocols are also adjusted so that the shrimp become accustomed to those utilized in the maturation facility. The moult stage is also observed and only females in the intermoult stage should be ablated when ready. In this way, the females to be transferred to the maturation unit will already be ablated and hence ready to begin production of nauplii almost immediately.

## 4.4 Maturation

The first step in larval production is the maturation and breeding of mature shrimp. The protocols to be adopted will depend to some extent on whether the hatchery operation is a component of a controlled breeding programme or if it is intended primarily for the production of postlarvae for commercial pond culture.

Depending on this distinction, the maturation system will be designed either to maximize the production of nauplii for commercial production of postlarvae or to allow for maximum control over mating and genetic crosses. Although it is possible to control mating in a conventional maturation unit, good control of individual parents requires unisex culture and artificial insemination, with larval culture and nursery systems designed for a large number of batches with relatively few larvae per batch. This presents operational challenges very different from a typical commercial hatchery or nursery system (Jahncke *et al.* 2002).

Appropriate infrastructure for broodstock handling consists of quarantine facilities, acclimatization facilities and the main production (maturation, spawning and hatching) facilities with their appropriate support systems.

**The maturation building must be large enough to contain sufficient maturation tanks and supporting infrastructure for the requirements of the hatchery**

The factors to consider in designing the facility are the level of naupliar production required, the stocking density and sex ratio of the broodstock to be used, the estimated spawning rate of the females, the estimated hatching rate, the estimated number of eggs and nauplii per female, and the production system (batch or continuous) employed.

**The conditions in the maturation room must be closely controlled**

The maturation room should be kept in low light, preferably with a system to control photoperiod. The photoperiod should be maintained at about 10–12 hours dark and 12–14 hours light, the light level changing between the two gradually over a period of one to two hours. Access to the maturation room should be restricted; noise (particularly loud or intermittent noise), movement and other disturbances should be kept to a minimum.

Preferably, the maturation room should have round tanks that are dark-coloured, smooth-sided, and of approximately 5 m diameter. The broodstock should be held with flow-through (new and/or recycled) water exchange of a total of 250–300% per day and a continuous, but not too vigorous air supply. Water depth is generally around 0.5–0.7 m. The shrimp are stocked at a rate of around 6–8 shrimp per sq. m. bottom surface area with a male to female ratio of 1–1.5:1. Thus, a 5 m diameter tank can accommodate 60–80 females and 60–100 males. Water temperatures are usually controlled to be maintained in the range of 28–29 °C, with a salinity of 30–35 ppt and pH of 8.0–8.2.

**The feed preparation area should be adjacent to, but separated from, the maturation room**

It should be equipped with all feed preparation utensils (knives, spoons, bowls/buckets, cutting surfaces, mixers, pelletisers etc.), and a fridge and a freezer to store food items.

**The maturation tanks should be must be siphoned daily and cleaned regularly**

Due to the high feeding rates employed, the maturation tanks require daily siphoning of uneaten food, faeces and moults. The siphon consists of two parts, a PVC tube and a hose. Each maturation tank should have its own PVC tube, but the hose may be used for all tanks. The hose should be rinsed with clean treated water before each tank is siphoned.

Debris and waste siphoned from the tanks can be collected in a mesh bag placed at the end of the hose and incinerated after the cleaning operation. At the end of the working day, the hose should be washed and remain immersed inside a tank of calcium hypochlorite solution (20 ppm).

Intermittent scrubbing of tank walls and bottoms must also be undertaken if there is an excessive build-up of algae or other sedentary organisms, including protozoan fouling organisms. This can often be achieved through lowering water levels in the tank without removing the broodstock, but occasionally requires the transfer of broodstock to new tanks. It is a good idea to leave at least one tank empty for such procedures, which can then be programmed on a regular basis.

Care must be taken during these cleaning exercises that the broodstock are manipulated as little as possible, as excessive disruption of mature brooders will interfere with their spawning rhythms.

**The equipment used to capture the mature females should be washed before checking each tank**

The hand nets used to capture mature females should be maintained in recipient(s) containing iodine-PVP and/or hypochlorite solutions (20 ppm active ingredient).

**An optimal population density for natural mating should be maintained**

The preferred population density for natural mating of *P. vannamei* broodstock is about 6–8 animals per square meter. If artificial insemination is to be done, the number can be increased up to 16 animals per square meter. It is important also to consider the biomass in weight rather than the numbers of broodstock per square meter that can be held in the tank without causing deterioration of the water quality through the feed used. A biomass/unit area of 0.2–0.3 kg/sq m is recommended.

**An optimal stocking ratio for males and females should be used**

Most systems will stock females and males together, usually in a 1–1.5:1 ratio. Occasionally, the sexes are kept separately. This has advantages, including reduced feeding costs for male-only tanks, because they can be reared on cheaper diets (primarily squid and enriched artificial feeds), increased sperm quality through maintaining males at lower temperatures (25–27 °C) where possible, increased stocking density of males, and facilitating artificial insemination, if this technique is employed.

However, the separation of males and females entails the capture and movement of females twice each spawning night (once to transfer to the male tank and the second time to transfer to the spawning tank), which results in excessive stress during a very vulnerable stage. In addition, mating tends to be better with mixed sexes, due to excitation of the shrimp by high hormonal concentrations in the mixed tanks. As a guide, wild broodstock usually produce spawning rates of 4–8% of females per night, while domesticated stocks tend to be more productive, producing 10–15% or more of females per night.

## 4.5 Spawning

**A separate spawning room should be used**

Spawning should take place in a separate room from the maturation area in order to keep the spawning area clean and to be able to carry out daily washing and disinfection of tanks without disturbing the broodstock. The spawning room should have sufficient and appropriate infrastructure for the level of naupliar production required.

**Where possible, spawning should be carried out individually**

This will reduce the risk of horizontal transfer of diseases between females. It has been shown that the tissues exuded during spawning and faeces can contain high levels of some viruses (IHHNV, HPV, BP, MBV etc.) and exposure to this can result in infection of uninfected females during collective spawning. If collective spawning must be carried out, the number of females per tank should be as low as possible to limit the number of females exposed to potential infection (i.e. one female to 200–300 litres of water).

**Spawning tanks can be any size from 300 litres up to 5-8 mt, depending on the type of spawning used (individual or collective)**

The tanks may be flat bottomed, but if they are slightly conical, or at least angled to the outlet, it allows easier and less damaging harvesting of all the eggs. Tanks should allow the harvest of the eggs in such a way that they can be subjected to washing or a disinfection bath after collection using formalin (100 ppm for 30 sec), or iodine PVP (50–100 ppm for 1–3 min). Treflan may also be added at 0.05–0.1 ppm to combat fungal infections. This disinfection will help to reduce the risk of disease transmission.

**Spawning systems should have the best water quality possible**

Water-purification steps should be taken for spawning and hatching tank water. This will typically include UV light treatment and passage through activated carbon and cartridge filtration to <1 μm. Preferably, water quality should be maintained with a temperature of 28–29 °C and salinity of 30–35 ppt, as in the maturation tanks. EDTA is often added to the spawning tank water as a chelating agent at a recommended dose depending on the heavy metal loadings of the location.

**As a general principle, broodstock should be handled only when necessary to avoid unduly stressing the shrimp**

Excessive chasing of individual shrimp should be avoided. When holding broodstock, grasp them firmly with the abdomen bent so that the uropods and telson are tucked between the walking legs to minimize flexing and the risk of dropping the shrimp. Avoid keeping the broodstock out of water for extended periods. For example, when transferring females to the spawning tank, they should be held as described while maintaining them underwater in beakers or buckets containing maturation water.

**Sourcing of gravid females should be done in the late afternoon/early evening**

Gravid females should be selected in the late afternoon or early evening (as soon as night falls), or at the most suitable time dictated by the photoperiod employed. When sourcing, use a strong, preferably waterproof, flashlight to see which of the females in the tank look gravid (those with the most highly developed, or stage IV ovaries). When a gravid female is found, use the scoop net to capture it as gently as possible and bring it to the side. The female is then inspected to see

if there is a spermatophore on the thelycum. If the spermatophore is present, the female is placed in a container and transferred to the spawning room. If there is no spermatophore present, the female is placed in another container and taken elsewhere for artificial insemination (if employed) before transferral to the spawning tanks.

**The fecundity, spawning rate (number of spawns per female) and length of time that the females are kept in maturation should be monitored**

To avoid deterioration of the naupliar quality, ablated females should typically be retired from the maturation unit after a maximum period of three months or 15 spawns, depending on the feeding regime used and health of the spawners. Nonablated females can be spawned for up to one year. This usually requires that females be identified individually by tagging or some other method.

**Egg and sperm counts should be made to determine good egg production and fertilisation**

As a guide, the quantity of eggs spawned per female should be in the range of 100 000 to 140 000 eggs for females of 30 to 35 g body weight, and up to 150 000 to 200 000 eggs for 40 to 45 g females.

To ensure good fertilization, sperm should be observed and quantified regularly through sperm counts using a high powered light microscope.

**A suitable system for egg collection should be employed**

Spawning may be either collective, with two or more females in the spawning tank, or individual. In either case, a suitable system for harvesting the eggs, excluding broodstock faeces and ovarian tissues (using a prefilter made from 300–500 μm mesh, for example) is required.

The eggs should be collected into a receptacle with a large, mostly submerged mesh of <100 μm pore size in order to retain them without damage. Once harvested, the eggs should be washed with adequately treated seawater (filtered and sterilized) and then disinfected using iodine-PVP (50–100 ppm/10–60 sec) before rinsing again with abundant clean seawater in another recipient.

**Fertilisation and hatching rates should be monitored**

Following collection, the eggs are then transferred to hatching tanks in the hatching unit. A sample of the eggs harvested should be examined to determine the fertilization rate and a count made to allow an estimation of the hatching rate. The fertilization rate should be at least 50% and is more typically >75%. Where fertilization rates fall below 50%, consideration should be given to discarding the entire batch and investigations begun to determine the cause of the problem.

## 4.6 Hatching

**Hatching should take place in an isolated and clean room**

Hatching tanks (300–1 000 litre) usually have pronounced conical bottoms to allow good water circulation and aeration and easy harvesting. Tanks vary in size from tens of litres to 1 mt, and can be stocked with up to 4 million eggs/mt. Water quality should be maintained at 29–32 °C and 32–35 ppt salinity for optimal hatching. EDTA (at up to 20 ppm) and Treflan (0.05–0.1 ppm) are usually added to the water in the hatching tanks for the same reasons as with spawning.

The tank is provided with enough aeration to keep the eggs moving in suspension. The nauplii should appear approximately eight hours after stocking the eggs. After this point (typically after 12–15 hours), the aeration is stopped in order to harvest the nauplii. A dark cover or lid cover having a small hole cut in its centre is then placed over the tank and a light bulb is suspended above the hole. The healthy nauplii are allowed to aggregate below this hole over a period of 20–30 minutes and are then collected by bucket or siphon into a separate bucket or nauplii collector, where they can be washed and disinfected. They are then held in separate tanks or buckets with aeration or sent directly to the larval rearing facilities. The unhatched eggs and weaker nauplii that remain in the hatching tank are then discarded and the tank cleaned and disinfected. The spawning and hatching tanks are washed daily with calcium (or sodium) hypochlorite solution (30 ppm active ingredient), and rinsed with abundant treated water before being refilled.

## 4.7 Broodstock health screening

**Besides screening for general health, broodstock selected for maturation should be screened for WSSV, IHHN, TSV and YHV**

Where numbers of broodstock are large, the tests may be carried out on pools of 10 individuals from different broodstock groups. A minimum sample of 150 animals for each group of 1 000 shrimp should be taken and divided into groups of 10 shrimp for each analysis. When selecting for genetic programmes, more stringent disease screening should be used to ensure freedom from pathogens. Although PCR testing should be conducted on broodstock upon arrival during their quarantine, it is worthwhile to conduct additional PCR testing (at least for WSSV) after spawning. This is because there is evidence that broodstock that tested PCR-negative for WSSV during quarantine may test positive if analysed following exposure to a stress such as spawning.

## 4.8 Broodstock nutrition

**A good diet and feeding protocol should be essential components of the maturation programme**

A good diet and feeding protocol for broodstock are key factors in the production of good quality nauplii. The appropriate quantity of feeds must be determined in relation to the biomass in the tank. In general, feeding should be at a rate of up to 20–30% of the broodstock biomass (wet weight basis or when using fresh or frozen feed). When dry feed is used, the rate will be less.

The exact quantity of feed given should be adjusted frequently based on the consumption rate of each tank. The feeding should continue until only a very small amount of uneaten food remains in the tank a couple of hours after each feeding. The diet fed should be balanced, concentrating on the use of feeds high in vitamins, minerals, pigments and fatty acids (such as 20:5n3 and 22:6n3), which are essential for the production of eggs.

**Cross-contamination of feed during its preparation must be avoided**

Feed preparation should be carried out using good hygienic standards. Utensils (knives, tables, mixers, pelletisers etc.) must be kept clean, washed before use with iodine-PVP solution (20 ppm) and rinsed with clean water.

**Fresh feeds should be really fresh, and certified free from important viruses or sterilised**

When using fresh feeds such squid, polychaetes, *Artemia*, krill, mussels, oysters, clams etc., efforts must be made to ensure that the material is as fresh as possible. To ensure that fresh feed is not a biosecurity risk, a certificate should be requested at the time of purchase stating that the feed is free of the viruses TSV, WSSV and YHV by PCR analysis. Alternatively, the feeds may be sterilized or pasteurized (recommended) to inactivate any virus, as long as this does not affect the acceptability or nutritional quality of the feed. Ideally, different types of frozen feeds should be stored in separate freezers.

**Fresh feeds should be chopped to a size suitable for ingestion**:

Fresh feeds need to be chopped to a size suitable for ingestion by the broodstock and washed with clean water and weighed prior to feeding. Feeding will typically be done every three to four hours during the day and night.

**Artificial feeds should be enriched with nutritional additives**

Artificial feed should be enriched with additives such as vitamins C and E, immunostimulants, astaxanthin, carotenoids, polyunsaturated fatty acids etc. if it is intended to complement fresh feed with dry or moist pelleted feeds. Several commercial companies produce artificial feeds to supplement the fresh feeds used in maturation, although none yet serve as full replacements. Dry or moist diets can also be economically cold-extruded (using a pelletiser or an extruder) on site using regular shrimp feeds ground to powder and incorporating the various additives mentioned above, plus a binder such as alginate or gelatine. Selection of an appropriate feed depends on the specific requirements of the maturation facility.

**Dry feeds should be fed separately from fresh feeds and two to three times per day (at up to 2-3% of shrimp biomass/day) using low feeding rates each time to ensure that they are completely consumed**

Domesticated *Penaeus vannamei* have an advantage over their wild counterparts or *P. monodon* because they have been raised on pelleted diets and are thus accustomed to consuming them.

Wild broodstock often prove reluctant to eat dry feeds and must be acclimated to them very gradually over time. As for all management practices with broodstock, any changes made should be minimized as much as possible to limit stressing the animals. Any changes to feeding regimes, types, quantities and times should be minimized as much as possible. Hence, stocks of all feed ingredients or types used should be maintained at all times.

# 5 The post-spawning process

The post-spawning process includes facility maintenance, water quality management; broodstock handling; washing, selection, holding and transport of nauplii; postlarval rearing, maintenance, health management, assessment of condition, selection and risk assessment for stocking, shipping and transfer; and documentation and record keeping.

## 5.1 Facility maintenance

**To achieve consistent production of high quality larvae, the production facilities must be maintained in optimal condition**

Facilities must be maintained so as to optimize the conditions for the growth, survival and health of the shrimp broodstock, larvae and PL, minimizing the risks of disease outbreaks. In order to facilitate this, a set of protocols must be drawn up by the hatchery management as part of the Standard Operating Procedures (SOPs) and followed strictly by all staff members at all times. The hatchery's SOPs should include procedures for a sanitary dry out following each cultivation cycle (for larval rearing), or at least every three to four months (for maturation facilities), with a minimum dry period following cleaning of seven days. This will help prevent the transmission of disease agents from one cycle to the next.

**All tanks and equipment should be thoroughly cleaned on a regular basis, cleaned and disinfected after use, and cleaned and disinfected again before starting a new production cycle**

Tanks used for broodstock spawning, egg hatching, and holding of nauplii and postlarvae should be thoroughly cleaned after each use. The procedures used for cleaning and disinfection are basically the same for all tanks and equipment. They include scrubbing with clean water and detergent to loosen all dirt and debris, disinfecting with hypochlorite solution (20–30 ppm active ingredient) and/or a 10% solution of muriatic[8] acid (pH 2–3), rinsing with abundant clean water to remove all traces of chlorine and/or acid, and then drying. The walls of tanks may also be wiped down with muriatic acid; outdoor tanks and small tanks can be sterilized by sun drying.

The following points should be considered:

- Tanks should be washed and disinfected at the end of every production cycle.
- All hatchery equipment should be regularly cleaned and disinfected.
- Concrete tanks painted with marine epoxy or plastic-lined tanks are easier to clean and maintain than bare cement tanks.
- After harvesting the larvae from a larval rearing tank, the tank and all of its equipment should be disinfected. Similarly, once all of the tanks in a room have been harvested, the room itself and all its equipment should be disinfected.

[8] In the past, muriatic acid was referred to 3:1 HCl and $HNO_3$, but currently it is referred to as 34-37% HCl.

- Tanks can be filled to the maximum level and hypochlorite solution added to achieve a minimum concentration of 20–30 ppm active ingredient. After 48 hours, the tanks can be drained and should be kept dry until the next cycle starts.
- All equipment and other material used in the room (filters, hoses, beakers, water and air lines etc.) can be placed in one of the tanks containing hypochlorite solution after first cleaning with a 10% muriatic acid solution.
- Broodstock maturation tanks and all associated equipment should be cleaned and disinfected following a typical operation period of three to four months.
- Water pipes, air lines, air stones etc. should be washed on a monthly basis (or during dry-out) with the same chlorine concentration and/or a 10% solution of muriatic acid (pH 2–3) by pumping from a central tank.
- All hatchery buildings (floors and walls) should be periodically (once per cycle is recommended) disinfected.
- All other equipment should be thoroughly cleaned between cycles.
- Before stocking tanks for a new cycle, they should once again be washed with detergent, rinsed with clean water, wiped down with 10% muriatic acid and once more rinsed with treated water before filling.
- Disinfection procedures may require adjustment according to the special needs of the facility.
- Appropriate safety measures must be taken when handling the chemicals used for disinfection. Procedures regarding chemical usage and storage, wearing of protective gear etc. should be included in the hatchery's Standard Operating Procedures (SOPs).

Recommended products, concentrations and frequencies for the disinfection of various hatchery items are also given in OIE (2003).

## 5.2 Water quality management

**The hatchery infrastructure should allow appropriate cleaning and disinfection of the incoming water**

Incoming water should be cleaned and disinfected through chlorination and filtration before being distributed to different working areas (hatchery, algal culture, *Artemia* etc.). Distribution should be designed to avoid the risk of cross-contamination. Water and air distribution systems should be designed to allow for pumping of disinfecting solutions through the system and to permit complete drying during dry-outs.

**Ideally, maturation and larval rearing facilities should be built to take advantage of a supply of oceanic water**

However, it is possible to use seawater brought into the facility from elsewhere or suboptimal seawater with the appropriate filtration and disinfection techniques. In general, a closed recirculating system is more biosecure than an open water system, but requires additional biological and mechanical filtration and disinfection to maintain optimal water quality.

**"Sub-sand beach wells" should be used for primary water filtration**

The most common system for filtration of raw seawater entering the hatchery from the sea is through the use of "sub-sand beach wells". These consist of a series of filter galleries, well-points, wells, tips etc. that allow primary filtration before entering the hatchery. They also limit fouling organisms, pathogen hosts, red tides and some pathogens, which direct intake systems do not.

**Water carrying a high sediment load should be passed through sedimentation tanks to remove suspended solids**

A minimum storage capacity of 50% of the total capacity is required when the reservoirs can be refilled twice daily.

**Incoming water should be disinfected to destroy any remaining pathogens and any heavy metals present removed by chelation**

Calcium (or sodium) hypochlorite (10 ppm active ingredient for not less than 30 min), and/or ozone, or UV light should be used to disinfect the incoming water after initial filtration and sedimentation. After treatment with chlorine, the water in the reservoir must be checked by ortho-toluidine (3 drops in 5 mL of water sample) to ensure no chlorine residual remains (indicated by a yellow colour) before the water is used. A chart or whiteboard must be provided giving the date and time of treatments and the results of these tests signed by the person who is responsible for the water treatment. Once the chlorine has dissipated or been neutralized with sodium thiosulfate (1 ppm for every 1 ppm of chlorine remaining), EDTA can be applied to chelate any heavy metals present (quantities depending on concentrations of heavy metals and use).

**The temperature of the water should be adjusted before it enters the production units**

A boiler and heat exchange system, typically located between the reservoir and production units, may be required to adjust water temperatures to within the range required (generally between 28 and 32 °C depending on area and stage, see Table 4).

The water filtration system following the reservoirs should consist of sand filters, activated carbon, and other filtering elements such as cartridge filters or membrane filters for water uses requiring fine filtration.

**Sand filters must be properly maintained**

Sand filters must be backwashed at least two times per day (or as required based on the suspended solids loading of the incoming water) for a sufficient length of time to assure the cleaning of the filter.

Being able to open the filters to check for channelling and thorough backwashing is an advantage. At the beginning of each production cycle, the sand must be replaced by clean sand

that has been previously washed with sodium hypochlorite solution at 20 ppm active ingredient or 10% muriatic acid solution (pH 2–3). Activated carbon should be replaced at least once every hatchery cycle to maintain efficiency.

**Cartridge filters must exchanged daily**

For cartridge filters, two sets of filtering elements must be available and these sets should be exchanged every day. Used filters are washed and disinfected in a solution of calcium (sodium) hypochlorite at 10 ppm active ingredient or 10% muriatic acid solution for one hour. Some filter materials are sensitive to muriatic acid and thus care must be taken when this disinfectant is used. The filters are then rinsed with abundant treated water and dipped in a recipient containing a solution of 10 ppm sodium thiosulfate to neutralize chlorine (if used). Two or more new sets of filters should be used for each hatchery cycle, depending upon the suspended solids loading of the seawater.

The recommended final size of filtration depends on the uses of the water as shown in Table 4.

**Table 4. Recommended water filtration standards and water temperatures for different hatchery needs.**

| WATER USE | FILTER SIZE (μm) | TEMPERATURE (°C) |
|---|---|---|
| Maturation | 15 | 28 to 29 |
| Hatchery | 5 | 28 to 32 |
| Spawning and hatching | 0.5–1.0 | 29 to 32 |
| Algae culture (indoor/pure) | 0.5 | 18 to 24 |

**If recirculation systems are employed, additional biological filtration should be used**

To prevent cross-contamination between different areas of the hatchery, separate recirculation systems should be used for each area requiring them Water recirculation systems are the most efficient systems for broodstock maturation, as they reduce the need for water replacement and residual water discharge. Recirculation systems help maintain stable physical and chemical parameters in the water and also help concentrate mating hormones in maturation, as well as providing better biosecurity.

If recirculation of seawater is required for any area of the hatchery, additional biological filtration will be required to remove dissolved organic material. There are many types of biofilters, all of which incorporate living elements (denitrifying bacteria) that must be cultivated or "spiked" (additional biological material added to the filter) prior to use, so that their effects are optimized at all stages of the cycle. They also require periodic cleaning in a way that does not kill their beneficial bacterial inhabitants.

**The water to be used in the spawning and hatching tanks and pure algal culture facilities must be the same quality**

The spawning and hatching tanks and pure algal culture facilities must receive water of the same quality and treated in the same way as the water used in the maturation and larval rearing units

(i.e. with the addition of UV light sterilization and filtration to 0.5 or 1 μm). Additionally, for hatching and spawning, EDTA is often required at up to 20–40 ppm to ensure heavy metals are chelated and made unavailable, and Treflan at 0.05–0.1 ppm is usually used to combat fungi.

**Water distribution should be designed so that each area of the hatchery can be disinfected separately**

Water distribution from the reservoir to the various areas of the hatchery should be designed so that each area can be disinfected without compromising the other areas. In this way, regularly scheduled disinfections can be accomplished at times appropriate to each area and cross-contamination between areas can be avoided. Temperature and salinity regulation may vary between different sectors and is facilitated by well-designed distribution systems. In addition, each area has specific filtration requirements, which can be established prior to point of use, appropriate to each area of the hatchery. Pumps, pipes and filtration equipment should be sized so that maximum expected water exchange rates can be maintained to ensure optimal conditions are met at all times.

## 5.3 Broodstock disinfection

After removing the spent females from the spawning tanks, they should be immersed in iodine-PVP (20 ppm/15 sec) before returning them to the tank of origin.

## 5.4 Washing of nauplii

Harvested nauplii at stage 4 can be treated by bath immersion in Treflan (0.05–0.1 ppm) to prevent fungal contamination, followed by a thorough wash in filtered and sterilized water and a dip in an iodine-PVP solution (50–100 ppm for 1–3 min) or chloramine-T solution (60 ppm for 1 min), followed immediately by further washes with clean seawater.

Other washing steps have also been described using formalin and iodine-PVP. Chen *et al.* (1992) and Brock and Main (1994) described a method in which the nauplii are given a 30 second dip in both formalin (300 ppm) and iodophore (100 ppm) before rinsing with filtered sterile seawater for three minutes prior to stocking. This can be effective in removing debris and fouling organisms such as bacteria and protozoa, and may minimize the transmission of viral diseases.

## 5.5 Selection of Nauplii

**Nauplii should be harvested using a light to attract them to the water surface**

As the nauplii display strong positive phototaxis, healthy nauplii can be harvested using a light to attract them to the water surface. Those that remain at the bottom of the tank are discarded, reducing the percentage of weak and deformed nauplii. After harvesting, the number of good nauplii is counted to provide the hatching rate. In good batches, the hatching rate should be >70%. If lower rates are encountered, a decision is made as to whether the whole batch should be discarded and investigations initiated to discover the cause of the problem.

**The activity and colour of the nauplii should be evaluated and the percentage of deformities estimated**

A deformity rate of <5% is generally considered acceptable. An estimate is made of the naupliar condition using the extent of the positive phototaxis. To carry out this test, a sample of larvae is placed in a translucent container next to a light source and the displacement of the animals is observed. If 95% or more of the larvae move strongly towards the light, the batch is good; it is intermediate if 70% or more respond, and poor if less than 70% move towards the light. Poor batches may be discarded, depending upon the selection criteria of each hatchery.

## 5.6 Holding of Nauplii

**Harvested nauplii must be held under optimal conditions until they are stocked**

The harvested nauplii can be held at a density of 20 000–40 000/litre, with continuous light, clean water and aeration until they are ready to be stocked in hatchery tanks. As with eggs, nauplii (stage 4) can be treated with Treflan and/or disinfected. The equipment and material used to harvest the nauplii must be washed daily with a calcium (sodium) hypochlorite solution (30 ppm active ingredient) to prevent contamination of subsequent batches.

## 5.7 Transportation of Nauplii

**The nauplii should be transported at densities of 15 000 – 30 000 nauplii/litre, depending on distance or time to the hatchery**

Transportation is normally done in double plastic bags containing 10–15 litres of water and filled with pure oxygen. The bags are then packed in cardboard and/or styrofoam boxes, although plastic buckets and tanks are sometimes used. The temperature of the packing and shipping water is adjusted from 28–30 °C down to between 18 and 25 °C (and sometimes not at all), according to the travel time and distance to the receiving hatchery. Salinity is maintained at 32–35 ppt. Upon arrival at the purchasing hatchery, the nauplii should again be disinfected.

If possible, the transport vehicle should first be disinfected before entering the hatchery facilities. After unpacking the nauplii, the packing material must be incinerated.

## 5.8 Larval Rearing and Maintenance

**Larval rearing should produce the best quality, high-health postlarvae possible**

In many cases, where hatcheries and farms form distinct economic units, larval quality is often sacrificed for economy. However, in reality the most economic strategy is to produce postlarvae that will grow quickly, are free from disease and that will give a high survival and production rate in the grow-out facilities. In order to achieve this, all areas involved in larval rearing must be designed for optimal efficiency, cleanliness and productivity.

**Entrance to the larval rearing area(s) should be restricted**

Entrance to the larval rearing area(s) should be restricted to the personnel that work in these areas. Sanitary mats or footbaths containing a disinfectant solution (e.g. calcium or sodium hypochlorite solution, >50 ppm active ingredient) must be placed at the entrance of each room of the hatchery. The disinfectant solution must be replaced as necessary. At each entrance to the larval rearing room(s), container(s) with iodine-PVP (20 ppm) and/or 70% alcohol should be available and all personnel must wash their hands in the disinfection solution(s) on entry to, and exit from, the rooms.

**Each room should have a complete complement of materials for routine operation**.

Each room should have a complete complement of materials for routine operation (filters, meshes, buckets etc.). A tank (500–600 litres) containing disinfectant (hypochlorite solution, 20 ppm active ingredient) should be provided to disinfect hoses, buckets, etc. Common-use equipment can be placed in this disinfecting tank at the end of every day and rinsed before re-use the following day. The disinfectant in this tank should be replaced daily or as required.

**All materials and equipment should be for the exclusive use in each room, and should not leave the room or be used elsewhere**

Additionally, beakers, nets etc. used for each tank should be maintained in a bucket filled with sodium hypochlorite solution (20 ppm active ingredient) and dedicated to that one tank to prevent cross-contamination between tanks within the same unit.

**Larvae and postlarvae should be routinely checked for quality**

Samples of larvae and postlarvae for routine checking should be taken in disposable plastic containers (paper cups or 300 mL plastic beakers) that are disposed of once used. After the daily check is complete, the larvae or postlarvae should be discarded into a plastic container with sodium hypochlorite (20 ppm active ingredient) or another suitable disinfectant. Larvae and postlarvae used in the daily checks must never be returned to the larval rearing rooms or larval tanks.

**The infrastructure for larval culture should include of one or more units of conical or "V"-shaped larval rearing tanks**

The infrastructure for larval culture consists principally of one or more units of conical or "V"-shaped larval rearing tanks (the tanks are sometimes in two phases: one from nauplius to PL4–5, then larger, flat bottomed tanks or raceways for postlarvae or nursery culture). Supporting infrastructure (discussed in more detail elsewhere) includes a water storage, treatment, heating and distribution system; an aeration system; live feed production facilities for algae and *Artemia* (and others); laboratories for health checks, bacteriology and feed preparation; offices and an area for packing and shipping postlarvae.

## 5.9 Larval nutrition and feed management

**High standards of feed preparation must be maintained**

All feed preparation, *especially* of live feeds (algae, *Artemia* and others), is a critical control point (CCP), because feed can be contaminated through inappropriate handling. All sources of live, fresh or frozen food should be considered from the point of view of pathogen risk. The source, treatment, storage and use of feed items should be reviewed and steps taken to ensure that they are safe and properly handled.

**Entry to the algal culture and *Artemia* culture rooms must be restricted to authorised personnel**

Staff from these areas should not be able to enter other production areas. At the entrance of each room a footbath containing a disinfecting solution (calcium (sodium) hypochlorite, >50 ppm active ingredient) should be placed. This solution must be replaced as often as necessary. As in other areas, container(s) of disinfectant solution (20 ppm of iodine-PVP and/or 70% alcohol) should be placed at the doors and all staff should wash their hands on entering and leaving the room.

It is beyond the scope of this manual to detail exact feeding protocols for larval rearing. The feeding regimes should be based on the specific requirements of the various larval stages, backed by frequent and detailed examinations of the feeding activity of the larvae in each tank. Indications are given in this section of significant points to bear in mind.

### *Algae*

**An extremely high standard of hygiene must be maintained for microalgal cultures**

Microalgal culture requires extreme hygiene in the laboratory phases, including thorough disinfection and filtration (to <0.5 μm) of all water and air supplies, through the use of sterilizers for all equipment and water, to the use of pure laboratory-grade fertilizing chemicals and air-conditioning to maintain temperatures between 18–24 ºC.

**Pure cultures of algae must be maintained using appropriate sanitary and microbiological procedures**

Single-celled algae such as *Chaetoceros*, *Thalassiosira, Tetraselmis, Isochrysis* and *Dunaliela* are most commonly used. Pure cultures of all the algal species used should be maintained and cultured and subcultured on site, at all stages (from agar plates and tubes/bottles in the laboratory to massive on-growing outside). Appropriate sanitary and microbiological procedures should be used to ensure the quality of the culture. Contamination with protozoans that feed on algae, other species of algae, and bacteria (in particular harmful *Vibrio* spp.) should be avoided. Alternatively, pure starter cultures can be purchased from reputable algal culture laboratories and be on-grown in the hatchery's massive tanks using sanitary procedures. The procedure of buying one lot of pure algal culture and continuously subculturing it throughout each larval culture cycle is not

recommended, as it can easily lead to contamination of the algae and eventually, of the larvae themselves.

**All algal culture tanks must be washed and disinfected after each harvesting**

Following disinfection of the algal culture tanks with calcium (sodium) hypochlorite solution (10 ppm active ingredient), they should be rinsed with clean, treated water and washed with a 10% muriatic acid before being left to dry.

Planktonic microalgae are usually offered to the larvae from the last naupliar stages, so that upon metamorphosis to the first feeding stage (zoea 1), the larvae will be able to begin feeding immediately. Concentrations are usually maintained at 80 000 –130 000 cells/mL throughout the zoea l and mysis stages, and then decline through the postlarval stages as the larvae become more carnivorous. During postlarval or nursery culture, benthic algae are often used, as the postlarvae will begin grazing algae from the walls of the tanks.

## *Artemia*

**Measures should be taken to ensure that *Artemia* do not pose a risk of disease introduction**

Certification may be requested for freedom from TSV, WSSV and YHV viruses by PCR analysis for all *Artemia* cysts purchased.

***Artemia* should be decapsulated**

Although *Artemia* cysts may not carry major viral pathogens (Sahul Hameed *et al.* 2002), they are certainly significant sources of bacterial, fungal and protozoan diseases. Therefore. decapsulation of the cysts is recommended to avoid contamination of the *Artemia* culture water and the possibility of resulting contamination of larval rearing water.

Decapsulation is carried out using 40 g of caustic soda (NaOH) and 4 litre of chlorine liquid (8–10% active ingredient) in 4 litres of seawater per 1 kg of *Artemia* cysts to be decapsulated. During decapsulation, the decapsulation mixture must be maintained below 20 $^{o}$C using ice to prevent damage to the cysts. As soon as the cysts begin to turn orange (indicating successful decapsulation), they are rinsed with 100 g of sodium thiosulphate added to the water containing decapsulated *Artemia* to stop the chlorination. The decapsulated cysts can then be washed with clean fresh water and stored in a super-saturated brine solution until needed for hatching.

***Artemia* should be hatched at 1-2 kg cysts/mt of seawater under constant light and vigorous aeration for 24 hours or until fully hatched**

*Artemia* nauplii are then harvested, disinfected with a 20 ppm sodium hypochlorite solution, or better, chloramine-T at 60 ppm for 3 min, and washed with fresh water. They can then be fed live, or frozen and fed when needed, or placed into separate tanks for enrichment (for 3–12 hours), or for on-growing for feeding to postlarval stages.

**After harvesting the *Artemia*, the hatching tanks should be thoroughly cleaned**

After harvest, the tanks used to hatch *Artemia* must be washed with detergent and water, and then disinfected using a sponge dipped in sodium hypochlorite solution (20 ppm active ingredient), rinsed with abundant treated (filtered and sterilized) water and washed again with a 10% solution of muriatic acid.

Frozen *Artemia* nauplii or adults should be stored in a separate, exclusive freezer. Basic hygienic protocols (SOPs) must be implemented at all times.

### *Artificial feeds*

**Although artificial feeds generally do not pose a health risk, they must be properly used and stored**

Many kinds of artificial or formulated feeds are available for use during larval rearing. These types of feeds generally do not pose the same health risks as live feeds, because they can be maintained free from contamination quite easily.

Artificial feeds include dried algae, liquid feeds, microencapsulated feeds, flakes and crumbled pellets, and mineral and vitamin supplements and enrichments. These are used in various sizes according to the stage of larval development and in various combinations, depending upon hatchery preferences, water quality and nutritional requirements. However, they are usually used primarily as supplements to live feeds.

Generally, as long as high quality feeds are selected and they are stored correctly in cool, dry conditions, used promptly once the container is opened, and not used excessively (as this can lead to water quality issues), they should not present any health-related difficulties.

## 5.10 Larval health management

**If good numbers of high quality larvae are to be produced, tight control must been maintained on the many factors involved in managing larval health in the hatchery**

There are many factors involved in managing larval health in the hatchery. A tight control must be maintained on all of these factors throughout the larval rearing cycle if good numbers of high quality larvae are to be produced. Some of the more common factors affecting larval health during the larval culture cycle (assuming that high quality nauplii have been stocked according to the methods outlined earlier in this section) are shown in Table 5.

**Table 5. Some factors affecting shrimp larval health and possible control measures.**

| FACTOR | EFFECTS | CONTROL MEASURES | STANDARD |
|---|---|---|---|
| Excessive Stocking Density | Stress<br>Cannibalism<br>Poor water quality | Reduce stocking density | 100 to 250 nauplii/litre |
| Poor Water Quality<br>♦ Sea water (A)<br>♦ Tank water (B) | Mortalities<br>Late moulting<br>Deformities | Improve water quality by filtration, chlorination and/or sterilization (A)<br>Increase water exchange (B) | Filter < 5 μm<br>Activated carbon<br>Chlorination (10 ppm) followed by nutralization<br>Ozone and UV<br>20–100% water exchange per day |
| Long Stocking Period | Increased infection rates of later stocked larvae | Limit number of days in stocking hatchery | 3–4 days per unit |
| Poor Feeding (Quality and/or Frequency) | Cannibalism<br>Malnutrition<br>Epibiont fouling<br>Poor water quality | Appropriate feeding programme,<br>Frequent checks on feed consumption and water quality | Feed every 2 to 4 hours to satiation with high quality feeds |
| Quality and/or Quantity of Algae | Mortality in zoeal stages<br>Fouling of larvae | Routine counts and quality checks | *Chaetoceros* or *Thalassiosira* 80 000 to 130 000 cells/mL |
| *Artemia* Nauplii | Source of bacteria leading to mortality | Disinfection of *Artemia* nauplii | hypochlorite at 20 ppm active ingredient |

## *Stocking density*

**The density at which nauplii are stocked should not be excessive**

Overstocking can result in stress and in later stages, and may lead to cannibalism and reductions in water quality, especially when survival rates are high. In general, stocking rates for nauplii should be in the range of 100–250 nauplii/litre (100 000 – 250 000 per mt) of water. Lower stocking densities are typically used where larvae are grown to harvest size in a single tank, while higher densities can be used where a two-tank system is used. In the latter system, the larvae are typically cultured in a conical or "V" or "U"-bottomed tank at high density until PL4–5 and then transferred to flat-bottomed tanks for the later, benthic stages at reduced densities of up to 100 PL/litre.

Poor survival may reduce the density of larvae in a larval rearing tank to a level below where it is cost-effective to feed (because larval rearing tanks are generally fed according to volume of water rather than number of larvae).

## *Water quality*

**Good water quality must be maintained**

Water quality has a major impact on the health and performance of larval batches. Poor water quality can lead to poor growth, low survival, late moulting/staging, increased epibiont fouling and deformities. Water for larval rearing should be filtered to around 5 μm and disinfected with chlorine, ozone and/or UV. The temperature should be maintained between 28 and 32 °C and salinity above 30 ppt, at least until postlarval stages are reached. Dissolved oxygen levels should be maintained as close to saturation (6.2 ppm at 30 °C) as possible, but at least above 5 ppm. A pH of around 8 should be maintained. Overfeeding is one of the major causes of water quality deterioration and should be avoided.

Water quality is also maintained through aeration that is sufficient to prevent uneaten food and faeces from settling on the tank bottom (normally necessitating tanks with angled bottoms) and regular siphoning of the tank to prevent a build up of anaerobic sludge on the bottom.

**Water exchange should be carefully handled**

Generally, no water is exchanged until the mysis stage is reached, although water levels are generally increased throughout the zoeal stages, because nauplii are usually stocked in tanks that are only half full. After the mysis stage, typically 20–100% of the water is exchanged daily, depending upon the stocking density and water quality parameters. Care should be taken to make sure that the water used to top-up the tanks and all exchange water is at a similar temperature, salinity and pH to the water in the tank, and free from chlorine to avoid unduly stressing the larvae.

**Hatcheries should also consider the use of probiotics and bacterial enzymes**

In an effort to maintain water quality, prevent bacterial blooms and reduce or eliminate the requirement for antibiotics during larval culture, hatcheries are turning increasingly towards the use of probiotic powders or solutions of beneficial bacteria and bacterial enzymes. As for all these products and supplements, care must be taken in selecting those which do not pose their own health risks and which are efficacious and cost-effective.

## *Stocking period*

**Each separate unit of larval rearing tanks within a hatchery or, preferably, the whole hatchery should be stocked with nauplii in as short a time period as possible**

Each separate unit of larval rearing tanks within a hatchery or, preferably, the whole hatchery should be stocked with nauplii in as short a time period as possible, usually limited to three to four days. Prolonging this stocking period often results in increased incidence of disease for the later-stocked larvae, presumably through bacterial contamination from the older to the younger tanks.

This phenomenon is often associated with the so-called "zoea-2 syndrome", where late zoea 1 and early zoea 2 stage larvae refuse to eat and suffer high mortality with associated bacterial problems. This problem may be controlled through restricting the time of stocking to less than four days, using probiotics and maintaining good cleanliness in all areas of the hatchery at all times.

### *Nutrition and feeding*

**The quantity, quality and management of feed should be closed monitored**

The quantity, quality and management of feed can have an important impact on larval health and survival. Failure to provide sufficient feed of the right quality can lead to stress, poor growth, mortality, increases in cannibalistic behaviour, deformity and increased levels of epibiont fouling. This is especially true when a large proportion of the feed used is formulated diets. When using formulated diets as a supplement to live feeds, it important is to feed small amounts of high quality, appropriately sized, nonpolluting diets frequently. As a guide, particle sizes should be 10–50 μm for zoea, 100–200 μm for mysis, and 200–300 μm for early postlarval stages. A feeding frequency of every two to four hours is generally regarded as sufficient.

**For the majority of the larval feed requirements, reliance should still be placed on high quality live feeds, including algae and *Artemia***

However, insufficient or poor quality algae can also have severe consequences for larval health. Heavy mortality in the zoea stage, for example, has been linked to algal quality, and insufficient algae during this stage (<80 000 – 130 000 cells/mL) can result in the larvae having insufficient reserves to complete the stressful moult to mysis. Algal concentrations and quality should be regularly monitored to ensure that they are sufficient for the stage being fed.

## 5.11 General assessment of larval condition

**Assessment of larval condition should be one of the main activities carried out in the hatchery**

Assessment of larval condition is usually done in the morning, and decisions on water exchange, feeding and other management activities made so that action can be taken in the afternoon. The larvae in each tank should be inspected two to four times each day. Initially, a visual inspection of the larvae, the condition of the water in the rearing tank and the feed is made. A sample of larvae can be taken with a beaker and inspected with the naked eye. Observations are made on the larval stage, health, activity, behaviour and abundance of feed and faeces in the water. Records may also be taken of water quality parameters, and the amount of food in the tank.

The same, or a separate sample of larvae, should also be taken to the laboratory for a more detailed microscopic examination. This will provide information on the stage, condition, feeding and digestion and presence of any disease or physical deformity.

Samples may also be sent once or twice during the cycle for analysis in a PCR laboratory for screening for viral diseases.

The type of observations that are made can be categorized into three levels, based on the health assessment levels described in Table 2.

## *Level 1 Observations*

Level 1 observations are based on simple visual features of the larvae and water condition that can be easily seen with the naked eye in a glass beaker of animals taken from the tank. Special attention is paid to the behaviour or activity of the larvae, their swimming behaviour (according to the larval stage), water quality, presence of feed and faeces and later on, size disparity and homogeneity. These observations and the scoring system used are summarized in Table 6.

**Table 6. Summary of Level 1 assessments of larval health.**

| CRITERIA | SCORE | STAGE | OBSERVATION |
|---|---|---|---|
| *Swimming activity*<br>Active (> 95%)<br>Intermediate (70–95%)<br>Weak (on bottom) (< 70%) | <br>10<br>5<br>0 | All stages | Daily (2–4x) observations |
| *Phototaxism*<br>Positive (>95%)<br>Intermediate (70–95%)<br>Negative (< 70%) | <br>10<br>5<br>0 | Zoea | Daily (2–4x) observations |
| *Faecal string (cord)*<br>Present (90–100%)<br>Intermediate (70–90%)<br>Absent (<70%) | <br>10<br>5<br>0 | Zoea | Daily (2–4x) observations |
| *Luminescence*<br>Absent<br>Present (<10%)<br>Abundant (>10%) | <br>10<br>5<br>0 | Mysis | Night observation of the tank |
| *Homogenous stage*<br>High (80–100%)<br>Intermediate (70–80%)<br>Low (< 70%) | <br>10<br>5<br>0 | All Stages | Daily (2–4x) observation |
| *Intestinal contents*<br>Full (100%)<br>Half full (50%)<br>Empty (<20%) | <br>10<br>5<br>0 | Mysis | Daily (2–4x) observation |

### *Swimming activity*

The swimming activity of the larvae changes dramatically but characteristically through the larval cycle. Zoea l stages will swim rapidly and consistently forwards, usually in circles, filter feeding on phytoplankton. Mysis, by comparison, swim backwards with intermittent flicks of their tails, maintaining themselves in the water column and feeding visually on phyto- and zooplankton. PL, again turn to swimming rapidly and consistently forward, initially planktonically, but at least from

PL4–5 onwards, benthically, searching for food, unless maintained in the water column by strong aeration. Within these distinct modes of swimming, if >95% of the larvae are observed to be swimming actively, they are given a score of 10; if 70–95% are active, they are given a score of 5; and if <70% are active, they are given a score of 0.

***Phototaxis***

Zoea stage larvae should retain a strong positive phototaxis and move towards light. To test this, a sample of larvae is placed in a translucent container next to a light source and the displacement of the animals is observed. If 95% or more of the larvae move strongly towards the light, the larvae are good and given a 10; if 70–95% respond, they are acceptable and given a 5; and if less than 70% move towards the light, they are considered weak and given a score of 0.

***Faecal string (cord)***

During the zoea 1 stages, when the zoea are feeding almost exclusively on algae, long faecal strings can be seen projecting from the anus and loose in the water column. When 90–100% of the larvae have these long, continuous strings all along the digestive tube, through their bodies and continuing outside, they are considered well fed and given a score of 10. When 70–90% have these strings, or they are short or discontinuous, they are given a score of 5; and when <70% of the larvae have these strings, the larvae are not eating and they are given a score of 0.

***Luminescence***

This factor is observed directly in the larval rearing tank in absolute darkness. Larval luminescence is generally due to the presence of luminescent bacteria such as *Vibrio harveyi*. If no luminescence is observed, a score of 10 is given; if the observed luminescence appears low (up to 10% of the population), the score is 5; and if above 10% of the population are luminescent, the score is zero.

***Stage homogeneity***

This indicates the uniformity of larval stages in a tank. If 80% or more of the population is in the same stage, a score of 10 is given; if between 70 and 80% are at the same stage, the score is 5; and if less than 70% are in the same stage, the score is zero.

It should be noted that when larval shrimp moult, it is normal to see a decrease in the stage homogeneity, so the time at which the stage homogeneity is determined has to be taken into consideration. This is also true for postlarvae when they are moulting.

***Intestinal contents***

The intestinal contents can be observed in older larval stages. The intestine is visible as a dark line from the hepatopancreas in the larva's head region that is easily observed in larvae held in a clear container, such as a glass beaker. This is useful as a guide to larval feeding and feed availability. If most of the larvae observed are full, a score of 10 is given; if half of the larvae have food in the intestine, a score of 5 is given; and if <20% of the larvae have food in the intestine, the score is zero.

## *Level 2 Observations*

Level 2 observations are based on microscopic examination and squash mounts, if necessary, of a randomly taken sample of at least 20 larvae per tank (more for larger tanks). Special attention is paid to the state of the hepatopancreas and intestinal contents, necrosis and deformity of limbs, fouling organisms and the presence of baculovirus in the faeces or hepatopancreas of older larvae. These observations and the scoring system used are summarized in Table 7.

**Table 7. Summary of Level 2 assessments of larval health.**

| CRITERIA | SCORE | STAGE | OBSERVATION |
|---|---|---|---|
| *Hepatopancreas (lipid vacuoles)*<br>High (>90%)<br>Moderate (70–90%)<br>Low (< 70%) | <br>10<br>5<br>0 | All stages | Daily (2–4x) observations |
| *Intestinal content*<br>Full (>95%)<br>Moderate (70–95%)<br>Empty (< 70%) | <br>10<br>5<br>0 | All stages | Daily (2–4x) observations |
| *Necrosis*<br>Absent (0%)<br>Moderate (<15%)<br>Severe (>15%) | <br>10<br>5<br>0 | All stages | Daily (2–4x) observations |
| *Deformities*<br>Absent (0%)<br>Moderate (<10%)<br>Severe (>10%) | <br>10<br>5<br>0 | All stages | Daily (2–4x) observations |
| *Epibionts*<br>Absent (0%)<br>Moderate (<15%)<br>Severe (>15%) | <br>10<br>5<br>0 | All stages | Daily (2–4x) observations |
| "*Bolitas*"•<br>None<br>1 to 3<br>>3 | <br>10<br>5<br>0 | All stages | Daily (2–4x) observations |
| *Baculovirus*<br>Absent (0%)<br>Moderate (<10%)<br>Severe (>10%) | <br>10<br>5<br>0 | Mysis | Daily (2–4x) observations |

• Sloughed cells of hepatopancreas and intestine expressed as number of *"bolitas"* in the digestive tract)

### *Condition of the hepatopancreas and gut contents*

The condition of the hepatopancreas gives an indication of larval feeding and digestion. It is observed using a wet mount of a sample of larvae on a microscope slide at a magnification of 40X. In healthy larvae showing active feeding and digestion, the hepatopancreas and midgut will be full of small, easily observed bubbles (digestive or "lipid" vacuoles) and strong peristalsis will be seen in the intestine. If 90% or more of the animals sampled show abundant lipid vacuoles

and/or a full gut, a score of 10 is given; if the sample shows 70 to 90% of individuals with lipid vacuoles and/or a moderately full gut, a score of 5 is given; and if it is less than 70% and/or the intestine is empty, the score is zero.

***Necrosis***

Necrosis of the larval body and limbs, which is an indication of cannibalism or possible bacterial infection, can be observed by light microscope under low power. If necrosis is absent, a score of 10 is given; where <15% of the animals show some necrosis, a score of 5 is given; and where >15% show necrosis, indicating a severe infection is present, a score of 0 is given.

***Deformities***

Deformities may indicate poor quality nauplii, if in the early stages, and bacterial infections or mishandling and stress later on. Typically, the fine setae on the limbs of the larvae and/or their rostrums may appear bent, broken or missing; the tail may appear bent; or the gut may terminate before the anus. Typically, no remedies exist for these problems (unless due to rough handling), and such deformed larvae will die. In severe cases, it may be preferable to discard the whole tank as soon as possible to prevent infection of other tanks. Where deformities are absent, a score of 10 is given; ff <10% have deformities, a score of 5 is given; and if >10% present deformities, a score of 0 is given.

***Epibiont fouling***

The larvae may become host to a range of fouling organisms ranging from bacteria and fungi through to protozoans of many species. These will typically attach to the exoskeleton on the head and body, and particularly around the gills of the larvae. Where the infections are slight, the next moult may remove the fouling without further problems, but in severe cases, the fouling will persist or reoccur in the next stage, indicating poor water quality and necessitating action. Where fouling is absent, a score of 10 is given; if <15% have temporary or permanent fouling, a score of 5 is given; and if >15% are fouled continuously, a score of 0 is given.

***Baculovirus***

Baculoviruses can usually be detected in whole or squashed (stained with malachite green for *Monodon baculovirus*) preparations of hepatopancreas or faecal strands from larger-sized larvae, using a high powered light microscope to spot the characteristic viral occlusion bodies (which, in the case of MBV, are dark coloured and tetrahedral) . The expression of bacculoviruses is often mediated by stress, and if seen, reductions in levels of stress can often reduce prevalence and the associated problems of growth depression. Where baculoviruses are absent, a score of 10 is given; if <10% have baculovirus, a score of 5 is given; and if >10% are infected, a score of 0 is given.

**"Bolitas"**

"Bolitas" is the Spanish name given to a syndrome involving the detachment of epithelial cells from the intestine and hepatopancreas, which appear as small spheres within the digestive tract. It is believed to be caused by bacteria and can be fatal. Some success in preventing "bolitas" condition has been achieved by rapid stocking of the hatchery (within three to four days), use of probiotics, and good health and feeding management practices.

**The value of Level 1 and 2 scoring**

When all of these level 1 and 2 observations are made and recorded for each tank of larvae at each stage and the appropriate scores given in each case, an overall picture of larval health can be derived, with higher numbers relating to healthier larvae and vice versa. With experience, it becomes easy to judge the overall health of each tank of larvae and to recommend courses of action to combat the problems encountered, depending on the scores obtained.

### *Level 3 Observations*

Level 3 observations utilizing molecular techniques and immunodiagnostics are not normally required until the postlarvae are ready to be transferred to on-growing facilities. PCR and/or dot-blot techniques are commonly used to test for major viral pathogens. However, PCR is recommended as it is relatively more sensitive than dot-blot.

## 5.12 Selection of postlarvae for stocking

**Good hatchery management should be practised to ensure high postlarval quality**

Many factors affect the quality of PL. Feed quality and quantity, moulting, water quality (temperature, salinity, ammonia, suspended solids, faeces), use of antibiotics, diseases and bad management practices can all have an impact on the quality of postlarvae produced by a hatchery. These factors can be regulated through the use of good hatchery management practices, and this will have a major impact on the quality of the postlarvae produced.

As mentioned previously, the larval production plan should be aimed at producing the highest quality animals possible, because subsequent on-growing performance is directly related to postlarval quality. It is thus in the on-growing phase where postlarval quality is of most importance.

There are many indicators of health and quality that can be used to determine postlarval selection. These indicators fall under the previously mentioned (see Table 2) three categories or levels and are detailed in Tables 8, 9 and 10.

***Moulting***

The postlarvae should be checked that they are moulting easily, but that they are not moulting when intended for harvest and transport, because this will reduce survival rate during this critical time. Also check that there are no moults stuck to the heads of the PL, resulting in bent antennae and impediment of feeding, and ultimately starvation and death. This is usually caused by inadequate feeding, poor food quality and/or bacterial disease usually related to poor water quality. Thus, increased water exchange and revision of feeding protocols can be used to combat this problem.

## *Postlarval quality assessment using Level 1 procedures*

### *Swimming activity*

The vigour of swimming activity should be assessed as a general guideline of postlarval health using the techniques described for larvae in Section 7.12.1. The larvae can also be put into a bowl and the water swirled with a finger. Healthy postlarvae should orient themselves facing the current and not fall into a pile at the bottom of the bowl, being unable to resist the current. They should also respond to tapping the side of the bowl by jumping.

**Table 8. Summary of postlarval quality assessment using Level 1 procedures.**

| Criteria | Observations | Qualitative Assessment | Score |
|---|---|---|---|
| Moulting | Moults in the water Moults not sticking to head of PL | < 5% | 10 |
| | | 5–10% | 5 |
| | | >10% | 0 |
| Swimming Activity | Activity level of postlarval swimming behaviour | Active | 10 |
| | | Intermediate | 5 |
| | | Low | 0 |
| Direct Observation of Luminescence | Night-time observation of the tank | <5% | 10 |
| | | 5–10% | 5 |
| | | >10% | 0 |
| Survival Rate and Clinical History of Tank | Estimation of survival rate in each tank | >70%<br>40–70%<br><40% | 10<br>5<br>0 |

### *Luminescence*

The prevalence of luminescence as an indication of potentially pathogenic *Vibrio* spp. infections should be determined using the techniques described for larvae in Section 7.12.1 or using Level 2 techniques described below. Presence of luminescence requires immediate treatment (probiotic use can sometimes be successful) in order to prevent more severe infections.

### *Survival rate*

The survival rate of postlarvae in each tank should be estimated as an indication of the general state of health, clinical history and lack of problems during the cycle.

Each of these Level 1 postlarval quality assessments are carried out visually on randomly taken samples of >20 animals (where appropriate) and the scoring system detailed in Table 8 applied.

## *Postlarval quality assessment using Level 2 procedures.*

Level 2 assessments are carried out on a randomly selected sample of >20 postlarvae per tank which are examined using low- and high-power light microscopy. The scoring system detailed in Table 9 is then used to score the quality of each batch of postlarvae produced.

### *Muscle opaqueness*

An examination should be made of the body of the PL, concentrating on the bend of the tail around the $4^{th}$–$5^{th}$ abdominal segments. The normally transparent muscles turn opaque due to various reasons, including bacterial infection. This problem can be quite serious and potentially fatal if left untreated.

***Deformities***

Postlarvae should be examined for various deformities such as bent rostrum, enlarged head due to moulting problems, or missing or damaged limbs due to bacterial infections, to estimate general health. Some deformities are fatal.

***Size variation***

To determine the size variation, measure individually the length of at least 50 postlarve and calculate the mean length and the standard deviation. The coefficient of variation (CV) is obtained by dividing the standard deviation by the mean. If the CV is equal to or less than 15%, the size variation is considered low (score 10); if the CV is between 15% and 25%, the size variation is moderate (score 5); and if it is greater than 25% the size variation is high (score 0).

When postlarvae moult, it is normal that the CV will increase, so the time at which the CV is determined has to be taken into consideration. If the CV is found to be high, the test should be repeated after a day to give time for the whole population to complete the moult.

***Gut content***

Examinations of the intestinal tract for its contents and appearance (not just the colour) should be made to assess the PL's feeding level according to the criteria shown in Table 9. The presence of empty guts may be the first sign of disease, or may just be due to inadequate feeding. In either case, it should be investigated immediately. It is important to examine postlarvae immediately following sampling.

***Colour of the hepatopancreas***

The hepatopancreas should not be transparent and should have a good coloration. Typically, it should be dark yellow ferrous or ochre in colour, however, the colour of the hepatopancreas can be greatly influenced by the quality and colour of the diets fed and tanks used. A darker coloured hepatopancreas generally indicates better health. Care must be taken when using some flake feeds, as these may contain dyes that stain the hepatopancreas almost black, without necessarily contributing to the animals' health.

***Condition of the hepatopancreas***

The hepatopancreas of the postlarvae should be examined for its general condition, which is primarily indicated by the number of lipid vacuoles and its overall size. The presence of a relatively large hepatopancreas with a large number of lipid vacuoles is considered a sign of good health. Postlarvae with a small hepatopancreas containing few lipid vacuoles is a sign of under feeding, and improved feeding prior to harvest may be required in order to enhance their quality.

### *Epibiont fouling*

Postlarvae should be examined for any epibiont or organic matter fouling on the exoskeleton or gills (usually consisting of protozoans such as *Zoothamnium*, *Vorticella*, *Epistylis* or *Acineta*, filamentous bacteria or dirt and organic matter). Fouling can normally be moulted off or treated with formalin at up to 20–30 ppm for one hour (with full aeration).

### *Melanization*

Postlarvae should be examined for melanization, which often occurs where limbs have been cannibalized or where bacterial infections have occurred. Excessive melanization is a cause for concern and requires treatment through water quality and feeding regime enhancement, and sometimes reductions in stocking density, to prevent cannibalism and reduce bacterial loads.

### *Gill development*

The state of gill development should be examined, as it gives a good idea of when the postlarvae are able to tolerate salinity changes, which often occur when the shrimp are transferred to the on-growing facilities. When the gill lamellae have become branched like Christmas trees, approximately around PL9–10, they are generally able to tolerate fairly rapid changes in salinity (up to 1 ppt/hr down to 5 ppt, or 0.1 ppt/hr below 5 ppt) and can easily be acclimated to on-growing conditions. Where the gill lamellae remain unbranched, the shrimp should not be subjected to major or rapid salinity changes and should not be considered ready for transfer from the postlarval tanks.

### *Intestinal peristalsis*

A high-power microscopic examination of the intestinal tract of the postlarvae should be conducted in order to ascertain the peristaltic activity of the intestinal muscles. Strong gut peristalsis, in combination with a full gut, is an indication of good health and high nutritional status.

### *Baculovirus*

Refer to page 49.

### *Muscle to gut ratio*

A microscopic examination of the relative thickness of the ventral abdominal muscle and the gut in the 6$^{th}$ abdominal segment of the tail of the postlarvae should be conducted to determine the muscle to gut ratio. This gives a useful indication of the nutritional status of the animal. High muscle to gut ratios are preferable (see Table 9).

**Table 9. Summary of postlarval quality assessment using Level 2 procedures.**

| Criteria | Observations | Qualitative Assessment | Score |
|---|---|---|---|
| Muscle Opaqueness | Opaque muscle in tail of PL | <5% | 10 |
| | | 5–10% | 5 |
| | | >10% | 0 |
| Deformities | Deformities in limbs and head | <5% | 10 |
| | | 5–10% | 5 |
| | | >10% | 0 |
| Size variation (CV) | Calculation of CV of postlarval size | <15% | 10 |
| | | 15–25% | 5 |
| | | >25% | 0 |
| Gut content | Degree of fullness of digestive tract | Full | 10 |
| | | Moderate | 5 |
| | | Empty | 0 |
| Colour of the Hepatopancreas | Relative coloration of hepatopancreas | Dark | 10 |
| | | Pale | 5 |
| | | Transparent | 0 |
| Condition of the Hepatopancreas | Relative quantity of lipid vacuoles | Abundant | 10 |
| | | Moderate | 5 |
| Epibiont Fouling | Degree of fouling by epibionts | <5% | 10 |
| | | 5–10% | 5 |
| | | >10% | 0 |
| Melanization | Melanization of body or limbs | <5% | 10 |
| | | 5–10% | 5 |
| | | >10% | 0 |
| | | None | 0 |
| Gill Development | Degree of branching of gill lamellae | Complete | 10 |
| | | Intermediate | 5 |
| | | Slight | 0 |
| Intestinal Peristalsis | Movement of gut muscle | High | 10 |
| | | Low | 5 |
| Baculovirus | Daily (2–4x) observation of Mysis | Absent (0%) | 10 |
| | | Moderate (<10%) | 5 |
| | | Severe (>10%) | 0 |
| Muscle to Gut Ratio | Comparison of ratio between muscle and gut thickness | >3:1 | 10 |
| | | 1–3:1 | 5 |
| | | <1:1 | 0 |
| "Bolitas" (sloughed cells of hepatopanceas and intestine) | Number of bolitas in digestive tract | None | 10 |
| | | 1 to 3 | 5 |
| | | >3 | 0 |
| Stress Test | If < 75%, re-testing is recommended | >75% | 10 |

***"Bolitas" (sloughed cells of hepatopancreas and intestine)***

Refer to page 49.

***Stress test***

At harvest, or once the postlarvae reach PL10, a stress test can be carried out. There are several stress tests, and the most common method is to place a randomly selected sample of about 300 animals in a beaker with water at 0 ppt salinity, leave them for 30 minutes and then return them to 35 ppt (or ambient) water for another 30 minutes. Following this, the survivors are counted and the percentage of resistant individuals calculated. Stress tests should not be carried out when the postlarvae are moulting, as they are unduly stressed at this time. Some hatcheries have used 100 ppm of formalin for 30 minutes as the stress test, with similar success.

## *Postlarval quality assessment using Level 3 procedures.*

Level 3 assessments should be carried out on a statistically determined number of postlarvae (usually 150 for a population > 10,000) from each tank (in order to provide a 95% confidence level at 2% prevalence in the result) using PCR techniques for the detection of important viral pathogens. This testing must be done according to standard protocols by a competent health laboratory, following all the rules for sampling, preservation and transport of the samples. For a detailed discussion of sampling for disease detection, see OIE (2003).

The only acceptable result for any of these viral pathogens is a negative result (which scores 10 points – see Table 10), where both negative and positive controls have simultaneously given their corresponding expected results. All batches testing positive should be destroyed.

**Table 10. Summary of postlarval quality assessment using Level 3 procedures.**

| Analysis | Observations | Qualitative Determination | Score |
|---|---|---|---|
| PCR | WSSV/YHV | Negative | 10 |
| | IHHNV | Negative | 10 |
| | TSV | Negative | 10 |

## 5.13 Risk assessment for stocking

**A table summarising the three levels of postlarval quality and the points system should be used to determine the fate of the PL**

As with larval quality assessment, a summary table should be made of these three levels of postlarval quality and the points system employed (using some or all of the above indicators, depending on circumstances). This table then is used to determine which tanks of postlarvae are selected for on-growing, which may require treatment before selection, and which will be rejected. As before, experience will guide the manager in his selection of indicators to use and of a cut-off point for points scored, below which the postlarvae batch will be treated or rejected.

**The risk of stocking a given batch of postlarvae must be carefully assessed.**

The decision to stock or not to stock a batch of postlarvae is ultimately an assessment of risk. No fixed guidelines or standards can be provided, as this generally comes from experience, but the following guide can be used to reduce the risk of experiencing mortalities or poor growth in pond culture of *Penaeus vannamei*. In this risk analysis, the order of importance of assessment is Level 3 > Level 2 > Level 1.

The following criteria can be used:

- Postlarvae must pass Level 3 assessment.
    - Postlarvae must be PCR or dot-blot negative for YHV, IHHNV, WSSV and TSV.
- Provided that postlarvae passed Level 3 assessment, the following guide can be used for Level 2:
    - A score greater than 100 represents a low risk of severe disease problems, therefore recommended
    - A score of 65–100 represents a moderate risk of severe disease problems.
    - A score less than 65 represents a high risk of severe disease problems, therefore not recommended.
- Provided that animals pass Level 2 assessment, the following guide can be used for Level 1:
    - A score greater than 30 represents a low risk of severe disease problems, therefore acceptable.
    - A score of 20–30 represents moderate risk of severe disease problems.
    - A score less than 20 represents a high risk of severe disease problems, therefore not recommended.

## 5.14 Shipping and transfer of postlarvae

**Postlarvae must be carefully and appropriately packed for shipping to grow-out facilities**

Postlarvae can be transported in large tanks or in boxes with plastic bags and at densities that may vary from 500 to 1200 PL/litre, depending upon duration and method. Two plastic bags (one inside the other) of 25–30 litre capacity are commonly used, filling with 10–15 litres of filtered water, adding the desired quantity of postlarvae and then filling with pure oxygen, bubbled into the water. As food source, live *Artemia* nauplii are typically added at about 15–20 nauplii per postlarvae for every four hours of transport. A few granules of washed, new, activated carbon may also be added to each bag to assist in maintaining low ammonia levels during long transportation times. The bags are then sealed with elastic bands and placed into sealed cardboard cartons for short distances and/or polystyrene for added insulation over long distances.

The temperature used and the stocking densities employed during transportation will vary according to the travel time and distance to be shipped. Typically, no temperature reduction is needed if the hatchery is close to the farm site, but temperatures will be reduced to 25–28 °C for transportation times of one to three hours, to 23–25°C over transportation times of 3–12 hours and 18–23 °C for over 12 hours. Such temperature reduction is used to lower the metabolic rate

of the larvae so that they will use less oxygen, excrete less waste and remain calm during transportation. The salinity of the water should be that to which the postlarvae have been acclimated, which should be similar to that expected in the grow-out facility.

**Strict biosecurity measures should be followed**

All shipping containers and equipment (nets, air stones, air lines etc.) should be disinfected before and after use (see appropriate sections in this document for procedures). If plastic bags are used, they should be incinerated after use; they should not be re-used for shipping postlarvae or broodstock shrimp.

The vehicles that deliver the postlarvae are a potential source of contamination, as they may visit several farms and hatcheries in the course of making deliveries. If possible, postlarval packing should take place at a point isolated from the production facilities, and the transport trucks (at least the wheels and tires) should be disinfected before entry to the hatchery.

## 5.15 Documentation and record keeping

**A comprehensive and up-to-date system of documentation and record keeping should be established**

Good documentation and record keeping are fundamental to any system of good management practice. It is important that a detailed set of written standard operating procedures (SOPs) is developed and a system of regular employee training programmes be established. It is also important that the SOPs are reviewed regularly and kept up to date.

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This publication provides technical guidance for the effective and responsible operation of shrimp hatcheries in Latin America. The document was compiled through an extensive consultative process undertaken from 2001 to 2003 that involved inputs from government-designated national coordinators, regional and international experts, representatives from several intergovernmental organizations, private sector representatives and FAO. This process was made possible through the FAO Regional Technical Cooperation Programme project – Assistance to health management of shrimp culture in Latin America: TCP/RLA/0071 (A) – which involved the participation of 14 countries of the region, several intergovernmental organizations, shrimp hatchery operators and farmers, and individual experts. It is envisaged that this document will provide a firm basis for the improvement of the health and quality of hatchery-produced *Penaeus vannamei* postlarvae in Latin America.

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