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Compete Caribbean

DELIVERABLE D-3-B
UPDATED BIOSECURITY AQUACULTURE ZONE MANAGEMENT PLAN

Improving Sustainable Production in the Belize Shrimp Cluster

Developing a Biosecurity Aquaculture Zone Management Plan for Belizean shrimp growers.

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SOFRECO

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ABBREVIATIONS / ACRONYMS

Abbreviations and acronyms			
CCP	Critical Control Points	TLB	Total Load Bacteria
CFU	Colony Forming Unit	TLV	Total Load Vibrios
EDTA	Ethylene Diamine Tetra Acetic acid	TRO	Total Residual Oxidant
FAO	Food and Agriculture Organization	TSV	Taura syndrome virus
FR	Food Ratio?	TVC	Total Viable Count
HACCP	Hazard Analysis Critical Control Plan	UV	Ultra Violet
HHGI	High Health Genetically Improved	WSSV	white spot syndrome virus
HTHT	calcium hypochlorite	YHV	yellow head virus
IPN	Infectious Pancreatic Necrosis	Z	Zoea
ISA	Infectious salmon anemia		
IHHNV	Infectious Hypodermal and Haematopoietic Necrosis Virus		
IMNV	Infectious Mionecrosis Virus		
LB	Load of Bacteria		
M	Mysis		
N	Nauplius		
OIE	World Organisation for Animal Health		
ORP	Oxidation-reduction potential		
PCR	Polymerase Chain Reaction		
PLx	Postlarva x		
PVP	Polyvinylpyrrolidone		
QA/QC	Quality Assurance/ Quality Control		
SOP	Standard Operating Procedures		
SPF	specific pathogen free		
SPR	Specific Pathogen Resistant		
TBC	Total Bacteria Count		

1 INTRODUCTION

Improvement of shrimp production depends on many factors such as design of facilities, progressive technification of process, genetic selection, feeding protocols and health management. One of the key elements related to health management is biosecurity which is defined as a "set of practices that will reduce the probability of a pathogen introduction and its subsequent spread from one place to another" (Lotz, 1997).

According to FAO, biosecurity is a strategic and integrated approach that encompasses the policy and regulatory framework for analyzing and managing risks to the life and health of people, animals and plants, and the associated risks to the environment. In consequence, it must include all the activities that aim at preventing, controlling and eradicating risks to life and health of shrimp populations. In order to **minimize risks of introducing and spreading diseases**:, we need to find the appropriate level of protection for each particular area:

- Identification and use of reliable, high quality and healthy sources of stock,
- Application of best management practices,
- Disease recognition and diagnosis based on effective surveillance systems,
- Identification of effective measures to adopt in case of an outbreak or an unknown mortality,

Biosecurity has a direct importance in:

- Reducing Mortality that translates into cost reduction.
- Reducing variability in production that allows us better planning.
- Reducing risks resulting in better return on investments.
- Reducing inputs (ie. drugs) which translate into an improvement in product quality and reduction of environmental impact.
- Improving the animal welfare (necessary for the new product labels) that makes possible an improvement in the image of the product and the sector.

The **basic elements of a biosecurity plan** include the physical, chemical and biological methods necessary to protect the shrimp farm from the consequences of all diseases that represent a relevant risk, and the appropriate level of biosecurity to be applied should take into account the ease of implementation and relative cost (assessing the impact of the disease on the production operations).

First step to develop a feasible biosecurity plan is to identify the **Critical Control Points (CCPs)** for each *production* area (quarantine, maturation, hatchery, grow-out ponds, algal culture, brine production (*Artemia* spp.), etc), taking into consideration different elements related with the risks:

- Access to facilities: people (workers and visitors), vehicles and animals.
- Water, considering and adequate treatment depending on origin.
- Shrimps: incoming broodstock, disinfection of eggs, nauplii and postlarvae.
- Ponds and equipment: cleaning and disinfection procedures, dry-out periods.
- Feed: fresh feed, algae and *Artemia*.

Once the CCPs are compiled, it is necessary to prepare the **Standard Operating Procedures (SOPs)** as a comprehensive document that covers each process carried out in each production area. The document should include details of all of the CCPs and describe the procedures to avoid each risk. The document should be accessible for all the shrimp farm staff, with adapted versions according with the training level of the personnel.

Owners of aquaculture production companies are responsible for biosecurity within their enterprises and for educating and informing workers and visitors. Each facility needs to develop its own biosecurity plan, but according with homogenous standards and guidelines at national level. This will require full understanding of the design and operation of the facility, knowledge of the animal´s health status and the pathogen´s transmission mode in order to be able to identify the risk to develop meaningful biosecurity measures according with relevance of detected pathogen.

Biosecurity is closely linked to epidemiological surveillance, the main objective of which is to establish and maintain disease-free status in a population. For this purpose, it is essential to detect the introduction of a pathogen at an early stage in order to be able to quickly establish the appropriate control and eradication measures, even before it has a health and/or economic impact on the population. In the case of endemic situations, the objective will be to estimate prevalence.

In this context, it is very important to identify a **biosecurity manager** (veterinarian or fish health consultant, with experience in epidemiology and risk analysis), with the responsibility to ensure the application of all preventive measures.

There are three rules that need to be kept in mind regarding evaluation and defining biosecurity measures. Measures should be:

- Justifiable: They should be supported by scientific evidences;
- Practical: They should not affect to the routine farming practices;
- Economically viable: The cost and benefits of the biosecurity measures should be well balanced.

A well thought biosecurity plan implemented and ‘owned’ by committed staff can limit risks of spreading diseases to and from a farm and therefore affect negatively its risk level and the surveillance necessary. This may act as a further incentive.

The plan should be read in conjunction with plans at the compartment, zone and territory levels (in the case of Belize, the whole country could be considered as a unique compartment) in order to ensure optimum benefits. It can also be used to form partnerships and agreements between factions of industry and also potentially with official services provided by government.

2 ELEMENTS OF A BIOSECURITY AQUACULTURE MANAGEMENT PLAN

In this document we are going to outline the biosecurity measures that should be implemented in order to reach an adequate risk control. This paragraph is related to introduction and spreading of main pathogens of shrimp and is grouped in five sections:

1. Physical barriers:
   1.1. Vehicles
   1.2. People (visitors and staff)
2. Production units and equipment:
   2.1. Production units
   2.2. Equipment
3. Water
4. Feed
5. Animals:
   5.1. Shrimps
   5.2. Other animals (vectors and carriers)

2.1 Physical barriers

Production sites should maintain barriers to control and restrict access of vehicles and people to facilities.

2.1.1 Vehicles

2.1.1.1 Introducing risks

Restricted access

The pathogens could be transmitted from farm to farm (and from pond to pond) using mechanical carriers like vehicles and people and the access to farms must be restricted. So farms should have a perimetral fence to avoid uncontrolled

entrances. Only the vehicles that are necessary should access to farms facilities (for example, the access to visitor's cars must be banned).

One or more than one facility entrance can exist, however in all cases it must exist a control point with a barrier to limit accesses, equipment for disinfection and a record book to register all accesses (driver, date, hour, identification of vehicle, origin, and reason for the visit).

Disinfection of vehicles

The likelihood of vehicle contamination will be determined by their use, e.g. transportation of mortalities, live aquatic animals, harvested aquatic animals. All potentially contaminated internal and external surfaces should be disinfected. Special consideration should be given to areas likely to be contaminated such as the internal surface of containers, pipes, transportation water and waste.

Strict disinfection protocols should be implemented for vehicles before accessing to the farm facilities. All potentially contaminated surfaces should be disinfected and special consideration should be given to external surfaces likely to be contaminated: wheel arches, undercarriages and wheels (tyres).

The wheel arches, undercarriages and wheels should be cleaned outside the farm using a high-pressure cleaner (preferably with heated water) to remove dirt, mud and organic matters, and then the surfaces should be rinsed with clear and clean water before being sprayed with a suitable disinfection solution. Oxidative chemicals such as chlorines are the most used disinfectants for vehicles with sodium or calcium hypochlorite at 100-200 ppm of active ingredient.

In the case of wheels, instead of spraying the disinfection solution, it is possible to use a tyre or wheel bath with dimensions such as to ensure that entire perimeter of the wheels (tyres) is completely submerged. If the tyres are dirty with mud, disinfection could not adequately work because disinfection solution could be inactivated. Evaporation is also another factor to look at.

In some cases, it could be desirable to install disinfection arches at the entry point of the farm to spray the whole outside parts of trucks and cars with a disinfection solution.

In all cases, it is necessary to register a detailed record of preparation of disinfection solution (date, responsible people, product, number of batches and concentration used), and the solution should be regularly replaced (even daily) depending on temperature and number of uses, in order to maintain its efficacy.

The cleaning-disinfection procedure should not be corrosive and should not damage the paintwork. Additionally, residual water from the process should be safely disposed avoiding the contamination of nearest water bodies. The application of corrosive disinfectants to vehicles should be avoided or, if used, corrosive residues removed following disinfection by thorough rinsing.

Disinfection of containers and auxiliary equipment (pumps, pipes…)

Usually the containers are made using a non-porous material (plastic, stainless steel…) that allows an easy cleaning and disinfection. Depending on the use of containers (transport of harvested shrimps, dead animals…), the disinfection protocols could vary.

Like the external surfaces of the vehicles, the internal and external surfaces of the containers should be washed with high-pressure cleaner or mechanical scrubbing

(preferably with heat water and/or detergents). The order for cleaning is important, starting from the top of the internal surface and moving downward, and finishing with the external surface (from top to bottom). After removing all organic matters, the containers should be rinsed with clear water (particularly if a detergent has been used), allowing the complete drainage of water.

Suitable chemical disinfectants are calcium or sodium hypochlorite (>30 ppm), chloramine T (20 ppm, left to dry or for a minimum of 30 min before rising) and iodophors (>200 mg/L of available iodine during 1-2 min). In the case of porous surfaces, it is recommended a solution of peracetic acid 2% duration at least 1 h.

The disinfection of pumps and equipment of the vehicle is also necessary (preferably at the origin of the travel), using a disinfection solution circulating throughout the pipes for 30 min (ie. sodium hypochlorite 20-30 ppm or muriatic acid 10% solution).

For more details about disinfection: Australian Government. *Operational procedures manual: Decontamination (Version 1.0).* Australian Aquatic Veterinary Emergency Plan (AQUAVETPLAN), Australian Government, Department of Agriculture, Fisheries and Forestry, Canberra, Australia. 2008; 122 pp.

2.1.1.2 Spreading risks

The movements of vehicles inside farm should be limited, even the vehicles of exclusive use in the farm. In each farm, if it is necessary, different zones can be defined according to a risk level. The zones should be separated by internal fences with gates, or barriers on the roads and pathways. When a vehicle crosses the boundary between two zones, specific disinfecting procedures should be carried out to minimize the risk of pathogens transfer.

2.1.2 People (visitors and staff)

People are also responsible of the transmission of pathogens, usually with the shoes and clothes.

2.1.2.1 Introducing risk

Restricted access

The perimetral fence of the farm (completely necessary for maturations and hatcheries) should have enough height to stop the entrance of unauthorized people.

The access to the farm is limited only to authorized staff (operational workers, administrative employees, managers…) or approved visitors (health officers, consultants…). In the entrance points, a record book should be available to register all people accesses (date, hour, name, institution, reason for visit, recent visits to other shrimp farms…) to ensure the traceability of people.

Furthermore, the staff responsible of the entry point should ensure that visitors are aware of biosecurity measures providing a leaflet with detailed instructions.

Clothes and shoes

It is very important that staff have specific clothes and shoes for exclusive use in the farm (even in some cases it could be necessary for different zones of the farm). Non-absorbent and easy to clean equipment should be selected. All staff entering a production area should use clean and uncontaminated protective clothing.

At the entry point there should be facilities for the exchange of personal clothes and shoes, for clean and disinfected clothes and waterproof footwear. Disinfection of personal equipment should consider the likelihood and degree of contamination associated with previous uses. The clothes will be preferably washed and disinfected inside the farm facilities. After each use, the footwear (rubber boots…) should be cleaned with a sponge or a low-pressure cleaner (from top to toe, and finally the footwear sole) before being disinfected by immersion (sodium or calcium hypochlorite >50 ppm) and then allowed to dry completely before the next use.

Disposable clothing items and disinfected waterproof footwear should be available for visitors.

Disinfection of shoes and hands

In the case of high biosecurity areas, it should be necessary to install showers before accessing to the facilities. Warm water and soap are to be provided to staff and visitors on entry and exit of critical areas (quarantines, maturations, hatcheries…).

At the entry point of the farm there should be a system to wash and to disinfect the hands. First of all, the staff and visitors will wash their hands with clean water and a detergent agent (soap), and after rinsing, the disinfection will be carried out using a hand hygiene dispenser unit (or a bucket) with iodine-povidone 20 ppm or alcohol 70%. In high risk situations, gloves could be used to minimize the risk of introduction of pathogens. Same cleaning and disinfection procedure should be carried out at the exit of the farm.

A very important way of introducing and spreading pathogens is with the footwear, and for this reason footbaths are a very valuable tool to restrict the transport of pathogens.

On entry and exit of production areas, the boots should be cleaned and disinfected. When footbaths are used, they should incorporate a cleaning procedure to remove accumulations of organic material and mud, and, be sufficiently deep to cover boots. A disinfectant solution that is not inactivated by organic matter should be used and be regularly refreshed with a new solution.

The effectivity of the footbaths depends on several factors:

- Placed in a well-ventilated area protected from direct sunlight (to avoid evaporation), rain and flooding (to avoid dilution).
- Placed at the entrance of the farm (or the building entrance), without the possibility of going around the pond and minimizing the risks of falling.
- Constructed with hard and well-drained surfaces, with a minimum area of 50x50 cm and 25 cm in height (large enough to allow a person to stand with both boots in the solution and deep enough to cover the top of the boot).
- When the pathways before the footbaths are dirty (mud, earth…), a previous footbath or area should be available with clean water and specific devices to remove accumulations of debris (i.e. brushes...)
- Disinfectant solutions should be resistant to organic matters: sodium or calcium hypochlorite solution 50-100 ppm, iodophors (Betadine® 200-250 ppm) or chloramine T 2%.
- Disinfectant solution should be emptied and renewed at least weekly (preferably, daily) and a detailed record book should be maintained about

maintenance (date, staff responsible, product, number of batches, concentration…)

For more details about disinfection: Australian Government. *Operational procedures manual: Decontamination (Version 1.0).* Australian Aquatic Veterinary Emergency Plan (AQUAVETPLAN), Australian Government, Department of Agriculture, Fisheries and Forestry, Canberra, Australia. 2008; 122 pp.

2.1.2.2 Spreading risks

Movements inside farm and areas with restricted access

The different production units should be physically separated or even isolated (i.e. quarantine). With this objective, internal fences or other barriers are constructed with control points where preventive measures similar to the ones at the entry of farm are adopted (record of accesses, shoes and hand disinfection…).

The workers accesses must be restricted to their specific area of work, and a good practice is to consider the use of different color clothes (T-shirts or overall) and boots for particular areas. This will allow identifying people in zones where they are not allowed.

Disinfection

At the entrance of each zone (building, room…) there should be a footbath and hand hygiene unit, similar to what is described in section 2.1.2.1. All persons must wash and disinfect their shoes and hands when entering or leaving the room.

Staff training

One of the most effective measures to reduce the spreading of pathogens related with staff is to provide training in shrimp health management and disease recognition. It is a key element of a passive surveillance system. So, training should be based on continuous learning with short courses, biosecurity protocols, disease recognition leaflets and posters… All staff should be trained in suitable husbandry techniques, clinical signs of diseases, pathways of introduction and spreading of relevant pathogens.

It is desirable to have a team of lecturers with high technical training and experience that supervise and train the workers. This team could be created from association of producers.

2.2 Production units and equipment

Aquaculture establishments include buildings for culture, harvesting and processing of aquatic animals, and other buildings associated with storage of feed and equipment. The approach to disinfection may vary depending on the structure of the building and degree of contact with contaminated material and equipment.

Buildings should be designed to allow effective cleaning and thorough application of disinfectants to all internal surfaces (rounded corners, smooth materials…). Some buildings will contain complex piping, machinery and tank systems that may be difficult to disinfect. Wherever possible, buildings should be cleared of debris and emptied of equipment, prior to disinfection.

2.2.1 Production units

2.2.1.2 Introducing risks

The introduction of a pathogen in a production unit is produced throughout the people (as already described before), the water, the equipment, the animals and the feed. In next sections we describe all these risks; however, it is very important that each production unit keeps up to date records related to all these elements.

2.2.2.2 Spreading risks

All the production units (ponds, tanks…), and buildings containing them, should be cleaned and disinfected after each production cycle in order to avoid the transmission of pathogens to next population.

The procedures to prepare a production unit depend on material of bottom and internal surfaces, and size of the unit.

An effective disinfection requires the removal of organic matter (that reduces the activity of many chemical disinfectants), the cleaning of internal surfaces of the unit (when it possible), the application of disinfectant (avoiding chemical residues) and application of dry out periods.

In the case of plastic and fiberglass tanks (usually used for broodstock spawning, egg hatching, and holding for nauplii and postlarvae), it is possible to apply any chemical disinfectant suitable for hard surfaces like calcium or sodium hypochlorite (>30 ppm), chloramine T (20 ppm, leave to dry or for a minimum of 30 min before rising), iodophors (>200 mg/L of available iodine during 1-2 min) or muriatic acid 10% solution (pH 2-3).

For tanks made from unpainted and/or cracked concrete surfaces is better to use alkaline disinfectants like a mix of sodium or calcium hydroxide with a detergent agent (as Teepol), sprayed onto the surfaces (0.1 l/m^2) and left for 48 h. Other option is to fill the tanks to the maximum level with a calcium or sodium hypochlorite solution 20-30 ppm during 48 h.

The most complicated scenario corresponds to the earthen ponds, due to their high content in organic load (including pathogens). The most effective disinfection procedure is to drain the pond and then to apply burnt lime (calcium oxide) at 100 ppm with the aim of increase the soil pH above 10 and thus kill the pathogens. Chlorination can also be used applying calcium hypochlorite 500 ppm because the organic matter in the pond soils reduces the effectivity of chlorine residuals.

In practical terms, the application of lime is more economical than chlorination. The treatment rates for lime are 500 or 1,000 kg/ha of burnt lime (calcium oxide) (for dried and wet ponds respectively) or 1,500 kg/ha of hydrated (slaked) lime (calcium hydroxide), while for chlorination 1,000 kg/ha of calcium hypochlorite should be applied to reach an effective concentration.

For more details about disinfection: Australian Government. *Operational procedures manual: Decontamination (Version 1.0).* Australian Aquatic Veterinary Emergency Plan (AQUAVETPLAN), Australian Government, Department of Agriculture, Fisheries and Forestry, Canberra, Australia. 2008; 122 pp.

Additionally, it is necessary to dry pond bottoms. Usually a drying period of 2 to 4 weeks is sufficient until the soil is dried and cracks by the effect of sunlight, and then the lime is applied at 500 kg/ha. However, during rainy season, it may be

impossible to completely dry the soil then the lime should be applied at greater rates in wet areas (1,000 kg/ha). In any case the pond bottoms should be dried completely at least once per year.

Other options are to plough the pond bottom or to remove the pond sludge (with an appropriate disposal, especially when there are dead shrimps and other crustaceans), followed by the application of lime.

If any disease outbreak or a pathogen detection occurred during production cycle, the sludge retired from soil bottom could be used to reinforce pond borders after an adequate drying period (particularly if it will not be covered with a liner).

2.2.2 Equipment

The use of shared equipment implies a high risk of introduction and spreading of diseases and adequate disinfection procedures should be implemented before re-use.

Some considerations should be taken into account in order to design a correct and effective cleaning and disinfection protocol:

- Increasing water temperature also increases the effect of chemical disinfecting products (approximately double for every 10ºC rise in temperature).
- The water hardness reduces the efficacy of some disinfectants (especially anionic detergents and bicarbonate-based cleaners). Sometimes, calcium deposits can be generated, and the application of an acidic detergent could be necessary.
- Extreme pH values (extremely acid or alkaline waters) can affect the action of chemical disinfectants. Usually seawater is buffered to pH 9, but freshwater may show a wider pH range, for example areas treated with lime can reach pH 10.
- The water turbidity reduces the inactivation of pathogens by ultraviolet rays (including those produced by sunlight). So, is should be desirable to use filtered water that ensure that water clarity is as high as possible.
- The organic matter reduces the activity of many chemical disinfectants, so an effective removal of debris and gross contamination must be carried out. For example, a strong alkaline detergent can be used to remove the residual fouling.
- Disinfectant solutions must to be replaced before they lose its efficacy, and details of replacement must be recorded.
- The application of disinfectants should take into account the type of material requiring disinfection and how disinfectants should be applied. Hard non-permeable materials can be cleaned thoroughly and allow contact with the disinfectant because there is little opportunity for infective material to lodge in crevices. Some products are also corrosive to metals, rubber, plastic… and can shorten the equipment life
- Disinfection efficacy will decrease if the surface is corroded, therefore proper maintenance of surfaces and equipment is essential.
- For permeable surfaces and materials, a higher disinfectant concentration and a longer contact time are required because the surface area is greater, chemicals cannot penetrate easily and residual organic matter may be present.

The choice of the application method should ensure that the whole surface comes into contact with the disinfection agent for the required period of time. When disinfectants are applied to vertical surfaces, care should be taken to ensure that

the required contact time is maintained before the disinfectant drains away. To sum up, cleaning and washing of surfaces and equipment are necessary to remove solid waste, organic matter (including biofouling) and chemical residues as these may reduce the efficacy of disinfectants. The use of detergent is also important to break down biofilms, and the detergent used should be compatible with the disinfectant and the surface being treated. After cleaning, any excess water should be drained and before the application of disinfectants all surfaces and equipment should be inspected to ensure there is no remaining organic material.

All equipment (including buckets, bins, nets, pumps, pipelines…) should be for the exclusive use in each production unit (tank, pond…), and should not leave the facilities or be used elsewhere. However, large equipment (like graders) could be used in different areas and additional biosecurity measures should be implemented (including a register of use).

The disinfectant should be selected considering the following:

- Efficacy against the pathogenic agents
- Effective concentration and exposure time
- Ability to measure efficacy
- Nature of the items to be disinfected and the potential for them to be damaged
- Compatibility with the available water type (e.g. freshwater, hard water or seawater)
- Availability of the disinfectant and equipment
- Ease of application
- The ability to remove organic matter
- Cost
- Impacts of residues on aquatic animals and the environment
- User safety

In any case, all equipment must be washed and disinfected after each use, and different protocols can be used depending on the equipment:

- Tank equipment: calcium or sodium hypochlorite at 200 ppm by 5 min, or iodophore (with 0.5% of available iodine) by 5 min.
- Plastic containers, beakers, nets, hoses, water pipes, air lines, air stones: soaking in calcium or sodium hypochlorite at 20-30 ppm, or muriatic acid 10% solution (at least once each month).
- Sand filters: calcium or sodium hypochlorite at 20-30 ppm, or muriatic acid 10% solution (pH 2-3)
- Cartridge filters: calcium or sodium hypochlorite at 10 ppm, or muriatic acid 10% solution (pH 2-3) for 1 h.

It is recommended to fill pipework, pumps and filters with a disinfection solution, recirculating the solution during an adequate period of time.

For more details about disinfection: Australian Government. *Operational procedures manual: Decontamination (Version 1.0).* Australian Aquatic Veterinary Emergency Plan (AQUAVETPLAN), Australian Government, Department of Agriculture, Fisheries and Forestry, Canberra, Australia. 2008; 122 pp.

Finally, in some circumstances (windy areas, close ponds, absence of perimetral walls…), it is possible to spread pathogens in water droplets as consequence of an intensive use of paddle wheel aerators. To avoid this pathway of infection, submerged aerators could be used.

2.3 Water

All the water (intake and effluents) used in shrimp farms must be disinfected according of the use of the waters and its characteristics. The quality of the water supply can affect the efficiency of physical processes (sedimentation, filtration and UV radiation) and of chemical disinfectants (high organic loads inactivates chlorine and iodine, and hard waters reduce activity of some detergents and disinfectants).

2.3.1 Introducing risks

Water should never be taken and used directly from the sea, estuary and/or river, and only filtered and treated water should be used. The treated water is maintained in reservoirs with a minimum storage capacity in accordance with the availability of water and the rate of water renewal required by the farm.

So, prior to application of disinfectants, it is very important to treat the inlet water to exclude aquatic animals (such as zooplankton than can be potential vectors of pathogens) and to remove suspended solids. In order to achieve these objectives a primary filtration or settlement step of inlet water is needed.

The selected method to remove organic matter and suspended solids depends on the initial quality of water, volumes to be filtered and available resources, and usually a combination of several methods is required.

A common practice is to fill settlement ponds to allow the sedimentation of suspended solids, preferably with filtered water using “natural” methods (as sub-sand beach wells) and/or a mesh filter bag of 150-500 µm to exclude potential wild crustacean carriers. The use of sub-sand beach wells is a very effective primary filtration system for raw seawater, because they limit the amount of pathogens and hosts. In case of direct water intake, the use of one or more mesh bag filters is compulsory, with special care on the integrity of the mesh because tears or leaks could permit the introduction of unfiltered water in the ponds.

The water from the first reservoir (settlement ponds, settling tanks) should be filtered again with one or more finer filters (sand filters by gravity or pressure, cartridge filters, carbon filters, drum filters…) in order to exclude particles greater than 20 µm. Final size of filtration depends on the uses of water. Recommended sizes are: maduration (15 µm), hatchery (5 µm), spawning and hatching (0.5-1 µm) and algae culture (0.5 µm).

Filters must be frequently washed and disinfected (even two times per day) depending on the load of suspended solids in the intake water. Sometimes two sets of filters must be available in order to continuously treat water, by alternating cycles of filtration and disinfection. For the sand filters, the sand must be replaced at least once every production cycle with clean sand previously disinfected with a sodium hypochlorite 20 ppm or muriatic acid 10% solution. Cartridge and bag filters should be dipped in a disinfection solution of calcium or sodium hypochlorite 10 ppm for 1 h. They are then rinsed with abundant treated water and dipped in a recipient with a solution of sodium thiosulfate 10 ppm to neutralize chlorine. When it is possible the exposure of filter elements to sunlight can be recommended as a complementary disinfection method.

Filtered water is disinfected and maintained in secondary reservoirs ready to use. Ultraviolet light (UV) is the most common physical method used for water disinfection. UV allows for the continuous disinfection of inlet water, but radiation intensity should be periodically checked according to manufacturer’s specifications.

Commercial UV units for water treatment operate in the spectral range of 190-280 nm (254 nm is recommended) delivering doses of at least 130 mWs/cm^2. The efficacy of UV radiation decreases with water turbidity, so the UV should be placed after filtration system and they are suitable to use before or after the final reservoir.

Chlorination is the most suitable chemical method to treat big amount of incoming water. In the reservoir, the water is disinfected with calcium or sodium hypochlorite 10-30 ppm for not less than 30 min. After treatment with chlorine and before the water is used, the water must be checked to ensure no chlorine residuals remains (using 3 drops of ortho-toluidine in 5 ml of water sample, a yellow color indicates residuals). Chlorine residuals can be dissipated using strong aeration or neutralized with sodium thiosulphate (1 ppm for every 1 ppm of residual chlorine).

For hatching and spawning, the addition of ethylene diamine tetra acetic acid (EDTA) 20-40 ppm is often required to chelate heavy metals present and made them unavailable.

Finally, trifluralin (Treflan®) at 0.05-0.1 ppm is usually used in water for hatching and spawning in order to fight against fungi.

For more details about water disinfection: Australian Government. *Operational procedures manual: Decontamination (Version 1.0).* Australian Aquatic Veterinary Emergency Plan (AQUAVETPLAN), Australian Government, Department of Agriculture, Fisheries and Forestry, Canberra, Australia. 2008; 122 pp.

A combination of methods may be beneficial where they are synergistic or where a level of redundancy is required. It is very important to monitor the efficiency of water disinfection, testing for pathogens and the level of disinfectant residuals. This can be achieved by direct testing for pathogenic agents of concern, indirect monitoring of indicator organisms or monitoring of residual levels of disinfectants. All operations of related with maintenance of filters and disinfection procedures should be recorded in a book to ensure the traceability of the process.

Management of chemical residues is important to avoid toxic effects on aquatic animals. For example, residues formed between ozone and seawater such as bromide compounds are toxic to early life stages of aquatic animals and may be removed using charcoal filtration. Residual chlorine should be removed from water by chemical deactivation or off gassing.

2.3.2 Spreading risks

All wastewater discharged from the shrimp farm should be temporarily settled and disinfected, particularly in high-risk situations (water from quarantine, water from ponds suffering an outbreak…), to avoid the spreading of pathogens in the waters close to discharge location. But also, it is important to reduce the release of disinfectants that can adversely affect aquatic fauna.

Depending on the load of organic matter, chlorination protocols can vary using calcium or sodium hypochlorite solutions of different concentrations at different times: 200 ppm for 1 h, 30 ppm for 4 days, 20 ppm for 1 h, 50 ppm for 30 min. Treatment should be done in special concrete tanks or lined sedimentation ponds. In any case, after treatment the water should be dechlorinated through strong aeration or neutralization with sodium thiosulphate to achieve chlorine residuals lower than 5 ppm prior to be discharged in natural waters.

2.4 Feed

2.4.1 Introducing risks

Feed ingredients of aquatic origin used in shrimp production can be a relevant source of pathogens to shrimp species. Live feed (as algae and *Artemia* spp.) and moist feed (as bloodworms and squid) can contain shrimp pathogens, but also the pelleted feed can contain pathogens if it is contaminated after heat treatment.

As in the case of previous elements, a detailed record of feeds must be maintained to ensure traceability.

Two basic procedures should be taken into account: use of certified ingredients and disinfection procedures.

Certified ingredients

Some fresh food, such as squid, polychaetes, krill, mollusks and *Artemia* spp. cysts, can be a vector of pathogens, and we need to ensure that they are not a biosecurity risk. A certification can be requested at origin stating that the all feed (including pelletized food) is free of relevant viruses such as white spot syndrome virus (WSSV), Taura syndrome virus (TSV), yellow head virus (YHV) and other pathogens of compulsory notification by OIE (World Organisation for Animal Health, http://www.oie.int). This certification should be supported by PCR analyses carried out by an accredited diagnostic laboratory.

In any case fresh fish and shrimp waste should be avoided for fresh feed, and even crustacean meal should not be included in balanced pelleted feed.

Disinfection of fresh food

Despite certification of fresh food, the ingredients can be subjected to a disinfection process (when it is available) or sterilization/pasteurization to inactivate any pathogen (as long as these processes does not affect to nutritional quality and palatability of the feed).

In ideal conditions, different types of feed should be stored in separated freezers to avoid cross contamination.

Before use, the fresh food should be washed with clean drinking water. It is also possible to disinfect their surface (before being chopped to a suitable size for ingestion) with a calcium or sodium hypochlorite 20 ppm for 3 min. All the equipment used for feed preparation (knives, mixers, tables…) must be washed with an iodine-povidone 20 ppm solution and rinsed with clean water before use.

We should take special care with disinfection of *Artemia*. Decapsulation of *Artemia* cysts are carried out with sodium hydroxide (caustic soda) and chlorine 8-10%. The *Artemia* nauplii should be disinfected with solutions of sodium hypochlorite 20 ppm, choramine-T 60 ppm or both, for 3 min; and then rinsed with fresh water. After each harvest, the hatching tanks must be washed with detergent and disinfected using a sponge dipped in a sodium hypochlorite solution at 20 ppm.

2.4.2 Spreading risks

In madurations and larvicultures, the higher risk of pathogens spreading is with bacterial contamination of algae cultures. For this reason, the access to algal laboratory should be restricted, and it is necessary to implement disinfection protocols for equipment, water and air.

During the laboratory phases of microalgae culture, extreme hygiene measures should be adopted. All the water and air supplies should be disinfected and filtered using filter sizes <0.5 µm. All the equipment and containers should be disinfected using calcium or sodium hypochlorite 10 ppm, and then rinsed with treated water and disinfected with a muriatic acid 10% solution before being left to dry.

A continuous monitoring of microbiological quality of algae cultures should be implemented in order to detect contamination with protozoans and bacteria (especially pathogenic *Vibrio* spp.).

2.5 Animals

2.5.1 Shrimps

Shrimps are the main risk for introducing and spreading pathogens into farms, due to the trade between farms (purchases and sales of broodstock and nauplii) and internal transfers inside the farm.

All new animals (usually broodstock) introduced in a farm should be maintained in quarantine facilities (previous a disinfection process). Before passing to the production units, the health status of shrimps must be checked based on clinical signs and the results of an accurate diagnostic test able to detect low levels of infection. In case of infection of an OIE notifiable disease, or a severe disease, all the animals of quarantine should be destroyed immediately and adequately disposed.

2.5.1.1 Introducing risks

Disinfection at arrival

When a batch of broodstock arrives to the quarantine area, they should be disinfected previously in a dip of iodine-povidone 20 ppm, potassium permanganate 100 ppm or formaline 50-100 ppm for 30-60 s under strong aeration, and then they can be released into the destination tank.

Harvested nauplii can be treated to prevent fungal contamination by bath inmmersion in trifluralin (0.05-1 ppm). They are then washed with clean seawater and they are dipped in iodine-povidone 50-100 ppm (for 1-3 min) or chloramine T 60 pp (for 1 min). They are after washed again with clean water. Alternatively dips of 30 s in formaline 300 ppm or iodophore 100 ppm could be applied.

For the removal of epibionts from postlarvae, it is recommended a bath in a formaline solution 20-30 ppm during 1 h with strong aeration.

Be careful when formalin is used, a whitish sediment can appear at the bottom of the container: it is formaldehyde and it is highly toxic, and it should not be used.

Quarantine

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

The requirements for a quarantine (location, design, infrastructure, equipment, water treatment, staff and Standard Operation Procedures) should be defined by the Competent Authority and they depend on a previous risk analysis. Routine quarantine is a preventive measure that reinforces pre-border measures

(international health certificates of source, use of specific pathogen free (SPF) animals, on-site inspection of exporting facilities…) and post-border measures (surveillance programs and contingency plans).

The quarantine facilities must be located as a separated production unit and it should be used only for quarantine purposes. It is an indoor facility with restricted access only to authorized staff and veterinary officers. Structure of the building should prevent the entry of insects (screen at windows, self-closing doors…) and to avoid entry and exit of water (concrete floor, sealed junctions and gaps). In the access, a change room is necessary to allow the people to take a shower, wash their hands and change clothes and boots at entry and exit). A footbath with disinfectant must be placed at the entry door. Inside the quarantine building, all the holding tanks should be identified by permanent numbers, with specific equipment (nets, buckets, beakers…) with the same identification number and a record book for each tank. A suitable area for cleaning and disinfection of equipment should be located inside the building with an adequate draining of water. All wastewater from the quarantine should be strictly disinfected before being released.

For more details about quarantine: Arthur JR, Bondad-Reantaso MG, Subasinghe RP. *Procedures for the quarantine of live aquatic animals. A manual.* FAO, Fisheries Technical Paper 502. Rome, Italy. 2008; 89 pp.

Health status

Health status must be certified by the Competent Authority according to the requirements of national and international regulations (OIE Aquatic Animal Health Code). The stocking of pathogen free animals (broodstock, nauplii or postlarvae) is the main requirement, and the introduction of Specific Pathogen Free (SPF) animals is the most interesting option to achieve it. However, there are other denominations related to the health status, but without any official certification as Specific Pathogen Resistant (SPR) or High Health Genetically Improved (HHGI).

Diagnostic and disease identification

The diagnostic can be carried out at different levels as key part of the health inspections after any transport and routinely during the production cycle.

The first diagnostic level is based on the observation of shrimps, looking for gross characteristics. Broodstock are examined for external lesions, sex determination, moulting stage, stage of ovarian development and removal of diseased, moribund and dead shrimps (diseased and moribund shrimps are useful for third level of diagnostic). In non-adult stages we can observe photo-tactic response of nauplii, feeding activity of zoea and mysis by observation of fecal strands, activity and behavior of larvae and postlarvae. At this level, it is interesting to carry out stress test to check vitality of animals.

The second diagnostic level requires basic laboratory facilities and equipment (light microscope, staining jars, growth medium, incubation stove…) for a more detailed examination of squashed tissues and basic bacteriology. These diagnostic techniques make it possible to control the bacterial flora, diseased and moribound animals, quality of eggs, nauplii, larvae and postlarvae, and bacterial growth from rearing water and animals.

Finally, the third diagnostic level uses more complex techniques like PCR which requires dedicated facilities, advanced equipment and well trained technical staff. These techniques allow for the early detection of the pathogens even in

asymptomatic animals. It should be carried out according to procedures described in the OIE Manual of Diagnostic Tests for Aquatic Animals, in order to provide a rapid and accurate diagnosis of notifiable diseases.

In case of suspicion of a notifiable disease and/or abnormal mortalities the health manager of the farm must immediately report it to the Competent Authority.

Traceability

All animal movements should be recorded, including information about date, origin, destination, batch reference, health certificates, stage and quantity of animals. These records are very important since they allow tracing the origin and spread of a disease.

It is also desirable to record diseased and dead shrimps in each production unit, prescribed treatments, feeding practices and water quality parameters.

2.5.1.2 Spreading risks

Surveillance and early disease/infection identification

Surveillance methods are described in the OIE Aquatic Animal Health Code, and three main approaches could be combined: passive, active and targeted surveillance. The objective of surveillance programs is to establish a disease-free populations and further maintenance of the health status.

Passive surveillance is based on the observation of clinical signs during routine health inspections and the evidence of abnormal mortalities (for this reason, it is necessary to maintain an accurate mortality record). When problems arise, a laboratorial diagnostic is required in order to confirm or discard notifiable diseases.

Active and targeted surveillance complement the routine health inspections and data record examination, with collection of samples for laboratorial diagnostic (in active surveillance, the samples are collected in case of observed clinical signs, and in targeted surveillance, the samples are routinely collected). The recommended minimum number of specimens is defined by the OIE Manual of Diagnostic Tests for Aquatic Animals in order to obtain significant results.

Definition of surveillance programs should be done by the Competent Authority, but shrimp farmers can implement a syndromic surveillance system to reinforce the official surveillance, as it is developed in this project.

Transfers and harvest

Specific measures should be implemented to reduce escapes to natural waters during transfer between ponds and harvests, especially in ponds that had registered disease outbreaks.

Contingency plan

In the event of a problem, the health manager must immediately take appropriate actions as outlined in a contingency procedure. This contingency plan should be prepared before the problem arises, and it should be revised and updated regularly (at least once a year).

The document must contain detailed procedures for reporting, disinfection of pond water and production units, and also destruction and disposal of affected animals.

Disinfection

During the production cycle, it could be necessary to treat the animals depending on the results of microscopically observation of gills and skin, or with the aim to reduce pathogen loads.

After the females are removed from the spawning tanks, a dip in iodine-povidone 20 ppm for 15 s is recommended, before returning to the tank of origin. On the other hand, the eggs can be disinfected with a similar protocol based on washing and disinfecting eggs with iodine-povidone 50-100 ppm for 1-3 min, or formalin 100 ppm for 30 s. These treatments can be combined with trifluralin 0.05-1 ppm in order to reduce fungal infections.

Finally, a bath with copper sulphate 0.1 ppm and aeration is indicated when the gills show excessive fouling by algae if or there are filamentous bacteria, or the application of a bath with formalin 30-50 ppm for 1 hour in aeration to control epicommensal protozoans.

Destruction and disposal

When an outbreak of a serious exotic disease is confirmed, one of the preventive measures included in the contingency plan could be the destruction of all animals of a production unit.

In this case a suitable method for destruction (process of killing by euthanasia) should be applied and depends on several factors:

- Size and number of animals to be killed: depending on the final weight of the population to destroy, it is important to ensure that the slaughtering facilities (site, equipment, experienced staff and methods) are available to achieve deadline of destruction (it depends on the risk of further spread of the pathogen).
- Destination of destroyed animals: in case of infected shrimps without clinical signs no chemical treatments, or emergency harvest for human consumption could be carried out and the animals should be slaughtered using techniques in accordance with food safety regulations. In the rest of cases the animals should be disposed in a safe manner after their destruction.
- Potential impacts in the water and environment due to the use of chemicals.
- Coordination with the available disposal methods and the disinfection procedures of water and production units.

Different methods for destruction are available:

- Physical methods: they involved the removal of animals from water, and time between removal and destruction should be as short as possible in order to reduce animals stress. The most common methods are hypothermia (water suspension of crushed ice or in a freezer) and hyperthermia (boiling water during enough time to maintain a core temperature of 80ºC at least for 75 s, this method is controversial due to ethical reasons).
- Chemical methods: the most frequent method is based on the addition of sodium hypochlorite to water in order to form free residual chlorine that kills the shrimps (with the additional advantage of disinfecting pathogens). Chlorinated water should be neutralized before discharge. Another method is based on the application of rotenone at 0.15 ppm, but it is a toxic product for fishes and other aquatic invertebrates, so before water discharge an inactivation with potassium permanganate is compulsory.

Finally, is important to prevent the presence of scavengers, particularly birds, close to the destruction site to avoid spreading infection mechanically by ingestion of carcasses, because some pathogens can survive in the avian gut.

For more details about destruction: Australian Government. *Operational procedures manual: Destruction (Version 2.0).* Australian Aquatic Veterinary Emergency Plan (AQUAVETPLAN), Australian Government, Department of Agriculture, Fisheries and Forestry, Canberra, Australia. 2009; 41 pp.

After a destruction procedure or a mortality event, dead animals should be disposed using a safe method as deep burial, incineration or ensiling (depending on the characteristics of the shrimp farm).

- Deep burial is a very convenient, simple and easy method. However, there are significant potential risks for the environment, and some factors should be considered such as distance to watercourses, sea and wells, permeability of the soil and proximity to other buildings.
- Incineration (or cremation) is an expensive method and it can create air pollution and odours during the process. The most efficient system is a crematoriums located in a safe area of the shrimp farm.
- Ensiling using organic acids or molasses and lactic acid bacterial cultures, until the complete solubilisation of carcasses can be ensured.

For more details about disposal: Australian Government. *Operational procedures manual: Disposal (Version 2.0).* Australian Aquatic Veterinary Emergency Plan (AQUAVETPLAN), Australian Government, Department of Agriculture, Fisheries and Forestry, Canberra, Australia. 2009; 65 pp.

2.5.2 Other animals (vectors and carriers)

2.5.2.1 Introducing risks

Filters

As we have already commented in previous sections, the zooplankton could be a carrier of shrimp pathogens, and it is possible to remove those using nets filter of 60 µm mesh size, and then a further disinfection treatment with calcium or sodium hypochlorite 60 ppm can be carried out.

Fences and nets

Some wild crustaceans (ie. crabs), that are susceptible to shrimp diseases, can move across the ponds over land barriers, and to prevent the access to ponds a fence around the culture pond should be installed. A net with 2 mm mesh size and 30-50 cm high is sufficient for this purpose.

Birds are also a relevant risk for diseases introduction in a pond. Some birds are attracted by dead and moribund shrimps at pond edges (especially during disease outbreaks), and they can be mechanical carriers. A range of very cheap netting is available on the market, in order to install a fence in the perimeter and over the pond. Other options are to use the pyrotechnics and the automatic exploders to produce noise, and the use of real ammunition as last resort.

2.5.2.2 Spreading risks

Finally, it is important to prevent scavengers and predators, particularly birds, close to the ponds with mortality or to the destruction site to avoid mechanically spreading infection.

2.6 Biosecurity levels in the aquaculture shrimp production chain

The shrimp production is done through successive steps realized into a series of biosecurity zones. Specific biosecurity measures have to applied for each site according to its specificity.

Management plan for integrated project have to be designed to provide an escalating and manageable distinction between risk areas:

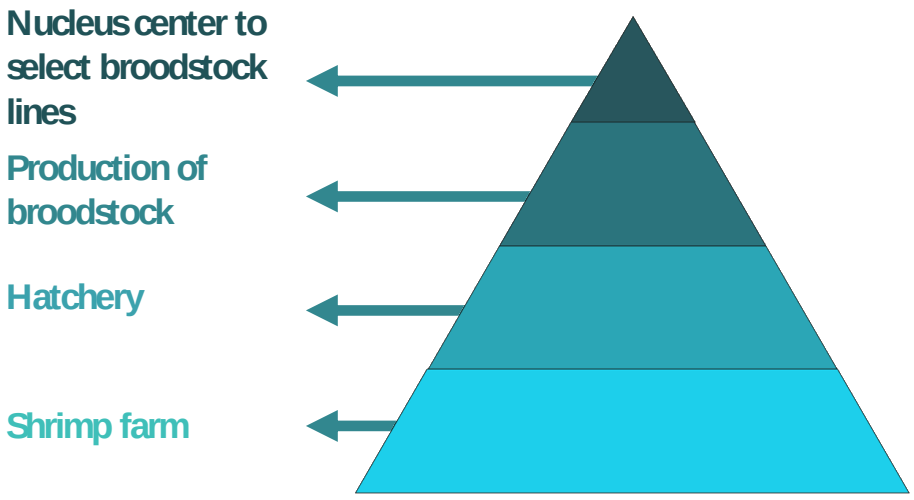
Zone 1	Central facilities**, accommodation and general use areas**
Zone 2	Processing plant
Zone 3	Transit zone between the central facilities, farms and supporting infrastructure
Zone 4	Farm, including water supply channels and intake settlement ponds; seawater and freshwater supplies, farm accommodation, warehouses, offices and storage facilities.
Zone 5	Hatchery
Zone 6	Broodstock production facilities

It is suitable to implement a consistent biosecurity plan for each established zone considering the seven principles of the HACCP (Hazard Analysis Critical Control Plan) system.

Hazard analysis	**Identify hazards, at each step in the process.**
Critical control points	Actions are taken to reduce or eliminate the hazard.
Critical limits	The limits to which the hazard must be reduced (i.e. level of water filtration)
Monitoring	Observation and measurement of cleaning and disinfecting to ensure the critical limits are met at each step.
Correction	Action must be taken if the critical limits are not met at each step.
Recording	Records must be kept showing that biosecurity program is in place and is being implemented correctly and continuously.
Verification	Tests and procedures to ensure that HACCP system is working properly. This should be carried out by QA/QC.

This plan has to be strictly respected by everybody, managers, teams and visitors, and it is very important to provide available documents and signals to alert and inform about specific measures in different areas.

External visitors need to be informed about the biosecurity needs. After approval by the site manager, they need to be accompanied them to visit the site and be provided with uniform and boots specific to the site.


Nucleus center to select broodstock lines
Production of broodstock
Hatchery
Shrimp farm

3 SPECIFIC BIOSECURITY AQUACULTURE MANAGEMENT PLANS

3.1 Hatchery

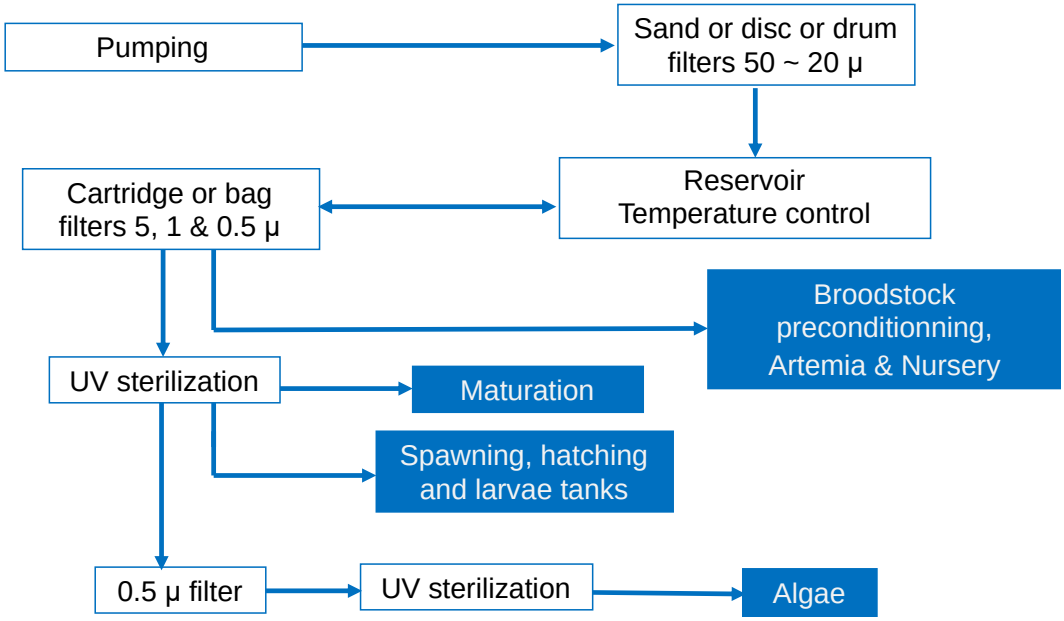
An advanced training in biosecurity and shrimp health management is required to allow for the competent day to day management of these topics. This training should apply to all hatchery workers, breeding program staff and production staff. All staff actively involved in hatchery will need to be trained.

3.1.1 Facilities and equipment

Element	*Minimum requirement*	*Optimum requirement*
Restricted access to hatchery	▪ Control point at entrance ▪ Sign in book for workers and visitors ▪ Last contact with shrimps? ▪ No children & no pets in the unit	▪ Perimetral fence
Disinfection facilities for vehicles	▪ Spraying portable device ▪ Feed truck should not enter production area ▪ Between each delivery ▪ For truck to transport PL's to farm: 1 day drying time ▪ Truck kept in designated parking area	▪ Wheel bath and permanent spray device
Disinfection facilities for people	▪ Clean clothing and footwear ▪ Foot bath filled with 20 ppm chlorine solution and spray with alcohol at entrance ▪ Wash bottles containing 100 ppm iodine solution should be strategically placed for hand disinfection between visits to different tanks	▪ Wear clean working uniforms at all times and remove before leaving the hatchery ▪ Hands should be washed with povidone
Water treatment/ reservoir	▪ Settlement pond + Filter with a mesh of 100 µm ▪ Enclosed reservoir area (both walls and roof).	▪ Replace sand filter by drum filter or disk filters

Element	*Minimum requirement*	*Optimum requirement*
Water treatment hatchery	▪ Cartridges and/or bag filters: ▪ Maturation, *Artemia* and nursery: 10 µ ▪ All other areas: spawning, hatching, larval rearing and algae: 5, 1 and 0.5 µ and then UV ▪ Bags and/or cartridges washed, disinfected in chlorine of iodine and dry every day before reused. This means need of at least 2 sets of bags and/or cartridges to operate the filters very day	▪ Optional: activated carbon before cartridges filters ▪ If use of ozonation ensure the value of ORP of water after ozonation process is in accordance to standards ▪ Ozonized water for broodstock: 500 mV ▪ Ozonized water for post larvae : 600 mV
All equipment's used in the hatchery as nets in tanks	▪ Separate color coding can be used for utensils for each section in the hatchery ▪ One set of equipment for each tank ▪ Each tank must have its own set of mesh nets as required for catching and/or checking larval or broodstock shrimp quality ▪ All equipment's must be disinfected with 50 ppm chlorine or with 100 ppm PVP povidone iodine before using it to another tanks ▪ The disinfectant solution should be changed daily for a new solution ▪ The area of the dike where sampling is done must be chlorinated 50 ppm after sampling ▪ All laboratory equipment's used for sampling as scissor and/or forceps must be disinfected with dettol and flame between individuals	
Dead shrimp and waste feed	▪ Feed spillage minimized ▪ Proper disposal of waste ▪ Garbage delivered to approved dump site ▪ Accordance with local regulations ▪ Infected shrimp incinerated	
Water discharged-	▪ Treat and filter the wastewater ▪ Avoid spread of contamination ▪ Discharge ponds not recommended	

3.1.2 Water treatment in hatchery



3.1.3 Routine procedures

3.1.3.1 Maturation department

Management of broodstock

Stage	*Minimum requirement*
Incoming broodstock	▪ Only certified SPF animals ▪ Disinfect broodstock bags with povidone iodine/ formalin 1,000 ppm ▪ Acclimatization if any difference on temperature, salinity or pH
Quarantine	▪ 5 days to 2 weeks for testing ▪ Water exchange: 300-400% per day ▪ Daily siphoning and dead removal
Ablation	▪ Recondition 3 to 4 weeks prior to ablation ▪ Disinfect injured eye with Iodine
Maturation	▪ Water exchange: 300-400% per day ▪ Daily siphoning ▪ Feeding frequency: 8 times with FR 28-32%
Fresh feed	▪ Maturation diets from disease free areas ▪ Ensure feed is within sell-by date ▪ Buy only from reputable companies with government-certified products ▪ CR testing of fresh feed ▪ Disinfection of fresh feed ▪ Do not overfeed as will reduce water quality, but do feed little and often ▪ Monitor feeding status of shrimp frequently and make records of feeds and health ▪ Store at cool temperatures ▪ Use new bags ▪ Bags should never go in production area ▪ Ensure feed cannot be contaminated by pest animals which could be disease vectors
Pelletized food	▪ Suppliers inspected regularly and registered in the country. ▪ Store al cool temperatures in dry conditions to avoid presence of fungi and pests

Broodstock treatments	▪ Daily or every other day probiotic dosing ▪ Every 2 weeks disinfection baths for animals ▪ Safe to use freshwater, chloramine T up to 10 ppm, formalin 25 ppm, copper 10 ppm, isatin 3 ppm
Spawning	▪ Dipping brooders with povidone iodine before transferred back to maturation tank ▪ Egg colleting and cleaning with clean sea water and dipping with povidone iodine 50-100 ppm for 2 min
Hatching	▪ Dipping nauplii with povidone iodine 50 ppm for 1 min, before transferred to holding tank
Holding	▪ Rinse nauplii for 6-8 h or minimum of 400% water exchange ▪ Dipping nauplii with 50 ppm povidone iodine for 1 min, seconds before transfer to larval rearing tank
Nauplii quality control	▪ For each holding tanks, check for - Activity (phototaxis) - Body cleanliness - Color - Fungus - Bacteriology - Setae - Deformity

Broodstock monitoring: PCR

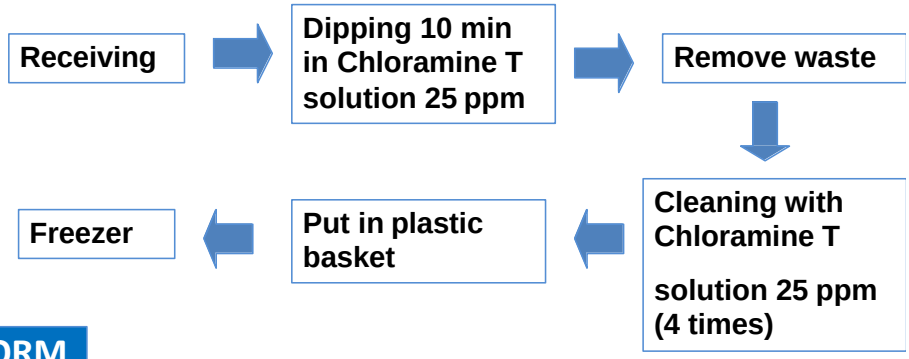
Area	Sampling point	Sample	Frequency	Pathogens				
				WSSV	IMNV	TSV	YHV	IHHNV
Maturation	New incoming broodstock (Quarantine)	Tissue	At arrival	X	X	X	X	X
	Dead broodstock	Tissue	Monthly & weekly	X	X			
	Hemolymph	Hemolymph	Monthly	X	X			
Fresh feed	Polychaetes	Tissue	Weekly	X	X			
	Squid	Tissue	Weekly	X	X			
	Krill	Tissue	Weekly	X	X			
	Clam, mussels, oyster, etc.	Tissue	Weekly	X	X			
	Artemia biomass	Tissue	Weekly	X	X			

Broodstock monitoring: microbiology

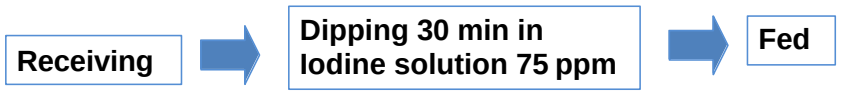
Area	Sampling point	Sample	Frequency	Pathogens				
				WSSV	IMNV	TSV	YHV	HHNV
Maturation	New incoming broodstock (Quarantine)	Water bag	At arrival	X	X	X	X	X
		Hemolymph	At arrival & Monthly	X	X	X	X	X
Fresh feed	Polychaetes	Tissue	Weekly	X	X	X	X	X
	Squid	Tissue	Weekly	X	X	X	X	X
	Krill	Tissue	Weekly	X	X	X	X	X
	Clam, mussels, oyster, etc.	Tissue	Weekly	X	X	X	X	X
	Artemia biomass	Tissue	Weekly	X	X	X	X	X

Fresh feed sanitation

SQUID

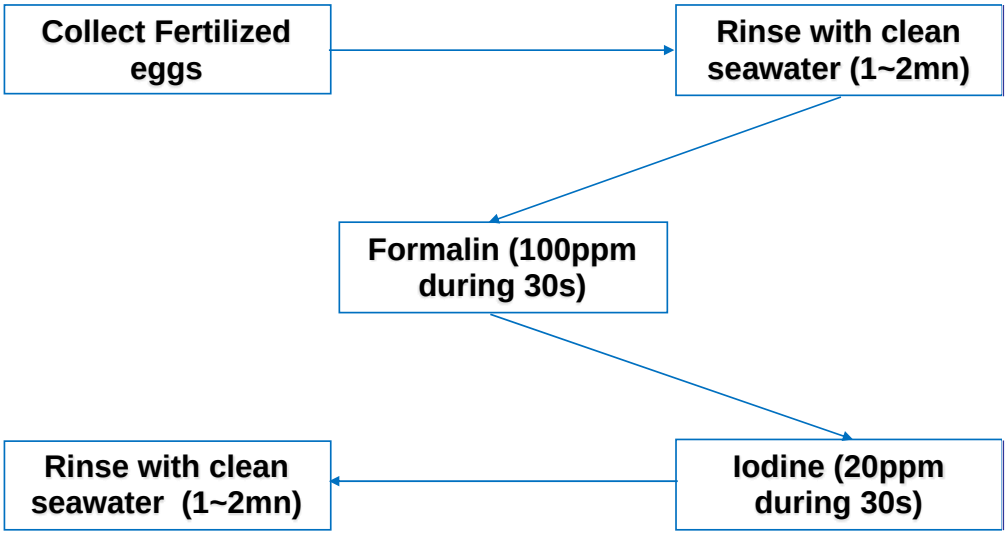


WORM



Eggs and nauplii washing

Stage	*Minimum requirement*
Tank	Conical bottom
Water filter	5, 1 & 0.5 µ + UV
Water flow trough	50% for 6-8 h
Density	max. 4,000 nauplii/L
Temperature	29°C



Microscopy check of nauplii

		Nauplii stage	
		N1	N6
Activity (%)		>75	>85
Tissue Condition (%)	Broken	<3	0
	Shrink	0	0
	Necrosis	0	0
Spine (%)	Breaking setae	0	0
	Dirty setae	<3	0
	Curly setae	0	0
Deformity (%)	Antennula	0	0
	Spine	0	0
	Body	0	0
	Tail	0	0

Microbiology check of nauplii

		Nauplii stage	
		N1	N6
TVC (cfu/mL)	Yellow	$<10^3$	$<10^2$
	Green	0	0
LB (%)		0	0
Fungus		Negative	Negative

3.1.3.2 Larval rearing department

Larval rearing

Stage	*Minimum requirement*
Stocking nauplii	▪ Use only sterilized water ▪ Dipping nauplii bags with povidone iodine 300-500 ppm ▪ Acclimatization before stocking
Water exchange	▪ 2.5-5% at larvae stage ▪ 5-25% at PL1 through harvest
Water quality parameter during rearing	▪ DO: Minimum of 4 ppm. ▪ Unionized NH_3: Maximum of 0.5 ppm ▪ Nitrite : Maximum of 1 ppm

Algae

Stage	*Parameters*
Check quality	▪ Color ▪ Dirty foam at surface ▪ Cell content ▪ Contaminants ▪ Amoeba ▪ Dinoflagellates ▪ *Spirogyra* ▪ *Nitchia* ▪ *Navicula*

Artemia

The *Artemia* department is the dirtiest area of the hatchery in terms of biosecurity. *Artemia* cyst shells may be loaded with bacteria, fungi, and even contaminated with organic impurities; bacterial contamination in the hatching medium can reach numbers of more than 10^7 CFU·mL^{-1} (= colony forming units).

Therefore, if no commercially disinfected cysts are used, it is recommended to apply routinely a disinfection procedure by using hypochlorite. This treatment, however, may not kill all germs present in the alveolar and cortical layer of the outer shell. Complete sterilization can be achieved through cyst decapsulation, described in 0.

Daily larvae health monitoring

Parameter	*Stage*	*Target*
Length	PL1, PL4, PL8 through harvest	PL1 minimum 4 mm
Gut Content	Z, M, PL1 through harvest	Minimum 50%
Hepatopancreas condition	Z, M, PL1 through harvest	Normal
Luminous bacteria (wet mount)	Daily (M2 through PL7)	Negative
	PL2, PL7 through harvest	Negative
Deformity	N, Z, M, PL1 through harvest	0%
Pigmentation	N, Z, M, PL1 through harvest	Normal
Activity	N, Z, M, PL1 through harvest	Minimum 80%
Density algae (AM)	Z, M, PL1	9,500-136,500 cell/mL
Density algae (PM)	N, Z, M, PL1	9,500-136,500 cell/mL

Parameter	Stage	Target
Ecto & endoparasite	Z, M, PL1 through harvest	Negative
Stage	N, Z, M, PL1 through harvest	18 days (PL9)
Necrosis	N, Z, M, PL1 through harvest	Maximum 20%
Fungi	N, Z, M, PL1 through harvest	Negative
Stress test	PL8 through harvest	Minimum 95%

N: Nauplii; Z: Zoea; M: Mysis; PL: Postlarvae

Microbiology check of larvae

Stage	Parameter				
	TVC	TLV	TBC	TLB	LB
N	X	X	X	X	
Z2	X	X	X	X	
M2	X	X	X	X	
PL2	X	X	X	X	PL2
PL5	X	X	X	X	
PL7	X	X	X	X	PL7

N: Nauplii; Z: Zoea; M: Mysis; PL: Postlarvae

PCR check of Larvae

Sampling point	Sample	Frequency	Parameters				
			WSSV	IMNV	TSV	YHV	IHHNV
Nauplii	Tissue	Stock	X	X	X		X
PL harvest	Tissue	Harvest	X	X	X		X

Quality control of PL 20

- Age: PL10 to PL20 (rostrum with 5 spines minimum)
- Average weight at PL10: ≤ 300 PLs/1 g
- Average weight at PL20: individual body weight >7.5 mg and size >12 mm
- Minimum survival 50%
- Maximum standard deviation of sizes: 10%
- Hepatopancreas and well-developed digestive tract
- Well branched gills, more than 3 branches
- No green colonies of *Vibrio* spp.
- Negative PCR
- Stress test

Parameter of PL Quality Control	Standard
PL age	PL 9-12 (17 -21 days)
PL length	Minimum 8,5 mm at PL9
Survival in larvae tank	Minimum 50%
Coefficient of Variance	Maximum 10%
Luminous bacteria	No infection
PCR Test (WSSV,TSV, YHV, IHHNV, IMNV)	Negative

3.1.4 Biosecurity in hatchery

3.1.4.2 Use of povidone iodine

- Immersion operational tools/ equipment's of hatchery (dose: 100 ppm)
- Hand baths (dose 100 ppm)
- Sterilize tanks before fill-up with water (dose: 100 ppm)
- For dipping nauplii (dose: 50-100 ppm)
- For dipping bags of broodstock upon arrival (dose: 1,000 ppm)
- For the case of necrosis in broodstock / PL (dose: 5-15 ppm)
- For the case of parasite in larvae tank (amoebas, dinoflagellates) (dose: 5 ppm)

3.1.4.1 Use of formalin

- Immersion harvesting equipment's and filter bags (dose: 300 ppm)
- For the case bacterial filamentous in larvae tank (dose: 5-25 ppm)
- Immersion broodstock (dose: 25 ppm)
- Stress test (dose: 100 ppm)

3.1.4.2 Use of chlorine

- For flush-out tanks due to infectious diseases, apply chlorine 1,000 ppm after 50% reduction in water in the tanks
- Foot baths (dose: 200 ppm)
- Disinfection floors (dose: 200 ppm)
- Disinfection tanks (dose: 200 ppm)

3.1.4.3 Use of potassium permanganate

- $KMNO_4$ (dose 200 ppm). Equipment, tire, and foot baths.

3.1.5 Periodical procedures

Element	*Minimum requirement*
Sample to detect pathogens	▪ At stocking & at transfer to nursery and to farm ▪ When abnormal mortalities are observed
Complete Dry out	▪ Al least 7 days after end of each production cycle

3.2 Shrimp farm

3.2.1 Personnel, material, vehicles and other logistics movement

Activity	*Risk*	*Mitigation measures*
Wearing of uniforms	Medium	▪ Wear the specific uniforms at all times while working in the farms and remove at the time of leaving the farm ▪ Uniforms are exclusively used inside farms during the working hours. No uniforms shall be allowed to wear after the working hours unless instructed by the farm manager or immediate supervisor ▪ Employees where transfers from farm to other farm are required in their job, uniform should be changed from farm to farm basis. Those are the staffs that are in contact with soil, water & shrimps only
Personnel movement	Medium/ High	▪ Workers who must enter in each pond to perform their duties should first disinfect his hands and boots or sandals ▪ Visitors will only be allowed to go to farms after thorough screening-that they should have gone through all the disinfection and quarantine measures; that they should not have visited prior

Activity	Risk	Mitigation measures
		to this visit any fish or shrimp farm within the 48 h period ▪ Any employee of the company must also be quarantined 48 h prior to entry to Company premises if he has visited any fish or shrimp farm, fish auction markets, fresh sea products supermarket
Vehicle traffic	High	▪ Vehicles that circulate throughout other areas of the farm must be subjected to a decontamination procedure ▪ Vehicles of one farm should not go to other farms or circulate between production units unless permitted and gone through with decontamination. Vehicles should be thoroughly cleaned and passing through chlorine baths upon entry. An alternative would be to spray the vehicles with a chlorine solution ▪ Fresh chlorine solution should be prepared whenever needed and checked periodically during periods of heavy traffic ▪ One official entrance/ exit gates for each farm

Activity	Risk	Mitigation measures
Disinfection of vehicles & other equipment	High	▪ Vehicle used in collecting various farm samples should go through tires wash into chlorine solution before entering to another farm. Inside part should be disinfected in case pond water is in contact with it. Maximum care should be observed particularly if staff is in contact with different pond water, soil & shrimps. In this case, hand disinfection is highly required ▪ Outboard engine (OBE) should be disinfected first prior to use in another pond. Disinfection is by dipping a portion of OBE (having in contact with pond water) into a prepared chlorine solution at designated shed-house. Since pond water is not drained totally inside OBE, it is therefore necessary to run such equipment while disinfection is in place to remove excess water ▪ Disinfection of heavy equipment's into 25-50 ppm chlorine solution shall be applied on the following cases. Note that chlorine concentration can be increased during the period of disease outbreak ▪ Excavator machine. Disinfect bucket portion ▪ Dump truck. Tires disinfection required ▪ Bulldozers, ploughing machine & compactors. Require disinfection on parts having in contact with pond soil
Loading of incoming materials (e.g. molasses, lime, fertilizers)	Medium	▪ Designated location and no entry of delivery truck in the farm ▪ If it is compulsory for the vehicle to go inside the farm, it must go through and subjected to decontamination procedure by taking a clearance from the bio-security section

3.2.2 Pond preparation

3.2.2.1 Earth ponds

Activity	Risk	Mitigation measures
Use of equipment in the pond	Medium	▪ Clean and disinfect with a power washer between ponds and between farms
Pond bottom drying & disinfection	Medium	▪ Dry until it crack & apply chlorine (25-50 ppm) in wet area to eliminate all crustaceans/ fishes thrive in this area ▪ In cases where there are large areas of the pond bottom which cannot be dried between crops, or where chlorine application would be excessively costly, hydrated lime can be applied at 1,000-1,500 kg/ha to

Activity	Risk	Mitigation measures
		kill any potential disease vectors ▪ During the dry-out, drain the sub-feeder canal, dry until it crack & apply chlorine/ hydrated lime ▪ Prepare pond well, line ponds maintain pH>7 with lime, remove old sediment or treat with disinfectants and/or probiotics
Filtration of incoming water	High	▪ Use of 500 µ mesh sock net or drum filters ▪ It is being reused, some treatment is essential, as: - Settlement pond - Filtration
Carrier protection	High	▪ Pond biosecurity is multilayered. Should include provisions for keeping birds, crabs, disease vectors out ▪ Filtration of the water and low water exchange ▪ Bird nets over the pond ▪ Crab fences around the ponds
Preparation of inlet gates for filtration of incoming water	Medium	▪ Installation of frame with 1,000 µ mesh and 250 µ bag nets in the inlet gates ▪ All screens and slabs should be inspected for leaks prior to filling pond ▪ All leaks must be sealed to prevent unfiltered water from entering the ponds and use sponge type of material to control water leakages
All equipment's used in the farm as nets; aerators, lab equipment's, etc.…	Medium /High	▪ Separate color coding can be used for utensils for each section in the farm ▪ One set of equipment for each pond ▪ Each tank must have its own set of mesh nets as required for catching and/or checking shrimp quality ▪ All equipment's must be disinfected with 50 ppm chlorine or with 100 ppm PVP povidone iodine before using it to another pond ▪ The disinfectant solution should be changed daily for a new solution ▪ The area of the dike where sampling is done must be chlorinated 50 ppm after sampling ▪ All laboratory equipment's used for sampling as scissor and/or forceps must be disinfected with dettol and flame between individuals

3.2.2.2 Intensive lined ponds

Activity	Risk	Mitigation measures
Use of equipment in the pond	Medium	▪ Clean and disinfect with a power washer between ponds and between farms
Pond bottom drying & disinfection	Medium	▪ Dry completely the pond for one week ▪ During rainy season clean the liner with high pressure washer and a disinfectant solution
Filtration of incoming water	High	▪ Use of 500 µ mesh sock net or drum filters
Carrier protection	High	▪ Pond biosecurity is multilayered. Should include provisions for keeping birds, crabs, disease vectors out ▪ Filtration of the water and low water exchange ▪ Bird nets over the ponds ▪ Crab barriers around the ponds

Activity	Risk	Mitigation measures
Preparation of inlet gates for filtration of incoming water	Medium	▪ Installation of frame with 1,000 µ mesh and 250 µ bag nets in the inlet gates ▪ All screens and slabs should be inspected for leaks prior to filling pond ▪ All leaks must be sealed to prevent unfiltered water from entering the ponds and use sponge type of material to control water leakages
All equipment's used in the farm as nets; aerators, lab equipment's, etc.…	Medium /High	▪ Separate color coding can be used for utensils for each section in the farm ▪ One set of equipment for each pond ▪ Each tank must have its own set of mesh nets as required for catching and/or checking shrimp quality ▪ All equipment's must be disinfected with 50 ppm chlorine or with 100 ppm PVP povidone iodine before using it to another pond ▪ The disinfectant solution should be changed daily for a new solution ▪ The area of the dike where sampling is done must be chlorinated 50 ppm after sampling. ▪ All laboratory equipment's used for sampling as scissor and/or forceps must be disinfected with dettol and flame between individuals

3.2.2.3 Chemicals for competitor/disease vector elimination

Product	Dose
Chlorine	30-100 ppm of water (before lime)
Iodine	5 kg/ha
Crustacide	1.5 to 3 ppm commercial brand formulations of dichlorvos, dipterex or trichlorfon
Tea seed meal (saponin)	To kill fish : 100-200 kg/ha

3.2.3 Transfer and stocking of PLs and/or juveniles from hatchery to farm

Activity	Risk	Mitigation measures
PLs or juveniles harvest	Medium /High	▪ Only disease free PLs passing quality control test & PCR negative must be received on the farm ▪ Strict biosecurity measures at the reception area to maintain a virus-free environment ▪ Only deputed employees should go inside hatchery to check PLs and supervise transport preparation ▪ Fry positive for potential virus should be discarded and follow decontamination procedures ▪ Any mortality observed during PL or juveniles harvest in hatchery tank, farm representative observation and comment must be attended. There must be information dissemination to the concerned departments and immediate decision should be made ▪ Avoid escapes to natural waters
PLs or juveniles transport to the grow-out pond/ nursery	Medium	▪ Fry tank and truck must be washed with disinfectant (chlorine solution at 25-50 ppm) before and after use ▪ In case where truck is used to other pond on same date, it has to be washed and chlorinated first before proceeding to

Activity	Risk	Mitigation measures
		fry harvest activities ▪ In this regard, there should be decontamination place inside hatchery and or farm in order not to disrupt stocking activities especially during the period of more than 2 trips/truck/day
Juvenile transfer	Medium /High	▪ Cleaning and disinfection of juvenile transfer facilities with 20-50 ppm chlorine before and after use ▪ Add a small amount of ascorbic acid (20 ppm plus) to the bags ▪ Cool bags to temperatures that slow the PL down depending on time of transport ▪ Acclimate them to your ponds as slowly as possible ▪ Use survival cages to ensure that PLs survive well up to 96 h post stocking ▪ Avoid escapes to natural waters

3.2.4 Pond management

Activity	Risk	Mitigation measures
Pond environment monitoring	Medium /High	For water quality this includes: ▪ Daily/twice daily: Oxygen, pH, temperature, salinity, Secchi ▪ Weekly: NH_3, NO_2, alkalinity, H_2S and bacterial levels in water + minerals in low salinity culture ▪ Monthly: Total N and Total P For sediments: ▪ Check percentage organic matter (at least during pond prep.) and size of sediment pile through probing during culture cycle ▪ Also note: Algal bloom crashes, filamentous algae, weeds, presence of fish/birds/insects, froth, flocks, etc. ▪ Additional: rainfall, air temperature
Stock monitoring	Medium /High	Daily analysis of shrimp health through checking of quality of shrimp caught on feed trays, noticed around pond banks or from weekly cast net samplings. Disinfect cast nets between ponds with iodine! (chlorine damages cast nets) Order for collection of samples: ▪ from ponds with young shrimps to old shrimps ▪ from healthy ponds to ponds with suspicion of disease ▪ Must look carefully and record in pond book the apparent health of shrimp in each pond ▪ These analyses critical for early detection of any problems ▪ Look for: vitality, color, molt status, fouling, deformity, spots or damage to carapace, gut fullness and color, feed consumption rates, presence of predators/pests on trays/in pond ▪ Staff must be vigilant and record all these and if necessary, communicate any abnormalities to the manager ▪ Animals need to be sampled as a population regularly. ▪ Animals also need to be looked at daily by feeders, water quality testing personnel for overt signs of problems ▪ Boats should be used on single ponds and not moved between ponds Look at outlet areas when water is exchanged ▪ Behavior is an important gauge of health that is often ignored until it is too late ▪ Presence of birds ▪ Feeders should be trained as to what to look for
Animal health	Medium	▪ Feed consumption is a useful indicator of animal health

Activity	*Risk*	*Mitigation measures*
proactive practices	/High	▪ Multiple small feedings instead of three or four large feedings ▪ Manage stresses ▪ Manage organic accumulation ▪ Water quality impacts growth and stress levels ▪ Look for early signs of problems ▪ Sample weekly for growth and sample shrimp for health ▪ Offers potential to harvest shrimp before acute disease kills large numbers at once ▪ Very rare that single pathogens are the cause of a problem ▪ Rarely is there mass die offs without evidence ▪ Some pathogens typically kill animals with no sign of dead shrimp ▪ Daily observations for disease/mortality ▪ PCR/histopathology tests conducted for viral diseases where required
Basic bacteriology for farms	Medium /High	▪ Since bacterial problems (especially with *Vibrio* sp. bacteria) are a major cause of stress, disease and death in shrimp farms, knowledge of bacterial populations within the ponds is essential ▪ This especially true with EMS caused by *V. parahaemolyticus* ▪ Simple bacteriology procedures can and should be used for the following reasons: - checking that the water disinfection procedures utilized in the farm are working - checking the evolution of bacterial populations within the ponds (to help determine preventative and curative management techniques) ▪ Selection and dosing rates of anti-bacterial chemicals, products and disinfectants for use in the farm
Flagging system	Medium /High	System to identify easily the problematic ponds and are of the farm: ▪ Green Flag: Normal status, no pathogen or disease detection ▪ Yellow Flag: Regular practices with normal movement of vehicles and staff. Detection of a pathogen/infection polymerase chain reaction (PCR, very light or light positive), but no clinical signs or disease outbreak observed ▪ Red Flag: Disease involving clinical signs/mortality or suspicion of a disease outbreak (PCR strong/medium positive) or histology positive. Allocation of personnel to monitor the strict implementation of biosecurity measures. Definition of buffer compartments. Isolation of affected and buffer compartments through further restrictions on the movement of staff, vehicles, etc. Histology monitoring of the affected stock and PCR monitoring of buffer zones
Contingency planning	Medium /High	▪ The objective of the contingency plan is to quickly recover production through rapid initial response and effective implementation of biosecurity measures at minimum cost with minimum disruption ▪ Plan must be written down and communicated to staff, with details the steps and timing of the responses ▪ This includes flag system and short, efficient reporting system ▪ The contingency plan should include: - Diagnostic procedures - Reporting procedures - Sanitary slaughtering procedures - Infected shrimp handling and disposal - Protocols for staff/vehicle movement and disinfection - Protocols for flagging farm compartments - Disinfection procedures - Fallowing procedures

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ANNEX 1. DISINFECTANTS

Clean surfaces with detergent and disinfectants prior to proper disinfection. The following list comprises the disinfectants recommended for use in shrimp farms:

- Desiccation
- Steam
- Hot water (60ºC)
- UV light (from natural sunlight)
- Chlorine (as calcium hypochlorite, HTHT or a bleach solution containing a sufficient concentration of hypochlorite)
- Lime (as calcium oxide or calcium hydroxide)
- Formaldehyde gas (from sublimated paraformaldehyde or concentrated formalin/potassium permanganate reaction)
- Iodine (as contained in iodophors)
- Ozone
- Concentrated acids

Physical disinfectants

Process	*Indication*	*Method of use*	*Comments*
Desiccation, sunlight	Pathogens on earthen bottoms	Dry for 3 months at an average temperature of 18°C	Drying period can be reduced by the use of a chemical disinfectant
Dry heat	Pathogens on concrete, stone, iron, ceramic surfaces	Flame blower, blowlamp	
Damp heat	Pathogens in transportation vehicle tanks	Steam at 100°C or more for 5 min	
Ultra-violet rays UV-C (254 nm)	Viruses and bacteria	10 mJ/cm^2	Minimum lethal dose

Chemical disinfectants

Preparation of disinfectant solutions require the use of clear water to avoid the inactivation of the product by contaminants and organic matter.

Process	*Indication*	*Method of use*	*Comments*
Acetic acid	Infectious salmon anemia (ISA)	0.04-0.13%	
Quaternary ammonia	Virus, bacteria,hands, plastic surfaces	0.1-1 g/L for 1-15 min	IPN virus resistant
Calcium oxide	Fish pathogens on dried earth-base	0.5 kg/m^2 for 4 weeks	Replace in water and empty disinfected pools keeping the effluents at pH <8.5
Calcium hypochlorite	Bacteria and viruses on all clean surfaces and in water	30 mg available chlorine/L Leave to inactivate for several days or neutralize with Na thiosulfate after 3 h	Can be neutralized with sodium thiosulfate. See special recommendations
Calcium cyanamide	Spores on earthen bottoms	3,000 kg/ha on dry surfaces; leave in contact for 1 month	
Carbamate	Broad spectrum invertebrate pesticide	0.1 ppm or as directed, approximately 100 L per 10 ha pond @ 1m depth.	For use in pond sterilization due to disease.
Chloramine T	Destroys ISA	1% for 5 min	
	Destroys IPN	1% for 30 min	
Chlorine dioxide	ISA	100 ppm for 5 min	In water of low organic loading (commercially available as dutrion, twin oxide, norahco)
Formic acid	Ensile fish waste	pH <4 after at least 24 h	Destroys bacterial fish pathogens and ISA but not IPN
Formalin	Fish pathogens in sealed premises	Released from formogenic substances, generally trioxymethylene. Comply with instructions	Nodavirus resistant
Hydrogen peroxide	ISA virus	0.02-0.06%	
Iodine (iodophors)	Bacteria, viruses on nets, boots and clothing	200 mg iodine/L for a few seconds	See special recommendations
	Hands, smooth surfaces	>200 mg iodine/L a few seconds	
Ozone	Sterilization of freshwater, fish pathogens	0.2-1 mg/L for 3 min	Costly and very toxic for fish and humans
	Surfaces, equipment in seawater	0.5-1 mg/L TRO (total residual oxidant) for 30-60 min	
Poroxy compounds, e.g. Virkon	IPN virus	1% for 1 min	
Peracetic acid	ISA virus	0.08-0.25%	
Sodium	Fish pathogens on	Mixture:	The most active

hydroxide	resistant surfaces with cracks	▪ NaOH 100 g ▪ Teepol®, 10 g ▪ $Ca(OH)_2$, 500 g ▪ Water, 10 L Spray, 1 L/10 m^2 Leave for 48 h	disinfectant $Ca(OH)_2$ stains the surfaces treated Teepol® is a tensio-active agent
Sodium hypochlorite	Bacteria and viruses on all clean surfaces and in water	30 mg available chlorine/L Leave to inactivate for a few days or neutralize with Na thiosulfate after 3 h	
	Nets, boots and clothing	200 mg to 1 g available chlorine/L for several minutes Leave to inactivate for a few days or neutralize with Na thiosulfate after 3 h	
	Hands	Rinse with clean water or neutralize with thiosulfate	
Trichloro-phenol	Broad spectrum invertebrate pesticide	0.5 ppm or as directed, approximately 500 L per 10 ha pond & 1 m depth.	For use in pond sterilization due to disease

ANNEX 2 NEUTRALISATION OF HALOGENS

Chlorine and iodine are highly toxic for aquatic animals and, in order to prevent serious accidents that could result from a manipulation error, it is recommended to neutralize these products with sodium thiosulfate (5 moles of thiosulfate neutralize 4 moles of chlorine). The molecular proportions are the same for iodine.

Accordingly, in order to inactivate chlorine, the amount of thiosulfate should be 2.85 times the amount of chlorine (in grams):

- Number of grams of thiosulfate = 2.85 x number of grams of chlorine
- For iodine, the amount of thiosulfate should be 0.78 times the amount of iodine in grams:
- Number of grams of thiosulfate = 0.78 x number of grams of iodine.

It is also possible to prepare a thiosulfate solution at 1% by weight, in which case the neutralizing volumes will be as follows (in mL):

- For chlorine: 28.5 x [number of liters of the disinfecting solution x concentration mg/L] / 100
- For iodine: it is necessary to multiply by 7.8 instead of by 28.5.

ANNEX 3 DECAPSULATION OF *ARTEMIA* CYSTS

The hard shell that encysts the dormant *Artemia* spp. embryo can be completely removed by short-term exposure to a hypochlorite solution. This procedure is called decapsulation. Decapsulated cysts offer a number of advantages compared to the non-decapsulated ones:

- Cyst shells are not introduced into the culture tanks
- Nauplii that are hatched out of decapsulated cysts have a higher energy content and individual weight (30-55% depending on strain) than regular
- Decapsulation results in a disinfection of the cyst material
- Decapsulated cysts can be used as a direct energy-rich food source for fish and shrimp
- For decapsulated cysts, illumination requirements for hatching would be lower

Cyst rehydration

The cysts are first rehydrated (1 hour) either in fresh water or in sea water (35‰ NaCl).

The cists are hydrated in buckets containing about 15 L of seawater at 30°C. No air bubbles to stir the cysts during hydration.

After the hydration time, the cists are concentrated and rinsed with seawater in a mesh pouch of 100 µ, then spin out until they form a compact mass.

Decapsulation

The decapsulation of *Artemia* cists is done using sodium hypochlorite (NaOCl) and caustic soda (NaOH).

In general, the activity of the available chlorine solution is 10% and the caustic soda is in crystallized form.

The decapsulation solution is prepared by mixing the NaOCl and NaOH solution, the doses of each of the two solutions being calculated according to the number of cysts to be decapsulated.

These quantities are determined as follows:

- The weight of cysts to be decapsulated in gram x 0.0035 - volume of NaOCl in liter
- The weight of cysts to be decapsulated in gram x 0,25 = gram of NaOH crystals
- The weight of cysts to be decapsulated in gram x 0,007 = liter of seawater

The caustic soda crystals are set to dissolve in the chlorine solution. Hydrated, rinsed and wrung-out cists are placed in a bucket containing a volume of seawater calculated according to the amount to be decapsulated.

The decapsulation mixture is poured into the bucket. Decapsulation begins immediately. To properly homogenize the medium, the contents of the bucket are mixed with a PVC tube for the duration of the decapsulation (about 3 min). The cists gradually change from a brown to an orange color indicating the end of decapitating.

The decapsulation itself lasts 3 to 5 min. The reaction causes a sharp increase in temperature and water or ice should be added as soon as it exceeds 35°C.

Too long decapsulation over time can compromise success results, with chlorine gradually attacking the egg membrane and killing it.

Rinsing - neutralization

Once the decapsulation is complete, the eggs are quickly transferred into a 100 µ mesh pouch and immediately rinsed with seawater until the smell of chlorine is no longer noticeable (about 5 min).

Then, they can be neutralized using a 1% sodium thiosulfate solution (10 mL for 450 g of cysts) and rinsed back with seawater for 15 to 30 min. This last operation is not mandatory if the rinse is effective.

Storage of decapsulated cysts

Once the eggs have been decapsulated and rinsed, they can either hatch immediately or keep them cold or in brine.

Cold storage

At the end of these operations, the *Artemia* eggs are again vigorously wrung out on a 100 µ mesh, until they form a compact mass. They are then put in a refrigerator at 4°C for use within 24 h.

Storage in brine

In this case, they are stored in brine (330 g NaCl / L H_2O) at a rate of 100 grams of cysts per liter of brine with strong aeration.

After 4 h, when the eggs are dehydrated again, the brine is changed and the decapsulated cysts can be stored without aeration for several weeks. Before hatching, however, they will need to be rehydrated as noted above.

Use of decapsulated cysts

Incubation

For hatching, the decapsulated cysts are placed in 700 L bins at a maximum density of 2 g of cysts per liter of seawater at 28-30°C.

The volume of seawater in the tank is adjusted according to the number of cysts to hatch to work as close as possible to this density. The decapsulated cysts are

stirred by a strong aeration and subjected to a light intensity of at least 2,000 lux on the surface of the tank.

Hatching and harvest

At 30°C, 80 to 85% of cysts are hatched after 18 to 20 h. It is best to harvest the nauplii as soon as possible after hatching, even if this results in some losses in the production of Artemii. In practice, a first harvest is made after 18 h of incubation (or even 14 h) to harvest small Artemii for the Mysis stages. The hatching rate is then only 35-40%.

Artemii from the second harvest (24 h of incubation) are distributed preferably to PLs aged at least 4 to 5 days.

Since the cists have been decapsulated, the harvest of the nauplii can be done directly without the need to perform shell-nauplii separation. They are harvested by draining the basin into a bucket with openings on which 100 µ mesh have been glued. Before the tank is harvested, aeration is removed to allow sediment of dead nauplii, non-hatch cists, any remaining hulls, and a flush is carried out.

The nauplii is then transferred to a 100 µ mesh pouch, suspended in a tray, to be thoroughly rinsed with seawater. During rinsing, an air diffuser is installed inside the pouch. The foam created by the aeration is removed using a fine mesh net. Then the nauplii are stored in a tray of up to 300 L volume to be counted.

Once harvested, the Artemii must be distributed quickly because their nutritional value decreases very quickly after hatching. However, nauplii can, without problems, be kept alive at low temperature (3-5°C) for 24 h at density of 2-3,000/mL. The nauplii are again rinsed with seawater before being distributed.

Counting

The Artemii are stored in 20 L buckets. After homogenization, a 1 mL sample is taken from the bucket and diluted in 100 mL of seawater. The number of Artemii "x" is counted in 3 times 1 mL of this dilution:

$$\text{Number of Artemii} = 100 * 20 * (X_1 + X_2 + X_3)/ 3$$