CHAPTER 1.1.3.

METHODS FOR DISINFECTION OF AQUACULTURE ESTABLISHMENTS

1. General principles

Disinfection is employed as a common disease management tool in aquaculture establishments. It may be used as a routine practice in biosecurity programmes designed to exclude specific diseases, as well as a routine sanitary measure employed to reduce disease incidence within farms, or it may be used in disease eradication (stamping out) efforts. The specific reason for disinfection will determine the disinfection strategy used and how it is applied.

The general principles pertaining to disinfection of aquaculture establishments involve the application of chemical treatments in sufficient concentrations, and for sufficient periods, to kill all pathogenic organisms that would otherwise gain access to surrounding water systems. As the inherent toxicity of disinfectants prohibits safe use in open water, or open water systems, disinfection can only reasonably be applied to hatcheries and tank holding facilities. In addition, as some aquaculture establishments are generally seawater based, compounds produced during seawater disinfection (residual oxidants) must also be disposed of carefully.

This chapter gives a general overview of disinfection methods. It is important to remember that there are other alternative methods in use. Please also refer to the individual *Aquatic Manual* chapters for specific methods where they exist.

The choice of disinfection procedures depends on the size, type and nature of the materials and facilities to be disinfected, and on the products that are legally available in a country. Surfaces to be disinfected may include fabric or woven material (clothes, nets), hard surfaces (plastic, concrete) or permeable materials (earth, gravel). Disinfection is more difficult on permeable surfaces and requires more time.

As the presence of organic matter will reduce the disinfection capacity of most disinfectants, filtering influent water is recommended. In addition, all surfaces must be thoroughly cleaned prior to disinfection. The detergent used must be compatible with the disinfectant and both must be compatible with the surface being treated (e.g. iodophor solutions are generally acidic so cannot be used on concrete, which is alkaline). Ensure that the waste produced from washing is disinfected before disposal. Complete coverage of the surfaces is required (e.g. using a high pressure spray or soak).

Disinfection procedures must be established and used according to the objectives of disinfection and any identified risks. Diseased aquatic animals, animal fluids and tissues (e.g. viscera, blood, mucus, faeces), and their contact with equipment and workers present a risk of transmission of pathogens that could eventually infect healthy aquatic animal populations.

Basic disinfection protocols include the removal of all aquatic animals (both dead and alive) from the facility, a cleaning programme that is designed to eliminate all the remaining organic matter adhering to the surfaces, the use of disinfectants on equipment and installations and a final neutralisation step using chemical products.

When removing aquatic animals from the facilities prior to disinfection, the direct disposal of diseased populations of aquatic animals of any life stage or age into receiving waters is a hazardous practice that facilitates the spread of disease from farmed to wild populations or to neighbouring farms using the same water supply. Such disposal should not be permitted. When the decision is made to discard a population due to the presence of disease, the stock should be harvested or humanely killed. In the case of land-based aquaculture establishments, the tank, raceway, or pond, etc., should be disinfected prior to discharge and again prior to restocking.

The washing and disinfection procedures should at least include the following stages:

a) Removal of solid waste, etc., followed by prewashing,
b) Deep cleaning and washing,
c) Disinfection,
d) Rinsing.

The process should be monitored throughout by a technically competent person and records need to be kept.

It is essential to provide protection from contact with hazardous substances by wearing protective clothing, face masks, eye protection, etc., as appropriate.

The disinfectants must be stored in a way that presents no direct or indirect danger to animal or human health and the environment.

2. Occurrence of listed diseases

When an OIE listed disease, or an important but unlisted emerging disease, occurs for the first time at a particular farm, at a particular site (i.e. at a quarantine facility), or within a region or country previously believed to be free of that disease, it may be advisable, if not required, to eradicate the disease by depopulating the facility and performing a thorough disinfection of all or part of the facility. Fallowing of the affected facility for a defined period of time may be warranted in some situations (see Chapter 4.4. Fallowing in aquaculture in the *Aquatic Code*).

3. Prevention of disease spread to wild populations

The direct disposal of diseased populations of an aquatic animal (any life stage; i.e. fertilised or unfertilised eggs, larvae, postlarvae, juveniles, or adults) or waste products derived from them (i.e. processing plant wastes such as blood, viscera, shells, broken shrimp pieces, etc.) into receiving waters (i.e. creeks, rivers, estuaries, bays, littoral areas) is a dangerous practice that facilitates the spread of disease from farmed populations to wild aquatic animal stocks or to neighbouring farms that use the same water supply, and it should not be permitted. With cultured stocks, when the decision is made to discard a population (i.e. that is being cultivated in a hatchery tank or grow out pond) due to the presence of disease (or poor culture performance, which may be due to an undiagnosed disease), the stock in the tank or pond should be harvested and/or humanely killed in the tank or pond. The water in the tank or pond should be disinfected prior to discharge. The emptied tank or pond should be disinfected prior to restocking.

4. Routine sanitation and biosecurity

Many aquaculture establishments, especially those cultivating early stages of aquatic animals, employ measures that use a number of disinfection methods for disease prevention and control. These measures may be part of a farm’s routine biosecurity programme that may be designed for exclusion of specific diseases as well as serving as general pest and disease exclusion measures.

4.1. Disinfection of eggs and larvae

4.1.1. Guidelines for disinfection of fish eggs

Disinfection of eggs with iodine can be carried out for the various fish species, but it is most commonly used for eggs of fish of the Salmonidae family (salmon, trout and char). Although generally effective for decontamination of surfaces of eyed and newly fertilised eggs, the use of disinfectants, such as iodophors, should not be relied upon to prevent vertical transmission of some bacterial pathogens (e.g. *Renibacterium salmoninarum*) and viral pathogens (e.g. infectious pancreatic necrosis virus) that may be present within the eyed and newly fertilised egg.

4.1.2. Eyed eggs of salmonid fish

There are a number of protocols regularly used to disinfect eyed eggs. In general, the pH of the solutions of the iodophor product must be between 6 and 8. At a pH of 6 or less, the toxicity for eyed and newly fertilised eggs increases, and at 8 or more, the disinfection efficacy decreases. It is therefore essential to control the pH, and 100 mg litre^{-1} $NaHCO_3$ must be added to the water. It is recommended that the eggs be rinsed in clean fresh water, or 0.9% saline, before and after disinfection and that an iodophor solution giving 100 ppm (parts per million) free iodine in 0.9% saline free of organic matter be used as the disinfectant solution. The contact time for a 100 ppm iodophor solution should not be less than 10 minutes and the solution should be used only once.

4.1.3. Newly fertilised salmonid eggs via a water-hardening process

For disinfecting newly fertilised salmonid eggs via a water-hardening process with iodophors, the active iodine concentration should be 100 ppm. One such procedure is as follows:

- Eggs should be stripped and separated from ovarian fluid, rinsed in 0.9% saline (30–60 seconds), sperm added and fertilisation allowed to proceed for 5–15 minutes,
- The eggs should then be rinsed in 0.9% saline (30–60 seconds) to remove excess sperm and other organic materials,
- The eggs should then be rinsed in a 100 ppm iodophor solution for 1 minute. Then the solution should be discarded and replaced with a fresh 100 ppm solution and the eggs disinfected for a

further 30 minutes. This solution, and the rinsing solutions, should be used only once. The ratio of eggs to iodophor solution should be a minimum of 1:4,

- The eggs should be rinsed again in fresh or sterile hatchery water for 30–60 seconds,
- Water-hardening should be finished using clean water.

It is important that eggs are not fertilised in the presence of the iodophor solution as this will kill sperm cells.

4.1.4. Eggs of other fish species

For other species, preliminary tests should be conducted to determine at what egg stage and iodophor concentration, disinfection can be carried out safely. Disinfection of eggs of marine species, such as plaice, cod and Atlantic halibut, for which some adverse effects of iodophors have been documented, may be achieved with 400–600 mg $litre^{-1}$ glutaraldehyde and a contact time of 5–10 minutes. However, this is not effective against nodaviruses for which the use of ozone at 1 mg O_3 $litre^{-1}$ for 30 seconds is recommended. An ozone concentration of 0.1–0.2 mg O_3 $litre^{-1}$ for 3 minutes inactivates most pathogenic fish bacteria as well.

4.1.5. Efficacy limits

Disinfection of eggs with iodophor may not always be effective in preventing vertical transmission of infectious pancreatic necrosis virus, *Renibacterium salmoninarum* and infectious haematopoietic necrosis virus, for which this method was developed initially. The ineffectiveness of iodophor disinfection in some instances has been proven by epidemiological studies and laboratory tests.

4.1.6. Mollusc eggs and larvae

Disinfection of eggs and larval stages is not considered practical for most molluscan culture systems. In addition, there is little information on specific disinfection procedures for pathogens of molluscs (i.e. *Marteilia* spp., *Haplosporidium* spp., *Bonamia* spp., *Perkinsus* spp., iridovirus and pathogenic levels of marine microbes) or seawater. Therefore, disinfectants and concentrations are based on related pathogens or seawater sterilisation. Three stages of disinfection can be applied to hatcheries:

a) pretreatment of influent water, e.g. filters (1.0 and 0.22 µm) or chemical disinfection for protection of mollusc stocks;

b) treatment within the facilities (especially recycling systems) = protection of mollusc stocks;

c) treatment of effluent water = protection of the environment.

4.1.7. Disinfection of eggs and larvae in penaeid shrimp hatcheries

Certain penaeid shrimp viral diseases (i.e. spherical baculovirosis, tetrahedral baculovirosis, and hepatopancreatic parvovirus infections) are transmitted by faecal contamination of spawned eggs. These diseases, as well as infections due to certain other shrimp viruses such as white spot disease virus, and certain bacterial and fungal disease agents, can be eliminated or have their incidence reduced through the routine use of disinfection protocols when used to surface disinfect eggs and/or recently hatched nauplii. A widely used method is given below:

For fertilised eggs[1]

Collect fertilised eggs. Rinse with running seawater for 1–2 minutes. Fully immerse eggs in 100 ppm formalin for 1 minute. Fully immerse eggs in iodophor (0.1 ppm iodine) for 1 minute. Rinse in running seawater 3–5 minutes. Transfer to disinfected larval rearing tanks.

For nauplii[2]

Using phototaxic response to light, collect nauplii with netting or screen. Rinse with running seawater for 1–2 minutes. Fully immerse nauplii in 400 ppm formalin for 30–60 seconds. Fully immerse nauplii in iodophor (0.1 ppm iodine) for 1 minute. Rinse in running seawater 3–5 minutes. Transfer to disinfected larval rearing tanks.

1 Fertilised eggs are more sensitive than nauplii to formalin.
2 Nauplii are much easier to collect than fertilised eggs in hatcheries.

4.1.8. Precautions

Certain precautions have to be taken prior to the use of iodophors, since the products on the market contain a variable quantity of detergents that can give rise to toxic effects. It is therefore recommended that preliminary tests be carried out among the products on the market. It is advisable to build up stocks of the most satisfactory product but the expiry dates should be observed. It is also important that any chemicals used for disinfection of eggs are used in compliance with the relevant health and safety and environmental protection regulations of the country that they are used in. Finally, in the case of eggs that have been transported, the packaging should also be disinfected or, better still, destroyed in a manner that will not pose a risk of water contamination and/or a health risk to other fish at the end destination.

4.2. Materials and equipment

All the equipment used for feeding, cleaning, and for removal of dead aquatic animals should be unique to each culture unit.

The food management containers in the different units should be different from the ones used for mortality collection and should be carefully washed and disinfected, following the established protocols, as required, with the products authorised.

The machinery used in an aquaculture establishment must never be transported to another aquaculture establishment, unless the latter does not contain aquatic animals. Only in exceptional cases is machinery allowed to be transported, and only after it has been washed and disinfected with authorised products.

The remaining machinery used should be washed and disinfected with authorised products before entry to and departure from the farm.

In the case of aquaculture establishments located in the sea, nets should never be changed from one aquaculture establishment to another, even if they have been washed and disinfected.

In the case of modules, floating store rooms, and automatic feeding systems, they should all be washed and disinfected before changing the location of the units. In freshwater facilities, these should never be moved between aquaculture establishments.

4.3. Nets

All nets need to be washed after being taken out of the water. Nets that have not been washed after being submerged in a body of water should not be re-immersed into another body of water. Once the net has been removed from the water, it should be transferred as soon as possible to the net washing site, thus avoiding these nets contaminating other nets, equipment and working areas. The transfer of nets from and towards the washing stations should be carried out using the appropriate watertight compartments or packaging, thus preventing any cross-contamination.

Farms requiring washing services and eventual impregnation of the nets with anti-scaling products ought to have this operation carried out by authorised net-pen cleaning services, when available.

The washing process should guarantee the elimination of all the elements adhering to the nets, such as eggs and/or larvae of parasites, molluscs, echinoderms, algae, organic matter, etc.

4.4. Vehicles

Regarding vehicles, the aquaculture establishment's manager (or whoever he or she designates) should ensure that the following requirements are met:

- only vehicles that serve the aquaculture establishment should be given access to the production areas or transit zones, and they can enter these areas for a determined purpose only; temporary service vehicles, visiting vehicles or workers' vehicles should not have access to these areas,

- all vehicles that enter the production areas or transit zones, without exception, must be disinfected when entering and leaving the aquaculture establishment, using products authorised for that purpose,

- the manager of the aquaculture establishment (or whoever he or she designates) should request a "disinfection certificate" or similar, before a transport vehicle is given access to the production units. The certificate should confirm that the vehicle's tanks into which the aquatic animals will be loaded have been disinfected,

- all people entering with a vehicle must follow the disinfection protocols established for the aquaculture establishment,

- vehicles and containers that serve more than one centre must be disinfected after each centre visited, and the "disinfection certificate" or similar that accredits the procedure should be requested periodically,

- a register of all the vehicles that enter the farm facilities or transit zones should be kept. The register ought to contain at least the date, hour, company, reason for the visit, name of the driver and last centre visited,

- vehicles for different services should not enter the farm facilities at the same time, especially vehicles used for the collection of dead aquatic animals ("mortality trucks").

The following should be considered when selecting products and procedures to be followed:

- the effectiveness of detergents and disinfectants in dealing with the different types of materials and surfaces,

- the location inside the farm of the areas or items to be disinfected,

- the manufacturer's recommendation regarding the detergent and/or disinfectant, and other variables that may be relevant to the type and level of contamination that the surfaces may have.

4.5. Staff

Before entering a production area, all staff should put on their protective clothing (e.g. overall, coat, gloves, boots, apron); then both boots and hands should be disinfected. It should be strictly forbidden to enter the area without the authorised protective clothing.

No person should exit the facility wearing a working outfit, even if they have transited through the sanitary barrier and disinfected their shoes and hands.

4.6. Pipelines and tanks

Routine disinfection of pipelines and tanks is highly recommended; the frequency of disinfection will vary according to the turnover of the aquatic animal stocks. High concentrations of aquatic animals should be rotated between disinfected tanks as often as practical and/or kept in water that has been disinfected with ozone or chlorine and subsequently neutralised. Each new batch of aquatic animals introduced into a facility should be placed in pre-disinfected holding units.

For routine sanitation, hatchery and broodstock tanks (i.e. tanks for broodstock maturation, mating, spawning, larval rearing and indoor nurseries) should be cleaned, disinfected and dried between uses. Tanks used for the above-named purposes are typically precast fibreglass tanks or they are constructed of concrete or wood and either coated or painted with resin-based coatings (e.g. epoxy or fibreglass resin) or lined with plastic liners manufactured for this purpose. After harvest of the stock from the tank, all loose objects and large-sized organic debris such as algae, faeces and left-over feed should be removed. With relatively small tanks, it is advisable after harvest of the stock to fill the tank to capacity, immerse all non-porous corrosion resistant equipment (i.e. airlines, air stones, stand pipes, screens, sampling containers, etc.) in the tank (see also Section 4.11.iii), and then add calcium hypochlorite to provide a minimum of 200 ppm free chlorine. This should be allowed to stand overnight. After the proper chlorinated soak-time, the tank can be drained and freshwater rinsed. Before draining the system, the treated water should be dechlorinated, unless appropriate effluent collection and treatment systems are in place. After the tank has been rinsed it should be allowed to dry completely. In the case of large tanks, an initial cleaning to remove loose debris should be followed by disinfection with a concentrated (~1600 ppm chlorine) solution of calcium hypochlorite. All inside and outside surfaces should then be sprayed with this chlorine solution. The tank should then be allowed to stand for several hours before being rinsed, filled and flushed. Surfaces should then be scrubbed free of all remaining material. After disinfection with chlorine, small or large tanks should be rinsed with clean water, then filled and flushed to ensure that no chlorine residues remain before the tank is restocked.

Alternatively, disinfection of re-circulating aquaculture facilities, concrete raceway systems and all non-porous tanks can be achieved by the use of a solution of sodium hydroxide and biodegradable detergent (e.g.

Teepol®[3]). All tank surfaces should be thoroughly pressure washed or steam cleaned to remove organic materials, the tank surfaces should then be sprayed with a 1% solution of sodium hydroxide containing 0.1% Teepol, at an application rate of 2.5 litres per square metre of tank surface. The wet tanks surface, following drainage of the solution, should remain at a pH above 12. Any residual water in the base of the tanks should be treated to raise the pH above 12 for a period of 24 hours. After the tanks surfaces have dried they can be rinsed and the tanks re-filled. Disinfection of pipe work and filter tanks in re-circulating aquaculture facilities can be achieved with the same disinfectant solution. Typically, following drainage and cleaning of the system, a reservoir tank, containing sufficient solution to fill all pipe work in the system is filled with the solution, which is then pumped around the system for 24 hours. The pH of the solution should remain above 12 at the end of this period. The reservoir solution can be used to disinfect all, thoroughly washed, filter materials, screens and ancillary equipment such as nets, bins and small tanks. Again these should be immersed in the solution and left for 24 hours. Following disinfection all tanks and equipment should be thoroughly rinsed and the recirculation system thoroughly flushed before being re-filled. Where necessary, neutralisation of a large volume of the disinfectant solution prior to disposal can be achieved using hydrochloric acid. Any persons applying, or otherwise likely to come into contact with, the disinfectant solution should wear appropriate eye and skin protection.

Regular air- or heat-drying of pipelines (daily), tanks and other equipment (e.g. algal culture carboys), in addition to disinfection of their surfaces, is also recommended (especially for disease outbreaks of unidentified aetiology).

a) Chlorine is usually applied as sodium hypochlorite (such as HTH®, Chlorox®, household bleach, etc.). Fill all pipelines with 50 mg chlorine litre^{-1} (= 50 ppm). Allow an exposure time of at least 30 minutes before flushing with clean water. This solution is effective against most microbial agents. Chlorinated water must be neutralised prior to release from the holding facility. Optimal neutralisation is achieved by passage through activated charcoal (this removes excess chlorine and chloramines). Reducing agents such as sodium thiosulfate (see Section 5.2.1 for example use) or aeration, which do not remove toxic chloramines, may also be used.

b) Iodophors are generally applied as alkaline solutions (such as Wescodyne®, Betadine®) at 200–250 mg iodine litre^{-1} (ppm) with a contact time of at least 10 minutes.

    NOTE: Iodophors are not effective against certain protozoans in suspension, e.g. over 1000 mg iodine litre^{-1} is tolerated by *Labyrinthuloides haliotidis* of abalone. Iodophors may be effective against protozoan parasites following air or heat drying of tank surfaces and pipelines.

4.7. Disinfection of source water

Since several of the listed diseases of aquatic animals in the *Aquatic Code*, as well as a number of other important diseases, can be introduced into farms with source water when it contains vectors or carriers (i.e. wild infected crab or shrimp larvae, wild fish, etc.), some farms operate biosecurity plans that include provisions for the disinfection of source water. This may be accomplished by a variety of means that may include one or some combination of the following procedures:

a) For filtration of source water – source water is pumped into a supply/settling canal where it first passes through coarse bar screens to remove larger aquatic animals and debris. Then, the water is passed through a series of progressively finer screens, and final filtration is accomplished by passing source water through a fine mesh (150–250 µm mesh size) bag screen before being introduced into a culture pond or storage reservoir.

b) Instead of using mesh nets, some farms place filtration structures in the supply channel. A series of compartments within these structures are filled with filter matrixes, beginning with coarse gravel for initial removal of larger aquatic animals and debris, an intermediate section that contains a finer matrix of sand and gravel, and the end section that contains fine sand.

c) For chlorination and de-chlorination, source water is pumped to a supply channel or directly into culture ponds or reservoirs (with or without filtration) and treated with sufficient chlorine to kill any potential vectors or carriers in the source water.

d) 'Zero' or reduced water exchange: Some farms use supplemental aeration and re-circulation of water in culture ponds and within the supply and discharge systems of a shrimp farm to reduce source water

3 Reference to specific commercial products as examples does not imply their endorsement by the OIE. This applies to all commercial products referred to in this *Aquatic Manual*.

requirements. This reduces the volume of source water that needs to be disinfected before use, as well as reducing nutrient loss from farms with effluent.

4.8. Disinfection of effluent water

a) Ozone has been used successfully in controlling the microbial content of effluent water from quarantine facilities. Residual compounds, formed as a result of the interaction of ozone with seawater (residual oxidants), at levels of 0.08–1.0 mg litre^{-1} are considered sufficient to significantly reduce live microbes (principally bacteria).

NOTE: The measurement of residual ozone in seawater is problematic due to the rapid and continuous formation of oxidant products in seawater. Residuals formed between ozone and seawater (hypobromite, bromine or hypobromous acid) are toxic to early stages of aquatic animals) and should be removed using a charcoal filter before passing through/out of a mollusc facility. UV treatment of seawater post-ozonation may be required for complete sterilisation, e.g. for quarantine.

b) Chlorine administered as sodium hypochlorite at a concentration of 25 mg chlorine litre^{-1} is effective against certain protozoans (*L. haliotidis*). However, 50 mg chlorine litre^{-1} is recommended for complete microbial sterilisation, although higher concentrations may be used under certain conditions (e.g. quarantine). Nevertheless, these require proportionately greater neutralisation treatments and exhaust systems to deal with the toxic fumes produced.

c) Iodophors are not as effective as the above two treatments for killing protozoans.

4.9. Disinfection of large ponds or lakes

Disinfection of large earth-bottomed ponds or small lakes (e.g. carp rearing facilities or fisheries) for pathogen eradication (e.g. SVCV) can be problematic due to their size and the volume of freshwater they contain. Nevertheless, subject to safe adequate drainage of such facilities, earthen bottoms can be treated with lime (see Section 5.1.2 for example method and dosage).

4.10. Buildings

The disinfection regime used should be building-specific and dependent upon the use pattern of that particular building.

i) *Office buildings:* these buildings would most often be subject only to foot traffic from people who have been in contaminated buildings or culture areas. Therefore, the greatest focus of attention should be the floors and cold storage units in the building. Floors should be thoroughly cleaned (if they are non-porous) with standard detergents and cleaning solutions, followed by thorough drying. If the floors are carpeted, they should be vacuumed and cleaned with a detergent appropriate for carpets, or ‘steam’ cleaned. All other areas within these buildings, such as walls, bathrooms, desks, refrigerators, freezers, etc. should be examined for potentially contaminated materials (i.e. frozen shrimp in freezers) and any such item found, and its container, should be cleaned and disinfected or disposed of in a sanitary manner.

ii) *Culture buildings:* it must be assumed that these buildings have had direct contact with the disease agents and they will therefore be handled in a different manner from that of the office buildings. The disinfection regime for these buildings will consist of two steps. First, the building should be thoroughly swept and/or vacuumed (where appropriate) to remove as much large-sized organic and inorganic debris as possible. This should be followed by the second step, washing with detergent solution, and finally the third step, treatment with chlorine. Chlorine solution (~1600 ppm) should be applied (by spraying) to all surfaces that are not prone to the corrosive actions of chlorine. Surfaces that should not be chlorinated can be sponged first with an iodophor solution minimally providing 200 ppm of free iodine. These can then be covered with plastic or any other protective material. Floor surfaces can be soak-chlorinated to a depth of 5 cm with a 200 ppm chlorine solution. This should be allowed to stand for a minimum of 48 hours. If any of the sprayed surfaces are susceptible to corrosion by chlorine, those surfaces can be freshwater-rinsed after the 48-hour treatment.

In buildings where disinfection with chlorine is not practical, fumigation with formaldehyde gas should be considered. NOTE: this is a potentially hazardous method and appropriate safety procedures and regulations must be observed. After a general cleaning, fumigation of a sealable building can be initiated. The entire process, from the time the building is first gassed until it can be occupied again, should take a minimum of 36–60 hours. The entire building has to be totally sealed off during the actual fumigation, and there should be no means by which the gas can escape once it is placed in the building. If possible, the electrical service for the building should be turned off. The required environment for formaldehyde gas disinfection is a minimum temperature of 18ºC with a high relative humidity (at saturation is best, i.e. floors should be wet, etc.). Generation of formaldehyde gas is accomplished by the addition of 17.5 g potassium permanganate to each 35 ml of 100% formalin (a 37–39% aqueous solution of formaldehyde gas) for each 2.83 m^3 (100 ft^3) of space. Ideally, each room in the structure should have its own source of formaldehyde gas to assure that all areas of the building are uniformly treated. The correct amount of each compound (potassium permanganate and formalin) should be weighed out into separate containers, and the formalin should be placed in a non-plastic container that is at least 10 times the combined volume of both the formalin and the potassium permanganate. (The person applying formaldehyde gas fumigation should wear waterproof outer ware to protect their skin, an approved formaldehyde gas mask, and goggles or a face shield for eye protection). The containers with the correct amounts of the two reagents should then be placed on the floor in the centre of the room on a large disposable protective (plastic) mat. The formalin and potassium permanganate should not be mixed at this time. Once all rooms have the correct amounts of the two compounds, the building has been completely sealed and the environment modified as necessary, the actual fumigation can begin. The mixing of the two compounds must be done very rapidly and carefully as the reaction is immediate and somewhat violent as formaldehyde gas is emitted. Starting with the room farthest from the exterior door, add the permanganate to the formalin and proceed to the next room. After all rooms have been completed, lock the exterior door and seal it from the outside with tape. The building should be kept sealed for a minimum of 12 hours. After this disinfection period, the building should be flushed with clean air for 24–48 hours. There should be no detectable odour of formaldehyde before people are allowed to reoccupy the building.

An alternate method for the generation of formaldehyde gas is the sublimation of powdered paraformaldehyde. For each 2.83 m^3 (100 ft^3) of space, approximately 28 g paraformaldehyde should be used. It can be sublimated by being placed in an electric fry pan, which has been set on high. This procedure is somewhat more dangerous, because formaldehyde is flammable and a spark from such a heating device could theoretically ignite the gas. The same procedures noted above for the formalin/permanganate mixture in regards to venting, etc. should also be followed for the use of paraformaldehyde.

iii) *Processing buildings:* these buildings are typically constructed to permit routine disinfection. For the most part, the procedures followed in the routine operation of such buildings are appropriate for a total clean up (TCU), provided that the building, its cold rooms, and its freezers are also disinfected and thoroughly dried. If considered necessary, fumigation with formaldehyde gas may be undertaken to ensure destruction of the disease agent(s) of concern.

iv) *Other buildings:* buildings such as feed stores, maintenance sheds, tool rooms, etc., should be treated as for an office building. Care should be taken to remove all the large-sized debris, which would normally be found in relative abundance within these types of buildings. Potentially contaminated surfaces within such buildings should next be spray-chlorinated and allowed to stand for 24–48 hours. This should be followed by a freshwater rinse. All equipment, which should not be exposed to the corrosive action of chlorine, should be removed before spraying, and should be disinfected by surface disinfection with 200 ppm iodophor. Once the equipment has been disinfected, it can be brought back into the building. Fumigation with formaldehyde gas is another option for this type of building.

4.11. Clothing and equipment

Clean surfaces with detergent and disinfectants prior to proper disinfection.

a) Iodophors (such as Wescodyne®, Betadine®) at 200–250 mg iodine $litre^{-1}$ can be used as a footbath. NOTE: Iodophors will stain clothing.

b) Chlorine (household bleach solution at 50 mg chlorine $litre^{-1}$) is also an effective footbath or equipment wash.

c) Sodium hydroxide (1% NaOH + 0.1% Teepol or other detergent) makes an effective footbath for rubber boots. NOTE: Do NOT use for dress shoes/boots. It can also be used for disinfection of hatchery buildings, re-circulating aquaculture facilities, concrete raceway systems and all non-porous tanks. All tank surfaces should be thoroughly pressure washed or steam cleaned to remove organic materials, the tank surfaces should then be sprayed with a 1% solution of sodium hydroxide containing 0.1% Teepol, at an application rate of 2.5 litres per square metre of tank surface. The wet tanks surface, following drainage of the solution, should remain at a pH above 12. Any residual water in the base of the tanks should be treated to raise the pH above 12 for a period of 24 hours. After the tanks surfaces have dried they can be rinsed and the tanks re-filled. Disinfection of pipe work and filter tanks in re-circulating aquaculture facilities can be achieved with the same disinfectant solution. Typically, following drainage and cleaning of the system, a reservoir tank, containing sufficient solution to fill all pipe work in the system is filled with the solution, which is then pumped around the system for 24 hours. The pH of the solution should remain above 12 at the end of this period. The reservoir solution can be used to disinfect all, thoroughly washed, filter materials, screens and ancillary equipment such as nets, bins and small tanks. Again these should be immersed in the solution and left for 24 hours. Following disinfection all tanks and equipment should be thoroughly rinsed and the recirculation system thoroughly flushed before being re-filled. Where necessary, neutralisation of a large volume of the disinfectant solution prior to disposal can be achieved using hydrochloric acid. Any persons applying, or otherwise likely to come into contact with, the disinfectant solution should wear appropriate eye and skin protection.

4.12. Crustacean culture support equipment and systems

These are operational units of a shrimp culture facility, which may be housed in a building.

i) Artemia *systems:* all *Artemia* decapsulation and cyst hatching units and tanks should be treated in the same manner as other tanks. They should be cleaned of all large debris, filled to the top with clean water and then have calcium hypochlorite added to achieve a final concentration of 200 ppm (free Cl_2). Chlorination should be allowed to continue for 24–48 hours. The outside of such tanks may be spray-chlorinated (1600 ppm chlorine). Treated tanks can then be dechlorinated with sodium thiosulphate, drained, freshwater rinsed, and allowed to dry for a minimum of one week. Unopened containers of *Artemia* cysts at the facility can be retained. These should, however, be surface disinfected with chlorine (200 ppm) or iodophor (200 ppm).

ii) *Algae systems:* containers, tanks, incubators and rooms used to produce algae for feeding the larval stages of shrimp may be handled and disinfected in nearly the same way as other tanks systems. The only major difference being that special care must be taken to ensure that all chlorine residues have been rinsed from the units before they are used again. In the case of the culture tubes, flasks, carboys, and flasks used to culture algae, a combination of an acid (10% HCl) rinse or steam sterilisation can be used in lieu of disinfection with chlorine or iodophor.

Disinfection of stock cultures of living algae is not possible. The use of disinfection is clearly out of the question, since any compound that would kill the disease agent would likewise kill the algae. Hence, there are two basic methods of minimising the chance of a disease agent being present in the stock cultures:

- Dilution: all stock cultures can be cloned from the existing stocks. Each culture should be diluted either by means of serial dilutions (for broth cultures) or streaked for single colonies (agar cultures). All dilutions should be performed using strict aseptic techniques with all media being properly autoclaved. Passages from the stock cultures should not occur until the algae culture room has itself been disinfected as per the above building procedures. Once a culture has been diluted and cloned by either of these methods, to the point where there remains only one cell of the original culture, the risk is negligible that a (shrimp) disease agent may be present.

- New stock cultures: if existing stock cultures are discarded in a TCU, new stocks should be purchased from algae supply laboratories, or obtained from other sources where contamination with (shrimp) disease agents is unlikely, such as isolating desired species from wild populations of algae. New stock cultures should not be obtained from any facility that also cultures shrimp in order to avoid contamination with (shrimp) disease agents of concern.

iii) *Farm equipment:* nets, seines, porous air-line tubing, etc., which are relatively inexpensive and easily obtainable, should be discarded and removed from the facility during a TCU rather than being disinfected, as they are not readily disinfected and chlorine is likely to damage them and shorten their useful life.

Non-expendable equipment such as large sized flexible plastic tubing, pumps and pipes, transfer tanks, cages, harvest cages, harvest tables, Secchi disks, laboratory glassware, etc. should be soak-chlorinated in 200 ppm solutions for 24–48 hours. This is most easily accomplished by placing these objects in the tanks that are filled with 200 ppm solutions of chlorine. Care should be taken to have all items completely submerged (use heavy items to weigh-down more buoyant objects). A good guide is to place everything (except items that are to be thrown away) that is loose, or can be unsecured from its point of attachment, into the 200 ppm chlorine solution in their respective tanks.

In the case of similar type items that are associated with ponds, these should be placed in a special series of tanks set up near their respective ponds. These tanks should be filled with 200 ppm chlorine solutions. Following soak-chlorination, these items should be allowed to dry and be exposed to natural UV (sunlight) sterilisation. They should be turned at least once to expose all areas of the items to direct sunlight.

Tools and machinery, such as tractors, trucks, portable and stationary power tools, etc., should be thoroughly cleaned with standard cleaning solutions. All traces of mud, shrimp feed, etc. should be removed from these items. Following this, disinfection of surfaces likely to have been contaminated in normal use should be rinsed with an iodophor solution (at a concentration of 200 ppm) or cleaned with steam.

Small tools and instruments, such as scales and balances, test instruments, small power tools, etc., should be gently wiped with 200 ppm chlorine solution if they are inert plastic or 200 ppm iodophor if they are otherwise. These should then be placed back in their respective buildings during the formaldehyde fumigation. High precision electronic test equipment should not be subjected to fumigation, especially if there has been little chance that it was ever contaminated.

iv) *'New-Water' plumbing:* all new-water plumbing that is contained within buildings, especially those that have blind ends or terminate in manifolds, should be filled with a minimum of 200 ppm chlorine solution. The chlorine solution should be held in the lines for 24–48 hours minimum, followed by clean water rinsing. Pipes may also be disinfected by recirculating hot water (>60ºC) through them for several hours.

v) *Uniforms, boots, etc.:* all items worn or used by employees should be either disposed of or thoroughly washed and disinfected. In the case of clothing, such as coveralls, normal washing, which incorporates chlorine bleach, is sufficient, especially if accompanied by sun drying. Other items, such as boots, gloves and other non-cloth items can be safely soak-chlorinated in a 200 ppm chlorine solution. This should be followed by a freshwater rinse. These items should also be contained within their respective buildings during formaldehyde fumigation.

vi) *Feed items:* all feed items, such as prepared feeds, fresh feeds (i.e. squid, bloodworms, frozen Artemia, bivalve molluscs, etc.) should be removed from the facility and replaced with new feeds from sources known to be free of contamination by shrimp disease-causing agents.

5. Additional requirements for crustacean hatcheries and broodstock rearing/holding facilities

Virtually all penaeid shrimp hatcheries and broodstock holding/rearing facilities use seawater that has been disinfected to remove potential pathogens, pests, and disease-carrying agents via mechanical filtration, UV irradiation, and/or chemical disinfection. This may be by passive source water filtration (i.e. by the use of seawater wells or well points) or by mechanical filtration using high pressure pumps and a variety of water filtration devices and pore sizes. Some facilities use filtration coupled with UV light disinfection of source water, while others use chemical disinfection methods, using either chlorination and de-chlorination or high doses of ozone and subsequent removal of residual oxidants. Chemical disinfection of source water typically requires the use of one or more water storage reservoirs in which the water is treated and detoxified before use in the shrimp hatchery or broodstock facility. Numerous manuals are available that provide specific details on hatchery and broodstock facility design and operation for shrimp culture, as well as source water disinfection.

5.1. Disinfection of grow out ponds

Following the routine harvest of a crop from a grow out pond (or from a large tank or raceway used for grow out of a crop), the pond (tank) bottom should be inspected. Large deposits of organic debris should be treated or removed. This is easily accomplished in lined tanks, raceways, or ponds (i.e. by flushing with a high pressure hose), but poses more of a challenge in large earth bottom ponds. However, many methods of pond bottom disinfection and treatment between crops are practiced. These methods are given in detail in a number of shrimp farming manuals, and some will be listed here only with minimal details:

5.1.1. Chlorination

Chlorination may be used for routine treatment of ponds between crops or when disease eradication is the goal. After draining the pond, remove (and dispose of as many animals from the system as possible (this may be difficult in pond systems where the removal of large numbers of dead shrimp would not be practical). Partially refill the pond (or fill to capacity if required), discontinue the addition of new water, stop the discharge of effluent water, and remove any internal or external sources of aeration or aeration devices, which might be subject to corrosion. Then, evenly distribute sufficient granulated calcium hypochlorite (such as Olin HTH™) to provide a minimum residual free chlorine concentration of 10 ppm within the entire system's water. (NOTE: The person(s) applying the chlorine should wear waterproof outer ware to protect their skin, an approved chlorine mask, and goggles or a face shield for eye protection). Redistribute additional calcium hypochlorite as often as required to maintain the residual concentration at or near 10 ppm. Allow the system to stand for a minimum of 24–48 hours (especially if applied to large systems) at this minimal chlorine concentration. The chlorine will kill all shrimp and most, if not all, of the other organisms occupying the water column or residing in the pond. After the pond has been treated with chlorine for the required minimum time and before any water is discharged, neutralise the chlorine either passively by exposure to sunlight and air for approximately an additional 48 hours (without the addition of new chlorine) or by the addition of sodium thiosulphate at a rate of five (5) molecules of sodium thiosulphate for each four (4) molecules of chlorine (or the weight of sodium thiosulphate being 2.85 times the weight of chlorine in the water, see example table below).

Pond size	Average depth	Volume	Chlorine dose	Chlorine required	HTH (65% active Cl)	Thiosulphate required
1 hectare	1 m	10,000 m^3	10 ppm	100 kg	154 kg	285 kg

Periodic testing should be carried out for residual chlorine, and water should not be discharged until it has reached 0 ppm. Once the chlorine levels have been ascertained to be at 0 ppm, the system's water can be safely dumped into the farm's effluent system. In some culture systems, in particular raceways, tanks and small lined ponds (i.e. those systems in which the majority of the shrimp were not removed prior to disinfection), the dead shrimp should be collected for proper disposal.

5.1.2. Liming

The lime, calcium oxide (quicklime), should be applied to dried ponds beds, as this allows the quicklime to attack any organic matter by desiccation/dehydration, at a rate of 4000–5000 kg ha^{-1}. Great care should be taken to ensure that the lime is spread evenly over the soil surface. The pond should then be allowed to stand for at least a week, or until the soil has dried to the point of cracking to a depth of approximately 10–20 cm. Once the lime has been *in situ* for a week, the ponds are slowly filled, at which point the full pH effect is realised and disinfection is completed. Quicklime is still effective when used on wet ponds but then works only through the effect of pH. Additional lime may be applied after ploughing (see below) at a rate of 50% of that normally prescribed. The pond should again be dried for at least a week, depending on the weather.

Calcium hydroxide is less effective in achieving an effective pH and does not have the same desiccating effect that quicklime has on organic matter.

5.1.3. Drying and ploughing

Whether or not a pond is treated by chlorination or liming or left to dry untreated, ploughing is a commonly used method of treating a pond bottom to reduce its organic content, improve nutrient recycling, buffer pH, eliminate pests, and achieve disinfection through a combination of microbial degradation, exposure to sunlight, aeration, and desiccation. In some regions, drying and ploughing of dry pond bottoms may only be possible during the 'dry season'. When pond drying is an option, the pond bottom should be allowed to dry until the surface has cracked to a depth of approximately 10 cm. Once this level of drying has been reached, the soil should be broken up to a depth of approximately 20 cm with a plough, tiller, disk harrow, tine harrow or other similar farm implement. Ponds treated in this manner should be left for at least a week before being refilled and restocked.

6. 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 neutralise these products with sodium thiosulfate – five moles of thiosulfate neutralise four 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 × 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 × number of grams of iodine.

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

1. for chlorine:

   28.5 × [number of litres of the disinfecting solution × concentration mg $litre^{-1}$] / 100

2. for iodine:

   it is necessary to multiply by 7.8 instead of by 28.5.

   Both chlorine and ozone produce long-lived residual oxidant compounds in seawater. Seawater at 35 parts per thousand (ppt) salinity contains 60 ppm bromide ion, which produces hypobromite in the presence of ozone. Disinfected artificial seawater, at the same salinity, produces bromine and hypobromous acid. As these, along with other residual compounds, are toxic to aquatic animals such as larval oysters (and possibly other molluscs), treated seawater must be passed through an activated charcoal filter before being used for live mollusc larvae.

   Alternative protocols for halogen neutralisation involve treatment with sodium or potassium thiosulfate.

   Monitoring of residual oxidants should be carried out regularly, especially where temperature fluctuations occur. As residual ozone cannot be measured accurately in seawater, alternative monitoring protocols, such as a feedback loop, may need to be installed.

   NOTE: Exhaust systems should also be in place to remove toxic fumes (produced during disinfection) from enclosed work areas. Ensure compliance with local atmospheric regulations when discharging toxic fumes.

7. Re-stocking of disinfected farms

Following a TCU, restocking of the disinfected facilities or farms should be accomplished only with stocks known to be free of the diseases listed in the *Aquatic Code* or other emerging or significant diseases of concern.

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