CHAPTER 4.3.

DISINFECTION OF AQUACULTURE ESTABLISHMENTS AND EQUIPMENT

Article 4.3.1.

Purpose

To provide recommendations on planning and implementation of *disinfection* procedures to prevent the introduction or spread of *pathogenic agents*.

Article 4.3.2.

Scope

This chapter describes recommendations for *disinfection* of *aquaculture establishments* and equipment during routine biosecurity activities and for emergency response. Guidance is provided on general principles, planning and implementation of *disinfection* activities.

For specific methods of pathogen inactivation refer to the *disease*-specific chapters in the *Aquatic Manual*.

Article 4.3.3.

Introduction

Disinfection is employed as a *disease* management tool in *aquaculture establishments* as part of a *biosecurity plan*. *Disinfection* is used to prevent entry or exit of target *pathogenic agents* to or from an *aquaculture establishment* or *compartment*, as well as the spread of *pathogenic agents* within *aquaculture establishments*. *Disinfection* may be used during emergency *disease* response to support the maintenance of *disease* control *zones* and for *disease* eradication (stamping-out procedures) from affected *aquaculture establishments*. The specific objective of *disinfection* will determine the strategy used and how it is applied.

When possible, the spread of *pathogenic agents* should be prevented by avoiding transmission pathways rather than attempting to manage them through *disinfection*. For example, difficult to disinfect items (e.g. gloves, dive and harvest equipment, ropes and nets) should be dedicated to a specific site rather than moved between production units or *aquaculture establishments* after *disinfection*.

Article 4.3.4.

General principles

Disinfection is a structured process that uses physical and chemical procedures to destroy or inactivate *pathogenic agents*. The process should include planning and implementation stages that take into account potential options, efficacy and *risks*.

The *disinfection* process may vary depending on whether the overall objective is *disease* prevention, control or eradication. Procedures addressing eradication will generally involve destocking of all *aquatic animals* as well as *disinfection* of *aquaculture establishments* and equipment, whereas *disease* control aims at limiting the spread of *disease* between or within *aquaculture establishments*. Although different approaches may be used to achieve the identified objective, the general principles described below should be applied in all cases.

1) The *disinfection* process should include the following phases:

   a) Cleaning and washing

   Cleaning and washing of surfaces and equipment should always precede the application of *disinfectants*. It is necessary to remove solid waste, organic matter (including biofouling) and chemical residues as these may reduce the efficacy of *disinfectants*. The detergent used should be compatible with the *disinfectant* and the surface being treated. After cleaning procedures, any excess water should be drained before application of *disinfectants*.

   Where treatment of water is required, the presence of suspended solids may also reduce the efficacy of some *disinfectants*. Removal of suspended solids through various processes such as filtration, sedimentation, coagulation or flocculation should be performed.

   Biofilms, often referred to as slime, are a thin film of microorganisms and extracellular polymeric substances that adhere to surfaces. Biofilms physically protect embedded microorganisms against *disinfectants*. In order to achieve effective *disinfection*, biofilms should be removed during the cleaning and washing stage prior to the application of *disinfectants*.

   All waste produced should be disposed of in a biosecure manner because it may contain viable *pathogenic agents* that have the potential to spread *infection* if not controlled.

   b) Application of disinfectants

   This phase involves the application of chemical compounds or physical processes that are appropriate to inactivate the *pathogenic agent*.

   The application of *disinfectants* should take into account the type of material requiring *disinfection* and how *disinfectants* should be applied. Hard non-permeable materials (e.g. polished metal surfaces, plastics and painted concrete) can be cleaned thoroughly and allow contact with the *disinfectant* because there is little opportunity for infective material to lodge in crevices. *Disinfection* efficacy will decrease if the surface is corroded, pitted or paint is flaking, so proper maintenance of equipment is essential. For permeable surfaces and materials (e.g. woven material, nets and soil), a higher *disinfectant* concentration and a longer contact time is 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 all surfaces come into contact with the agent for the required period of time. The application of *disinfectants* should be undertaken methodically (e.g. using a grid pattern) to ensure that complete coverage and adequate contact times are achieved. Each phase should start from the highest point and proceed downwards, commencing from the least contaminated areas. However for some equipment, rinsing of surfaces with the *disinfectant* may be sufficient. 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. Vertical surfaces may need retreatment or the addition of compatible foaming agents to prolong adherence to surfaces.

   For pipes and biofilters, complete filling with the *disinfectant* solution should be done to ensure contact with all surfaces. Difficult to access and complex areas may require fumigation or use of misting equipment.

   c) Removal or inactivation of the disinfectant

   Removal or inactivation of chemical residues is important to avoid toxicity to *aquatic animals*, corrosion of equipment and environmental impacts. Processes that may be employed for the removal or inactivation of chemical residues may include: rinsing of surfaces, dilution to acceptable levels, treatment to inactivate chemical agents or, time to allow deactivation or dissipation of the active compound. These processes may be used in isolation or in combination.

2) *Disinfectants* should be used in accordance with relevant legislation. *Disinfectants* may present *risks* to the health of people, *aquatic animals* and the environment. Chemical *disinfectants* should be stored, used and disposed of in accordance with regulations and manufacturer's instructions.

3) *Disinfection* should be monitored to ensure appropriate dose of *disinfectant* and *disinfection* efficacy. Depending on the application process and the *pathogenic agent* of concern, this may be done in different ways. Examples include measurement of the active agent (e.g. residual chlorine levels), indirect measurement of the active agent by an indicator process (e.g. monitoring oxygen reduction potential), and measuring efficacy using indicator bacteria (e.g. heterotrophic bacteria plate counts).

   In facilities that have undergone destocking and *disinfection*, the use of a sentinel population prior to restocking may be considered. The sentinel population should be susceptible to the pathogen of concern and exposed to conditions that would be conducive to the expression of clinical *disease* should viable pathogen remain.

4) *Aquaculture establishments* should keep records of the *disinfection* processes applied. The records should be sufficient to allow evaluation of the *disinfection* plan.

Article 4.3.5.

Planning

A *disinfection* plan should be developed that incorporates an assessment of the transmission pathways, the type of material to be disinfected, the *pathogenic agents* to be inactivated, the health and safety precautions and control measures required, and the environment in which the process is to be undertaken. The *disinfection* plan should be regularly reviewed and include a mechanism for determining efficacy. Any changes to the *disinfection* plan should also be documented.

The planning process should assess the critical control points where *disinfection* will be most effective. *Disinfection* priorities should be developed by considering potential pathways for spread of *pathogenic agents* and the relative likelihood of contamination. For effective *disinfection* of facilities containing *vectors* (e.g. ponds) the *vectors* should be excluded, removed or destroyed as part of the *disinfection* process.

An inventory of all items requiring *disinfection* should be developed when practical. An assessment should be made of the materials used in construction, their surface porosity and resistance to chemical damage, and accessibility for *disinfection*. Then, the appropriate *disinfection* method should be decided for each item.

The level of cleaning required prior to *disinfection* should be assessed for each type of equipment. If heavy soiling with solids and particulate matter is present, specific attention should be given to the cleaning process and the resources required. The physical or chemical cleaning process should be compatible with the *disinfectant* chosen.

Personnel, equipment and materials to be disinfected should be assessed taking into account the type and number of items to be treated and how waste material will be managed.

The ability to control water flow and water volumes should be considered at the planning stage and will depend on the enterprise type (recirculation, flow-through and open systems). Water may be disinfected using a variety of methods as described in Article 4.3.11.

Article 4.3.6.

Disinfection in an emergency response

Disinfection is essential part of any emergency response to support *disease* control activities such as *quarantine* of affected *aquaculture establishments* and stamping-out procedures. The conditions associated with an emergency response require different approaches for *disinfection* to those used in routine biosecurity. These conditions include a high level of *disease risk* (due to the significance of the *disease*), high pathogen loading, potential high volumes of infected *aquatic animals* and waste, large areas requiring *disinfection* and large volumes of contaminated water. Planning should consider these circumstances, incorporate an evaluation of *risks* and include methods for monitoring efficacy.

In an emergency response it may be preferable to avoid transmission pathways rather than relying on *disinfection*. Equipment should not be moved from an infected *aquaculture establishment* unless effective *disinfection* has been achieved. In some circumstances, equipment or material that is difficult to disinfect or has a high likelihood of contamination may need to be disposed of in a biosecure manner rather than be disinfected.

Article 4.3.7.

Types of disinfectants

Types of *disinfectants* commonly used in *aquaculture* include the following:

1. Oxidising agents

   The majority of oxidising agents are relatively fast acting and are effective *disinfectants* for a large range of micro-organisms. These compounds are inactivated by organic matter and therefore should be used following an effective cleaning stage. Organic matter consumes oxidising agents and the initial concentration (loading dose) may drop rapidly, making effective dosing levels (residual dose) difficult to predict. Therefore, residual dose levels should always be monitored to ensure that they remain above the minimum effective concentration for the required time period.

Oxidising agents may be toxic to *aquatic animals* and therefore should be removed or inactivated.

Common oxidising agents include chlorine compounds, chloramine-T, iodophores, peroxygen compounds, chlorine dioxide and ozone.

2. pH modifiers (alkalis and acids)

pH modifiers consist of either alkalis or acid compounds used to modify ambient pH. They have the advantage that they are not inactivated by organic matter and therefore can be used in areas where an effective cleaning phase is not possible such as in pipes and biofilters.

3. Aldehydes

Aldehydes act by denaturing protein. Two aldehyde compounds that may be used during decontamination of *aquaculture establishments* are formaldehyde and glutaraldehyde. They are highly effective against a wide range of organisms but require long exposure times. Aldehydes maintain their activity in the presence of organic matter and are only mildly corrosive. Glutaraldehyde is used in the liquid form as a cold sterilant, particularly for heat-sensitive equipment. Formaldehyde may be used as a mist or a gas for fumigation.

4. Biguanides

Of the many biguanides available, chlorhexidine is the most commonly used. However they are not effective in hard or alkaline water and are less effective against many *pathogenic agents* compared to other groups of *disinfectants*. These compounds are comparatively non-corrosive and relatively safe, thus they are commonly used in the *disinfection* of skin surfaces and delicate equipment.

5. Quaternary ammonium compounds (QACs)

The biocidal efficacy of QACs is variable and selective. They are effective against some vegetative bacteria and some fungi, but not all viruses. QACs are most active against gram-positive bacteria; action against gram-negative bacteria is slow, with some strains showing resistance. These compounds are not effective against spores. The advantages of QACs are that they are noncorrosive and have wetting properties that enhance contact with surfaces. QACs may be toxic to *aquatic animals* and should be removed from surfaces following *disinfection* procedures.

6. Ultraviolet (UV) irradiation

UV irradiation is a viable option for the treatment of water entering or leaving *aquaculture establishments* where there is some control of water flows in recirculation or flow-through systems. UV irradiation should be used following effective filtration because suspended solids reduce UV transmission and the effectiveness of this method.

7. Heat treatment

Susceptibility of *pathogenic agents* to heat treatment varies significantly. Therefore, the characteristics of the target *pathogenic agent* should be taken into consideration. Under most conditions, moist heat is more effective than dry heat.

8. Desiccation

Desiccation may be an effective *disinfectant* for susceptible *pathogenic agents* and may be used in circumstances where other *disinfection* methods are impractical or as an ancillary method to other *disinfection* methods.

Desiccation can be considered to be a *disinfection* method if complete drying of the item is achieved because the absence of water will kill many *pathogenic agents*. However, moisture content may be difficult to monitor in some circumstances. The effectiveness will vary depending on environmental conditions such as temperature and humidity.

9. Combined disinfection methods

Combined *disinfection* methods should be considered wherever they are synergistic and provide a higher assurance of effective *pathogenic agent* inactivation. Some examples include:

a) direct sunlight and desiccation as a combined *disinfection* method provides three potential *disinfection* actions, i.e. UV irradiation, heating and desiccation. It has no operational cost and may be used subsequent to other methods;

b) ozone and UV irradiation are often combined in series as they provide back-up systems and different modes of action. UV irradiation also has the advantage of removing ozone residues from treated water.

Antagonistic effects may occur when chemical agents or detergents are combined.

Article 4.3.8.

Selection of a disinfectant

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. fresh water, 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; and
- user safety.

Article 4.3.9.

Types of aquaculture establishments and equipment

Aquaculture establishments and equipment differ widely in their characteristics. This section presents some considerations for effective *disinfection* of different types of *aquaculture establishments* and equipment.

1. Ponds

   Ponds are generally large and may be earthen based or be fitted with plastic liners. These characteristics together with the large volumes of water make cleaning prior to decontamination difficult and high organic loads may affect many chemical *disinfectants*. Ponds should be drained of water and have as much organic matter as possible removed prior to *disinfection*. Water and organic matter should be disinfected or disposed of in a biosecure manner. Earthen ponds should be dried thoroughly and lime compounds applied to raise pH and aid the inactivation of *pathogenic agents*. Scraping, ploughing or tiling of the base of unlined ponds will also aid in incorporation of liming compounds and drying.

2. Tanks

   Tank construction material (e.g. fibreglass, concrete or plastic) will determine the type of *disinfection* method used. Bare concrete tanks are susceptible to corrosion by acids and potential damage by high pressure sprayers. They are also porous and therefore require longer application of chemicals to ensure *disinfection*. Plastic, painted and fibreglass tanks are more easily disinfected because they have smooth, non-porous surfaces that facilitate thorough cleaning and are resistant to most chemicals.

   Tanks should be drained of water and have as much organic matter as possible removed prior to *disinfection*. Water and organic matter should be disinfected or disposed of in a biosecure manner. Tank equipment should be removed for separate cleaning and *disinfection*, and all organic waste and debris removed. Tank surfaces should be washed using high-pressure sprayers or mechanical scrubbing with detergent to remove fouling such as algae and biofilms. Heated water may be used to enhance the cleaning process. Any excess water should be drained before application of *disinfectants*.

   When *disinfectants* are applied to vertical surfaces, care should be taken to ensure that adequate contact time is maintained before the *disinfectant* is drained. Following *disinfection*, tanks should be rinsed to remove all residues and allowed to dry completely.

3. Pipes

Disinfection of pipes may be difficult due to lack of access. Pipe construction material should be taken into consideration when selecting the *disinfection* method.

Pipes can be cleaned effectively through the use of alkaline or acid solutions, or foam projectile pipe cleaning systems. Effective *disinfection* in pipes requires the removal of biofilms, followed by flushing of the resulting particulate matter and thorough rinsing.

Once pipes are cleaned, chemical *disinfectants* or circulation of heated water can be used. All steps require pipes to be fully filled so that internal surfaces are treated.

4. Cage nets and other fibrous materials

Nets used in cage culture are often large, difficult to handle, have significant levels of biofouling and are usually made from fibrous materials that trap organic matter and moisture. Nets should be dedicated to a single *aquaculture establishment* or area because they have a high likelihood of contamination and may be difficult to disinfect.

Once the net has been removed from the water, it should be transferred directly to the net washing site. Nets should be thoroughly cleaned prior to *disinfection* to remove organic matter and aid in the penetration of chemical *disinfectants*. Cleaning of nets is best achieved by first removing gross biofouling and then washing with a detergent solution.

Following cleaning, nets may be disinfected by complete immersion in chemical *disinfectants* or heated water. Treatment duration should be sufficient to allow penetration into net material. The treatment method should be chosen considering the potential to weaken or damage nets. Following *disinfection*, nets should be dried before storage. If rolled nets are not completely dry they will retain moisture which may enhance survival of the *pathogenic agent*.

Other fibrous materials such as wood, ropes and dip nets have characteristics similar to cage nets and they require special consideration. Wherever possible, it is recommended that equipment is site specific if it includes fibrous material.

5. 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. The application of corrosive *disinfectants* to *vehicles* should be avoided or if used, corrosive residues removed following *disinfection* by thorough rinsing. Oxidative compounds such as chlorines are the most commonly used *disinfectants* for *vehicles*.

6. Buildings

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. 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*.

Misting or foaming agents are options for *disinfection* of complex areas and vertical surfaces. Fumigation can be considered for large or difficult to access areas if buildings can be adequately sealed.

7. Containers

Containers range from simple plastic bins used to transport harvested *aquatic animal products* or dead *aquatic animals* through to complex tank systems used for the transport of live *aquatic animals*.

Containers are generally manufactured using smooth non-porous material (i.e. plastic, steel) which can be easily disinfected. They should be considered high *risk* items because they are in close contact with *aquatic animals* or their *products* (e.g. blood, diseased *aquatic animals*). In addition the need to move them between locations makes them potential fomites for the spread of *pathogenic agents*. In the case of transport of live *aquatic animals*, *containers* may also have pipes and pumping systems and confined spaces that should also be disinfected.

All water should be drained from the *container* and any *aquatic animals*, faecal matter and other organic material removed by flushing with clean water and disposed of in a biosecure manner. All pipes and associated pumps should also be inspected and flushed. *Containers* should then be washed using appropriate chemical detergents combined with high-pressure water cleaners or mechanical scrubbing.

All internal and external surfaces of *containers* should be treated using an appropriate *disinfection* method. They should then be rinsed and inspected to ensure there are no organic residues and stored in a manner that allows them to drain and dry quickly.

8. Boats

All boats should undergo routine *disinfection* to ensure that they do not transfer *pathogenic agents*. The level of contamination of boats will be determined by their use. Boats used to harvest or to remove dead *aquatic animals* from *aquaculture* sites should be considered as highly likely to be contaminated. Organic material should be regularly removed from decks and work areas.

As part of the *disinfection* planning process, an assessment should be made to identify areas likely to be contaminated such as in and around machinery, holding tanks, bilges and pipes. All loose equipment should be removed prior to *disinfection*. Additional procedures should be developed for well-boats because of their potential to transfer *pathogenic agents* through the discharge of contaminated water. Contaminated effluent water should be disinfected prior to discharge (refer to Article 4.3.11.).

Where possible, boats should be placed on land for *disinfection* in order to limit waste water entering the aquatic environment and to allow access to hull areas. Biofouling organisms that may act as *vectors* and fomites should be removed.

Where boats cannot be removed to land, a *disinfection* method should be chosen that minimises the discharge of toxic chemicals into the aquatic environment. Divers should be used to inspect and clean hulls. Where appropriate, mechanical methods such as high-pressure sprayers or steam cleaners should be considered as an alternative to chemical *disinfection* for cleaning above and below the water-line. Fumigation may also be considered for large areas if they can be adequately sealed.

9. Biofilters

Biofilters associated with closed or semi-closed production systems are an important control point for *disease*. Biofilters are designed to maintain a colony of beneficial bacteria used to enhance water quality. The conditions that support these bacteria may also enhance survival of some *pathogenic agents* should they be present. It is normally not possible to disinfect biofilters without also destroying beneficial bacteria. Therefore potential water quality issues should be taken into account when planning strategies for *disinfection* of biofilters.

When disinfecting biofilters and their substrates, the system should be drained, organic residues removed and surfaces cleaned. *Disinfection* of biofilter systems can be undertaken by modifying water pH levels (using either acid or alkaline solutions). Where this is undertaken, the pH levels must be sufficient to inactivate the *pathogenic agent*, but should not be corrosive to pumps and equipment within the biofilter system. Alternatively, the biofilter can be completely dismantled, including removal of biofilter substrate, and the components cleaned and *disinfectants* applied separately. In the case of emergency *disease* response, the latter procedure is recommended. The biofilter substrate should be replaced if it cannot be effectively disinfected. Biofilter systems should be thoroughly rinsed before re-stocking.

10. Husbandry equipment

Aquaculture establishments will normally have a range of husbandry equipment items that come into close contact with *aquatic animals* and have potential to act as fomites. Examples include graders, automatic vaccinators and fish pumps.

The general principles described in Article 4.3.4. should be applied to *disinfection* of husbandry equipment. Each item should be examined to identify areas that come into close contact with *aquatic animals* and where organic material accumulates. If required, equipment should be dismantled to allow adequate cleaning and application of *disinfectants*.

Article 4.3.10.

Personal equipment

Disinfection of personal equipment should consider the level of contamination associated with previous use. Where possible, personal equipment should be site specific to avoid the need for regular *disinfection*.

Equipment should be chosen which is non-absorbent and easy to clean. All staff entering a production area should use protective clothing that is clean and uncontaminated. On entry and exit of production areas boots should be cleaned and disinfected. When footbaths are used they should incorporate a cleaning procedure to remove accumulations of organic material and mud, be sufficiently deep to cover boots, use a *disinfectant* solution that is not inactivated by organic matter and be regularly refreshed with a new solution.

Highly contaminated equipment such as dive equipment requires special attention and is often prone to chemical corrosion. Frequent rinsing of equipment will assist in reducing build-up of organic matter and make *disinfection* more efficient. Equipment should be allowed to dry thoroughly to ensure that moist microenvironments that may harbour *pathogenic agents* are minimised.

Article 4.3.11.

Disinfection of water

Aquaculture establishments may need to disinfect intake and effluent water to eliminate *pathogenic agents*. The most appropriate *disinfection* method will differ depending on the *disinfection* objective and the characteristics of the water to be disinfected.

Exclusion of *aquatic animals* and removal of suspended solids from the water to be treated are essential prior to the application of *disinfectants*. Pathogens are known to adhere to organic and inorganic matter and removal of suspended solids can significantly reduce loading of *pathogenic agents* in water. Removal of suspended solids can be achieved by filtration or settlement of suspended material. The most suitable filtration system will depend on the initial quality of water, volumes to be filtered, capital and operating costs and reliability.

Physical (e.g. UV irradiation) and chemical (e.g. ozone, chlorine and chlorine dioxide) *disinfectants* are commonly used to disinfect water. Suspended solids should be removed prior to the application of these *disinfectants* because organic matter may inhibit oxidative *disinfection* processes and suspended solids inhibit UV transmission and reduce efficacy of UV irradiation by shielding *pathogenic agents*. A combination of methods may be beneficial where they are synergistic or where a level of redundancy is required.

It is essential to monitor the efficacy of water *disinfection*. This can be achieved by direct testing for *pathogenic agents* of concern, indirect monitoring of indicator organisms or monitoring of residual levels of *disinfectants*.

Management of chemical residues is important to avoid toxic effects on *aquatic animals*. For example, residuals 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.