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Disinfection in aquaculture

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Summary: *The authors present information on the susceptibility to various disinfectants of some important pathogens in aquaculture, with special reference to the farming of salmonids. Practical disinfection procedures for use in the aquaculture industry are then described, on the basis of experience obtained in Norway and in other countries with a large salmon-farming industry. In addition to routine disinfection at aquaculture sites, the authors also describe disinfection procedures for use in outbreaks of notifiable diseases, as well as the treatment/disinfection of waste water and offal from the slaughtering and processing of aquaculture products.*

KEYWORDS: Aquaculture – Disinfection – Fish diseases – Management – Pathogens.

INTRODUCTION

For the purposes of this article, a chemical disinfectant is defined as any chemical substance which prevents infection by inactivation of microorganisms pathogenic to aquatic animals, except when these chemicals are used as therapeutic agents. Thus, technically, disinfection involves the use of chemical disinfectants to inactivate pathogens on all surfaces except living tissues. A single paper cannot cover all aspects of disinfection throughout aquaculture, and disinfection in salmonid fish farming has been chosen for the purposes of illustration. The procedures and principles described below for disinfection in salmonid farming are equally applicable to the other areas of fish culture, and to the farming of lobsters, shrimps and molluscs in sea-based or land-based aquaculture enclosures for commercial or scientific purposes.

Sections have been included on the susceptibility to different disinfectants of some important pathogens in aquaculture, although not all pathogens for aquatic animals are covered.

Routine disinfection may be reduced to a minimum if good management practices are implemented daily. When people or equipment or other materials enter or leave a farm, thorough technical disinfection should be performed. However, in the case of an outbreak of a notifiable disease, rigorous disinfection procedures are necessary. In addition, a period of quarantine is required before re-stocking. The procedures described below are primarily based on the principles set forth in Norwegian legislation, regulations and general practices.

No consideration is given here to the properties of the technical disinfectants in relation to the material to be disinfected, the temperature of the environment and the

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material, or the toxicological properties of the disinfectant for the animals and the user, as these aspects of disinfection are covered in other chapters in this double issue of the *Scientific and Technical Review*. However, the applications of various disinfectants in aquaculture are listed in Table I.

SOME MICROORGANISMS PATHOGENIC TO AQUATIC ANIMALS: SUSCEPTIBILITY TO DISINFECTANTS

Aeromonas salmonicida, *Vibrio anguillarum* and *V. salmonicida* (the causative agents of furunculosis, vibriosis and cold water vibriosis, respectively) are able to produce respiring but non-culturable cells which can survive for several months in free water or in sediments (14, 8, 9, 16). McCarthy (20) found that it was possible to disinfect both dried and wet cage nets contaminated with *A. salmonicida* using acriflavine (1:4,000) and 0.1% teepol in 1% NaOH, while 1% hypochlorite disinfected only wet cage nets. In the study by Sako and Sorimachi (29), ultra-violet (UV) light at a dose of 3.4 mJ/cm^2 killed all vegetative bacteria. Both Bullock and Stuckey (4) and Kimura *et al.* (18) reported the influence of particles in the water and the effect of filtration prior to UV irradiation. Without filtration, the UV dose had to be increased from 3-5 mJ/cm^2 to 15-30 mJ/cm^2 to obtain a 99.9-99.99% kill. A concentration of 0.1 mg Cl_2/l produced a 99.9% kill within 30 sec in both distilled and soft water, while 0.2 mg/l was needed in hard water (35). Ross and Smith (27) inactivated 10^7 colony-forming units/ml of bacteria within 5 min, using 25 mg/l of an iodophor.

Renibacterium salmoninarum, the causative agent of bacterial kidney disease (BKD), is a Gram-positive bacterium. There are few reports on the susceptibility of *R. salmoninarum* to disinfectants. However, Ross and Smith (27) reported that an iodophor concentration of 25 mg/l produced a total kill in 5 min at pH 7.0 and 8.0. Whipple and Rohovec (37) demonstrated that *R. salmoninarum* survived heating for 4 h at 50°C, for 3 h at 55°C, and for 15 min at 65°C. However, this pathogen survived for only 1 min at 55°C in fish silage (pH 3.8-4.3). *R. salmoninarum* survived for more than 4 h in citric phosphate buffer (pH 4.0), but for less than 30 min in fish silage.

The causative agent of infectious pancreatic necrosis (IPN) is a *Birnavirus*. IPN virus is stable in the presence of ether and chloroform, and is extremely stable in fresh water and sea water. It can survive for several months in both types of water and at various temperatures. According to Bylund *et al.* (5), IPN virus survived for several years in ensilage. IPN virus also survived drying for more than eight weeks (6). The virus did not replicate at temperatures above 40°C, and heating to 60°C produced a 99.99% kill in 30 min (21). Fløgstad *et al.* (11) reported a kill of more than 99.99 % in 1 min when IPN virus was heated to 65°C in the presence of organic material. IPN virus is very stable at low pH. According to Vestergaard-Jørgensen (34), several hours were required to produce a 99.9 % kill at pH 2.5, while the same effect was obtained after 10 min at pH 12.0. Fløgstad *et al.* (11) found that the use of sodium hydroxide at pH 11.6 produced a 99.9% kill in 24 h, while a kill of 99.9% or more was obtained after 6 min at pH 12.0. In the same study, they also found that when formic acid was used, a pH of 2.5 produced a 99% kill in 1 h, while a 99.9% kill was obtained at pH 2.0 in 6 min.

Sako and Sorimachi (29) produced a 99.9% kill using UV light at a dose of 150-200 mJ/cm^2, while Yoshimizu *et al.* (39) produced at least 99% kill at a dose of 100-150 mJ/cm^2.

In distilled water, hypochlorite at a concentration of 0.1 mg Cl_2/l inactivated IPN virus within 60 seconds (36). Desaultes and MacKelvie (6) found that a concentration of 25 mg Cl_2/l was needed to inactivate 10^5 tissue culture infective dose ($TCID_{50}$) per ml in 30 min, while a concentration of 40 mg/l was needed to inactivate $10^{7.5}$ $TCID_{50}$/ml in the same time. In the presence of organic material, the chlorine concentration must be increased significantly. Fløgstad *et al.* (11) reported almost no effect at an initial concentration of 250 mg Cl_2/l within 24 h when disinfecting waste water from a salmon slaughterhouse. The chemical oxygen demand (COD-value) of the waste water was 4,301 mg/l. If such waste water was pre-treated through chemical precipitation and then chlorinated, an initial chlorine concentration of 50 mg Cl_2/l for 15 min was sufficient to produce a 99.99% kill. The COD of the waste water was reduced from 3,501 mg/l to 1,121 mg/l following the precipitation step (12).

Iodophors are less sensitive than chlorine compounds to the presence of organic material. According to Amend (1), exposure to a concentration of 50 mg/l of free iodine for 6 min inactivated all viruses. These findings were not supported by Rosseland *et al.* (28), who found that a concentration of 100 mg/l free iodine produced a 99.9-99.99% kill in 10 min when disinfecting eyed eggs of salmonids. Elliot and Amend (7) were unable to produce total inactivation using 0.2% formalin for 1 h. They were unable to produce any virucidal effect using thimerosal (0.2% for 10 min) or malachite green (5 mg/l for 60 min), while acriflavine (500 mg/l for 20 min) produced some virostatic effect.

Torgersen (unpublished findings) has tested several commercially-available disinfectants against IPN virus. Disinfectants containing active substances such as halogens, oxygen-releasing compounds and acidic and alkaline compounds, were all able to produce at least a 99.99 % kill in 5 min. Quaternary ammonium compounds had no virucidal effect on IPN virus.

The causative agent of infectious haematopoietic necrosis (IHN) can survive for two weeks in distilled water, seven weeks in fresh water (36), and four times longer in distilled water than in sea water (25). IHN virus can survive drying at 4°C and 10°C for more than five days, and may survive for more than nine months at 4°C, six weeks at 21°C, 12 h at 32°C and 15-20 min at 60°C (19). The time required to produce a 99.9% kill was estimated as 8 h at 32°C, ten days at 21°C and more than twenty weeks at 4°C. The virus also survived at pH 4-10, but was relatively rapidly inactivated at pH 3 (25). Sako and Sorimachi (29) found that a 99.9% reduction in infectivity was obtained using UV light at a dose of 2 mJ/cm^2, while Yoshimizu *et al.* (39) produced a 99% reduction in infectivity using 1-3 mJ/cm^2. According to Wedermeyer *et al.* (36), chlorine compounds at a concentration of 0.5 mg Cl_2/l inactivated IHN virus within 10 min in hard water (containing $CaCO_3$ at 120 mg/l) and within 5 min in soft water (30 mg $CaCO_3$/l). In distilled water, a concentration of 0.1 mg/l for 30 sec was sufficient to produce complete inactivation. Amend and Pietsch (2) tested the susceptibility of IHN virus to malachite green (5 mg/l for 60 min), acriflavine (500 mg/l for 20 min), thimerosal (0.2% for 10 min), NaCl solution (3% for 30 min), benzethonium chloride (20 mg/l for 30 min), sodium hypochlorite (10 mg/l for 30 min), formalin (0.2% for 60 min) and iodine (27 mg/l for 15 min). Complete inactivation of the virus was obtained using only iodine and sodium hypochlorite, while formalin produced partial inactivation.

The causative agent of viral haemorrhagic septicaemia (VHS) is a rhabdovirus. The optimal temperature for replication of VHS virus in cell cultures is 10-15°C, while temperatures above 20°C will normally inhibit replication (38). The time required to produce a 99.9% kill was several years at –20°C, several months at 4°C, approximately

TABLE I

Disinfectants applicable to aquaculture and methods of use

Process	Indications	Method of use *	Comments
Physical			
Desiccation, light	Fish pathogens on earthen bottoms	Dry for three months at an average temperature of 18°C	Drying period can be reduced by the use of a chemical disinfectant
Dry heat	Fish pathogens on concrete, stone, iron, plastic surfaces	Flame-thrower, blow-lamp	
Damp heat	Fish pathogens in transportation vehicle tanks	Steam at 100°C or higher for 5 min	
Ultra-violet rays	Viruses and bacteria in water *Myxosporidia* spores in water IPN virus in water	5 mJ/cm^2 35 mJ/cm^2 125 mJ/cm^2	Minimum lethal dose
Chemical			
Quaternary ammonium compounds	Virus, bacteria, hands Gill bacteria, plastic surfaces	1 mg/l for 1 min 2 mg/l for 15 min	IPN virus resistant
Calcium oxide	Fish pathogens on dried earthen base	0.5 kg/m^2 for one month	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	Solution with chlorometric degree of 0.01 (i.e. 30 mg Cl_2/l) left to inactivate for several days	Can be neutralised with sodium thiosulphate **
Calcium cyanamide	Spores on earthen base	3,000 kg/ha on dry surfaces; leave in contact for one month	
Formalin	Fish pathogens in sealed premises	Released from formogenic substances, generally trioxymethylene. Comply with instructions	

Iodine (iodophors)	Bacteria, viruses on hands and smooth surfaces	> 200 mg/l for a few seconds	See special recommendations **
	Eyed eggs	100 mg/l for 10 min	
	Gametes during fertilisation	25 mg/l for several hours	
	Tanks		
Ozone	Fish pathogens, water, sterilisation	1 mg/l for 1 min	Costly
Sodium hydroxide	Fish pathogens on resistant surfaces with cracks (unpainted concrete, etc.). May be used in dried earthen ponds	Mixture: – sodium hydroxide 100 g – teepol 10 g – calcium hydroxide 500 g – water 10 l. Surfaces: spray 0.1 l/m^2, leave for 48h. Earth ponds: spray 2 l/m^2, leave for at least 2 weeks	The most active disinfectant ($Ca[OH]_2$) stains the surfaces treated; teepol is a tensio-active agent. Turn water on, checking pH
Sodium hypochlorite **	Bacteria and viruses on all clean surfaces and in water	Chlorometric degree of solution = 0.01 (i.e. 30 mg/l chlorine). Leave to inactivate for a few days or neutralise with sodium thiosulphate after 3 h	Based on bleach
	Nets, boots, clothing, hands	Chlorometric degree of solution = 0.06. Leave for 20-30 secs; rinse with clean water or neutralise with sodium thiosulphate	

IPN: infectious pancreatic necrosis

* Concentrations indicated are those for the active substance

** Chlorine and iodine are highly toxic for fish and, in order to prevent serious accidents which could result from a manipulation error, it is recommended to neutralise these products with sodium thiosulphate, as follows:

$$5Na_2S_2O_3 + 4Cl_2 + 5H_2O \rightarrow 2HSO_4^- + 8Cl^- + 4H_2O + 4S + 4SO_2 + 10Na^+.$$

Hence, five moles of thiosulphate neutralise four moles of chlorine; the proportions are the same for iodine. It is therefore possible to prepare a thiosulphate solution at 1%, in which case the neutralising volumes (in ml) will be as follows:

– for chlorine: 28.5 (number of litres of the disinfecting solution × concentration mg/l)/100

– for iodine: 7.8 (number of litres of the disinfecting solution × concentration mg/l)/100

four weeks at 20°C, and less than 1 min at 70°C. In a dry environment, the virus survived for about one week at 4°C. VHS virus survived for 10 min at pH 2.5 and for 2 h at pH 12.2. The virus was not stable in the presence of ether and chloroform. A total kill was produced by the use of 2% formalin or 25 mg/l of an iodophor for 5 min (34). At a temperature of 5-10°C, 0.2% NaOH or 2% formalin produced a total kill within 40 min (31).

Saprolegnia spp. often cause secondary skin infections on salmonids and may sometimes be lethal. Kimura *et al.* (18) demonstrated that UV light at a dose of 150-250 mJ/cm^2 was necessary to inhibit the germination of *S. parasitica*. Ross and Smith (27) found that 25 mg/l of an iodophor was without any effect on *S. parasitica*. This fungus is able to grow at 5-35°C, with optimum growth at 25°C. *Saprolegnia* spp. may also grow at pH 3.8-11. Malachite green at a concentration of 2 mg/l produced a total kill within 60 min, while malachite green and formaldehyde (1:625) produced a total kill in 75 min (24).

Exophiala spp., another genus of pathogenic fungi in salmonids, have optimal growth at 18°C, and may grow at pH 5.0-7.5. *Exophiala* spp. can survive at 3.5% salinity. Malachite green, a disinfectant traditionally used against fungi, has little or no effect. According to Håstein and Poppe (13), natamycin inhibited spore germination and produced a 99% kill after 30 min at 50 mg/l.

Aphanomyces astaci, the causative agent of crayfish plague, does not produce resistant spores, and the disease may be eradicated by removing all crayfish from the watercourse. The American crayfish (*Pacifastacus leniusculus*) may serve as a carrier of *A. astaci* (32).

Myxobolus cerebralis is the causative agent of whirling disease. Spores can survive in sediments for several years. Hoffman (15) reported that UV light at a dose of 35 mJ/cm^2 inactivated the parasite in water.

ROUTINE DISINFECTION IN AQUACULTURE

Visitors to an aquaculture establishment

Visitors to the farm should use disposable 'pull-on' shoes, or should disinfect their shoes/boots in an appropriate disinfectant, when both entering and leaving the aquaculture establishment. Also, if the establishment has different sites/areas with different levels of hygienic standards, a footbath containing an appropriate disinfectant should be provided at the entrance to each area, and footwear should be disinfected when passing from one area to another. The disinfectant should preferably have an active compound which is not sensitive to the presence of organic material, and it should not decompose rapidly. The ideal compound may be hard to find, but disinfectants based on phenolic compounds, an aldehyde-releasing compound or an iodophor are acceptable. The chosen disinfectant must be replaced regularly, at least every week. Visitors should not come into contact with water where aquatic animals are kept, nor with feedstuffs or automatic feed dispensers. If samples of water or feedstuffs are to be collected, these samples should be taken by the staff. If samples have to be taken by visitors, the visitor should disinfect his/her hands using an appropriate hand disinfectant, or use disposable latex gloves. For these purposes, 70% ethanol, 0.5% chlorhexidine in 70% ethanol, an iodophor (0.2% iodine) in water or 70% ethanol, or, indeed, any commercially-available hand disinfectant may be used.

The staff at the farm should wear clothing and boots/shoes which are used only on the farm. This clothing should be regularly washed in hot water (≤ 60°C), or disinfected.

Equipment entering and leaving the farm or in use on the farm

Under normal circumstances, no equipment should leave a farm without thorough disinfection. Small items of technical equipment should be washed using a high-pressure cleaning device to remove all visible contaminants, and should then be disinfected with a suitable disinfectant. The choice of disinfectant should be determined with regard to the material to be disinfected.

The wheels of land transport vehicles should be washed using a high-pressure cleaner followed by the application of an appropriate disinfectant.

Sea-based transport vehicles (boats) are difficult to disinfect routinely. However, the crew of the boat should never enter a farm without taking the same precautions as indicated previously for visitors.

Equipment which is used at separate sites, tanks or ponds on a farm should be disinfected before each new use. This is easily achieved by placing the equipment in a disinfection bath when not in use and during transport. Again, the choice of disinfectant must be considered in relation to the material from which the equipment is constructed.

Infrastructure at the farm

Unless the presence of a disease is suspected or has been demonstrated, it should not be necessary to disinfect buildings or outdoor areas on the farm. Technical installations, equipment or other structures should not normally be moved from one site to another. However, some equipment is too expensive to be duplicated, and disinfection will be needed if such equipment is used at different locations or sites. If boats and other equipment used in a watercourse are transported for use in another watercourse, this equipment must be completely dry before it is moved. Cage nets and accompanying equipment used at one site should be dried and disinfected using an appropriate disinfectant before being installed at another site.

Aquatic animal products entering and leaving the farm

Disinfection of eggs is recommended to prevent the spread of horizontally-transmissible diseases, but this cannot prevent the spread of vertically-transmissible diseases, such as BKD, IPN or IHN. An iodophor at pH 6-8 is recommended for the disinfection of salmonid eggs. Below pH 6 the toxicity for eggs increases, and above pH 8 the antiseptic efficacy decreases. The pH can be controlled by adding $NaHCO_3$ (at a rate of 100 mg/l) to water with low alkalinity. The eggs should be rinsed in fresh water before and after disinfection. The water used to prepare the iodophor solution should be free of organic matter. The iodophor should be diluted to give a final concentration of free iodine of at least 100 mg/l (22, 23). This concentration of iodine is sufficient for an efficient surface disinfection of eggs, with a contact time of at least 25 min (28). No increased mortality in eyed eggs of *Salmo salar* L. was observed in these experiments. The iodophor solution should be replaced when it turns pale yellow or the colour disappears. Finally, in the case of eggs which have been transported, the packing should be disinfected, or preferably destroyed.

Certain precautions must be taken prior to the use of an iodophor, as the products on the market contain a variable quantity of detergents which may give rise to toxic effects. Preliminary tests are thus recommended to determine the safe concentration and contact

time for disinfection; tests should preferably be conducted using several commercially-available products. Disinfection of the eggs of various fish species may be performed using iodine, but this procedure is most commonly used for fish of the family Salmonidae. However, iodine must not be used for disinfecting eggs of flatfish species (26).

DISINFECTION FOLLOWING AN OUTBREAK OF A NOTIFIABLE DISEASE, OR COMPLETE DISINFECTION IN 'ALL-IN/ALL-OUT' MANAGEMENT PRACTICES

Disinfection of earthen ponds

Under Norwegian regulations (3), earthen ponds and 'intake channels' should be dried out or pumped empty. Solid foreign particles should be removed and destroyed, and the uppermost 10-15 cm of sludge removed. This sludge may be contaminated and should be treated in a way which will prevent the spreading of pathogens. The ponds and corresponding channels are then disinfected using calcium oxide at a minimum rate of 0.5 kg/m^2. The areas treated with calcium oxide must be kept dry for at least one month at > 10°C, or at least two months at < 10°C. If it is impossible to empty the ponds and channels completely, due to the level of ground water or other factors, these areas should be pumped dry at least twice during the following period and treated with a minimum of 1 kg/m^2 calcium oxide each time. Before spreading the calcium oxide, foreign particles and sludge should be removed as described above. However, this amount of calcium oxide may affect fish in nearby areas when water is pumped into the pond again. According to Finlay (10), use of sodium hydroxide (1% + 0.1% teepol) is also recommended for disinfection purposes. This should be applied at a minimum rate of 2 l/m^2, and the pond should subsequently be left dry for several days.

Disinfection of fibreglass or concrete tanks

The use of high-pressure (> 5,000 kPa [50 bar]) cleaners on surfaces made of non-painted concrete should be avoided to prevent the surface from resembling a 'lunar landscape' of cracks and pores after washing (G. Schaller, personal communication). This type of surface will make thorough disinfection almost impossible, as the disinfectant does not penetrate sufficiently into the cracks and pores (even with the use of a detergent). If unpainted concrete is to be disinfected, the concrete should be washed using water at low pressure and an alkaline detergent, preferably in combination with mechanical brushing. The washing procedure must start at the top and proceed downwards. The disinfectant should then be sprayed onto the surfaces, starting from the bottom and proceeding upwards. After sufficient contact time, the surface should be rinsed using clean water. It is recommended to paint the concrete after disinfection, preferably using a latex or acrylic paint.

Disinfection of installations in sea water

Floating installations should preferably be dismantled and brought on shore for drying prior to disinfection. They must be cleaned using a high-pressure cleaner in combination with mechanical brushing, or other available equipment. When the installation is visibly clean, it may be disinfected. The preferred disinfectant would be an aldehyde- or phenolic-based disinfectant. These compounds have little corrosive impact on materials. When commercially-available products are used, the dilution rate recommended by the manufacturer should be respected. Formaldehyde-based products

should not be used below 10°C, as their efficacy is temperature dependent (30). Recommended concentrations for pure substances are listed in Table I.

In many cases, it is very difficult, laborious and costly to dismantle floating installations and bring them on shore. Such installations may be disinfected 'in place', at sea. The following procedure has been used successfully in Norway in recent years:

1. Remove and bring the cage nets on shore for treatment as described above; special washing machines are available for cage nets.

2. On all constructions made of metal, fibreglass, plastics or other hard synthetic surfaces, a high-pressure cleaner with an alkaline detergent should be used (if necessary in addition to mechanical washing) until all surfaces are visibly clean. For some constructions, the assistance of a scuba diver will be necessary to reach some areas of the installation.

3. All parts of the installation which are above the water level may then be disinfected as for ordinary surface disinfection. However, particular care should be taken in the choice of disinfectants, as some products are corrosive when used over a prolonged period.

4. Finally, the underwater part of the installation should be wrapped in a tarpaulin. The interior of the tarpaulin should be pumped dry and then filled with clean water containing a disinfectant at an appropriate dilution. Most disinfectants may be used, even hypochlorite, as this procedure is of relatively short duration and the surfaces are washed clean as soon as the tarpaulin is removed. In Norway, 500-1,000 mg/l of sodium hypochlorite is often used for this procedure and the tarpaulin is kept in place for approximately 24 h. When using sodium hypochlorite for such a purpose, central or local environmental authorities must be consulted.

Even after 24 h, the amount of residual hypochlorite will probably still be so high that it is toxic to fish. Care should therefore be taken when the tarpaulins are emptied in order not to stress or kill fish in nearby cages.

This procedure is not applicable on wooden constructions. These constructions should be dismantled and brought on shore to be dried and disinfected with an appropriate disinfectant before being returned.

Disinfection of pipelines, outdoor areas and buildings

Before pipelines are disinfected, algae and mussels attached to the inside of the pipelines must be removed as much as possible. Accumulation of algae and mussels in the pipeline system can be prevented or minimised by eliminating areas ('blind-side' pipes) where the water circulation is minimal. When disinfection is performed, it is almost impossible to reach these areas with sufficient disinfectant. Therefore, when constructing an aquaculture establishment, or when a renewal of the pipeline system is planned, efforts should be made to reduce the number of these non-useful sections of a pipeline. Where the unwanted presence of mussels is a problem, the water current may be reversed (simply by reversing the pumps) to enable fresh water to flow through the system. If algae are to be removed prior to disinfection, a well-known system from the oil industry may be adapted. Brushes may be placed inside the pipe to move through the system with the water current. Where this method is impossible (e.g. due to tight bends), a highly efficient detergent (e.g. 2 l of 15% NaOCl solution to 1,000 l of water) may be used before actual disinfection takes place. This will give a hypochlorite concentration of 300 mg/l. An equivalent amount of a chloramine may be used as disinfectant. After

the necessary contact time (30 min for NaOCl and 120 min for chloramine), clean water must be flushed through the pipeline system.

Disinfection of outdoor ground areas may have to be performed in cases of an outbreak of a notifiable disease, or whenever the veterinary or other governmental authorities decide. The most important areas are those where people, equipment and vehicles pass by. When the surface is asphalt, concrete or a similar substance, at least 0.4 l/m^2 of NaOH (2-3%), KOH (1%) or Na_2CO_3 (5%) should be used. On soil, dry $Ca(OH)_2$ may be applied, in addition to the alkaline solutions mentioned. In extreme cases, the upper surface layer of soil should be removed and/or covered by asphalt, concrete or other appropriate surface material.

Disinfection of buildings can be performed in one of several ways. The outside of buildings may be disinfected in the same way as any of the other surfaces described above. The surface should be cleaned using a high-pressure cleaner (except on unpainted concrete) or by mechanical washing. The disinfectant should be applied at a rate of > 0.4 l/m^2. After 24 h, surfaces should be repainted or, in the case of wood, either painted or treated with a wood preservative. For the inside of buildings, if application of a disinfectant is difficult or impossible, a room may be disinfected by fumigation with formaldehyde after rigorous cleaning and, if necessary, the temperature should be raised to > 15°C. If the air is too dry (< 70% humidity), water should be sprinkled on the floor and walls. Finally, the room must be sealed (air inlets, doors, windows and any other openings) using plastic foils, in order to keep the gas inside the room.

Two methods of fumigation may be employed, as follows:

a) Use 1 l undiluted formalin (commercial grade formaldehyde [30%]) and 300 g $KMnO_4$ per 20 m^3 volume. Place 1-2 l formalin in buckets made of metal or heat-stable plastic of at least 25 l capacity. Position the necessary number of buckets on the floor. Place 300 g $KMnO_4$ per litre of formalin **beside** each bucket. Beginning as far away from the door as possible, pour the $KMnO_4$ into the buckets, moving as rapidly as possible from one bucket to the next. A highly efficient disinfecting aldehyde gas develops in a few minutes. Keep the room sealed for at least 24 h.

b) Fumigation with formaldehyde may also be performed using paraformaldehyde at a rate of 1 kg per 100 m^3. Paraformaldehyde evaporates when heated to 190°C. The procedure to follow is the same as that described above under *a)*.

In connection with formaldehyde fumigation, sewage systems, pipelines and other conduits are disinfected using 0.3 l of commercial grade formaldehyde in 10 l of water. This mixture is poured into the systems, unless the pipelines can be dismantled and soaked in the disinfectant. After fumigation for 24 h, the room should be aerated for another 24 h before being used again.

Disinfection of transport vehicles

An important factor in the spread of diseases of aquatic animals is transport of aquatic animals by vehicles, which may act as vectors for disease transmission. This has been demonstrated in a study on the spread of infectious salmon anaemia (ISA) (33). In general, equipment used for transportation of aquatic animals should be specially constructed for this purpose. Surfaces which come into contact with transportation water or with aquatic animals or products must not be absorbent (e.g. not made of wood). All containers/tanks – whether these are permanent, temporarily mounted on a vehicle, or special containers for air transport – must be constructed in such a way that

cleaning and disinfection can be performed easily. The inside of the containers must therefore be without any of the blind areas which make cleaning and disinfection difficult. Pipes, gaskets and valves which come into contact with the transport water or aquatic organisms must be easy to dismantle for inspection, cleaning and disinfection. The material must be able to withstand either the chemical disinfectant to be used or damp heat at 100°C.

Cleaning and disinfection of 'well-boats'

For cleaning purposes, a high-pressure cleaner with a detergent should be used. Firstly, a 'clear deck' is established, consisting of the upper deck and rails from the stern to the aft, the exterior of hatches, the boom and the deck-crane. When this area has been cleaned, the 'well' system (with pipes, valves and other fittings) is cleared, beginning with the interior of the hatches and proceeding downwards to the bottom of the well.

The wash water collected in the bottom of the well should be continously emptied into the sea so that all areas are flushed with clean water.

An appropriate, non-corrosive chemical disinfectant may be applied to open surfaces. Disinfection of the interior of the pipes may be performed using either damp heat or a chemical disinfectant. The procedure should start with the interior and exterior pipelines, and valves and wells for the intake and outlet of water, followed by disinfection of the well from the bottom upwards to the top deck. Finally, the 'clear deck' is disinfected. After the necessary contact time, surfaces should be rinsed in the opposite direction with clean water so that all cleaning water is collected in the bottom of the well. The well is then emptied.

Disinfection of trucks/trailers and containers

The transport vehicle and the exterior of the transport container must be washed using a high-pressure cleaner, beginning at the top and front of the vehicle and proceeding backwards/downwards, including the exterior of any pipes. The bottom of the container, chassis, tyres and wheels should then be washed. Finally, the interior of the transport container should be cleaned from the top downwards, including the interior of any pipes, valves and pumps.

The disinfection procedure should begin with the interior of all pipes, valves and pumps, and may be performed using damp heat or a chemical disinfectant. On all other surfaces, the disinfectant should be applied in the opposite direction to the washing procedure. After an appropriate contact time, the transport system must be rinsed using clean water.

DISINFECTION IN RELATION TO THE SLAUGHTER OF FISH FROM AQUACULTURE

The necessity of disinfection in relation to the slaughter of aquatic animals has been discussed by several authors. Naturally, rigorous procedures for cleaning and disinfection must exist at a slaughterhouse which is preparing products for human consumption. In general, production premises and equipment should be cleaned daily. Cleaning and disinfection should be performed in accordance with an operational plan approved by the appropriate government authorities. In a slaughterhouse, clean and unclean sections must be carefully separated, as must waste and effluent. Separate,

closed drainage systems must also be present, leading from production areas, unloading areas and other areas exposed to unclean waste.

Results from epidemiological studies have demonstrated an increased risk of disease spread if a fish farm is located near a fish slaughterhouse (17). Under Norwegian legislation, all effluents must flow through a sieve arrangement (< 1 mm pore size) before further treatment. Material trapped in sieves, including sludge, is treated as waste or offal. All effluents are then treated by the use of approved methods and equipment to prevent the spread of an infection. Effluent treatment systems must be secured against blockages and/or other operational failures. In Norway, this procedure came into force in 1991, and the Central Veterinary Laboratory may approve (upon application) methods and technical equipment for the disinfection of waste water from fish slaughterhouses and related areas (Y. Torgersen, unpublished material). If the equipment is shown to be reliable, approval may be granted for a five-year period. The minimum requirement for approval of a method is that the method must demonstrate permanent inactivation of at least 99.9% of the causative agents of furunculosis and ISA. Infiltration into sand, gravel or similar material at a place and in a manner approved by the Regional Veterinary Officer (RVO) is also accepted. The currently-approved methods are listed in Table II. Table III shows the respective costs.

Waste or offal must be easy to collect and transport to a separate place of storage. Provisional storage may make use of chilling/freezing (< +4°C) or preservation with organic acid at pH < 4.0. All waste offal should be treated in accordance with the approved methods and equipment to prevent the risk of spreading an infection. Offal or other organic matter may be treated by incineration to ashes, burial at a place approved for the purpose by the RVO, or delivery to a rendering plant approved by the RVO. In general, the offal must be treated so that the causative agents of furunculosis and ISA are not detected in 1 g of offal. Otherwise, offal from the aquaculture industry is a valuable source of organic by-products which, after being ensilaged, may be used as ingredients in pet food, or in feed for livestock and fur animals. The principle of not recirculating organic by-products to aquaculture should be observed.

TABLE II

Methods approved by Norwegian veterinary authorities for disinfecting waste water

Method	Dose	Time required
Heat	60°C	10 min
	70°C	6 min
	75°C	5 min
	80°C	4 min
Formic acid	pH < 3.5	8 h
	pH < 4.0	24 h
Sodium hydroxide	pH > 12.0	24 h
Chemical precipitation + Cl_2	Initial chlorine concentration > 50 mg/l and residual chlorine > 5 mg/l	15 min
Chemical precipitation + ultra-violet light	> 25 mJ/cm^2	

TABLE III

Investment and daily cost of different methods of disinfecting waste water from fish slaughterhouses to a standard approved by Norwegian veterinary authorities

Method	Investment (US$)	Daily cost (US$) per ton of fish *
Precipitation + ultra-violet light	86,000	1.5
Precipitation + Cl_2	60,000	4.3
Heat	77,000	2.1
Formic acid (pH 3.5)	31,000	2.3
Sodium hydroxide (pH 12.0)	31,000	9.4

* Water consumption = 6 l/kg fish
Cost of 1 kWh = US$0.04
Cost of 1 kg NaOH = US$0.40
Cost of 1 l HCOOH (85%) = US$1.10
Cost of 1 kg $Ca(OCl)_2$ = US$7.00

Disinfection of intake water to farms raising aquatic organisms (hatcheries)

To reduce the risk of water-borne diseases, farms raising aquatic organisms should not be allowed to use water from a source which contains species equivalent to those raised on the farm, without thorough disinfection of the intake water.

In Norway, intake of such water is prohibited by legislation. However, the RVO may grant exemptions if the demands of the regulations for disinfection of intake water are fulfilled. These demands are as follows:

- Equipment must be approved by a competent body.

- Equipment must demonstrate permanent inactivation of at least 99.9% of the causative agents of furunculosis and ISA.

CONCLUSION

Due to the introduction of the measures described above – together with an understanding among fish farmers of the necessity of hygienic precautions and other disease prevention measures – the spread of infectious diseases between aquaculture establishments in Norway has been reduced.

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DÉSINFECTION EN AQUACULTURE. – Y. Torgersen et T. Håstein.

Résumé : *Les auteurs décrivent la sensibilité à divers désinfectants de quelques agents pathogènes importants en aquaculture et notamment en salmoniculture. Ils présentent certaines méthodes de désinfection applicables à l'aquaculture*

d'après l'expérience de la Norvège et d'autres pays où la salmoniculture est très développée. Outre la désinfection de routine sur les sites aquicoles, les auteurs exposent les procédures de désinfection réservées aux épidémies de maladies à déclaration obligatoire, ainsi que le traitement/désinfection des eaux usées et des déchets provenant de l'abattage et de la transformation des produits de l'aquaculture.

MOTS-CLÉS : Agents pathogènes - Aquaculture - Conduite d'élevage - Désinfection - Maladies des poissons.

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DESINFECCIÓN EN ACUICULTURA. – Y. Torgersen y T. Håstein.

Resumen: *Los autores describen la sensibilidad a diversos desinfectantes de algunos agentes patógenos importantes en acuicultura, y más particularmente en salmonicultura. Presentan ciertas prácticas de desinfección aplicables a la acuicultura a partir de la experiencia de Noruega y de otros países grandes productores de salmón. Además de la desinfección de rutina en los lugares en que se realiza esta producción, los autores también exponen los procedimientos de desinfección en caso de brotes de enfermedades de declaración obligatoria, así como el tratamiento/desinfección de las aguas usadas y de los despojos provenientes de la matanza y del procesamiento de los productos de la acuicultura.*

PALABRAS CLAVE: Acuicultura – Agentes patógenos – Desinfección – Enfermedades de los peces – Manejo.

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