

# Treatment of wastewater from fish slaugtherhouses

Evaluation and recommendations for hyginisation methods

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# Introduction

Prevention of fish diseases is essential for profitable operation of aquaculture facilities. Historically, pathogens (disease-causing bacteria, viruses, parasites or fungi) derived from fish processing companies have been involved in transferring infection. Not just the processing of Danish fish (mainly aquaculture fish) pose a significant risk, also increased globalization with escalating transport of fishery products enlarge the risk of transfer of pathogenic micoorganisms, which ae a risk not only for aquaculture fish but just as much to wild fish. In Denmark, especially infection with the highly loss-causing fish disease VHS has been crucial. Several disease outbreaks could be associated with release of infectious waste from cutting plants that had slaughtered/processed VHS infected fish. Based on this, the National Veterinary and Food Administration in 2005 introduced requirements for either the sanitisation or percolation of wastewater from fish processing plants. The claim was however introduced without that there was a final decision on whether the proposed methods were effective under Danish conditions and without assessing the business economics of the claim. The requirement would apply to existing companies from September 2008. It was, however, difficult to implement the required methods, as no one in the industry knew what methods were most successful based on financial, operational and disease prevention rationale. The Danish Veterinary and Food Administration has per August 2008 amended the order requiring that all fish processing companies now base their wastewater treatment of on percolation. This is far from possible at many of the existing Danish fish processing companies because of location and soil conditions. Based on this the order opens the possibility to apply for dispensation to sanitize the wastewater instead. However, there are no pre-approved methods, and it is therefore imposed on the industry to generating knowledge in the field. All companies must by the end of 2010 meet the requirements.

# Objectives of the project

- To provide knowledge about existing and new methods to sanitize wastewater from cutting plants.
- To evaluate the disinfecting effect of wastewater perculation under laboratory conditions.
- Through the above to achieve the best possible security measures to avoid spread of fish diseases to both aquaculture and wild fish.

#### Content of the project

- Desribe and evaluate the current methods for disinfection of wastewater from fish cutting plants.
- Recommend methods which are acceptable based on disease transfer risks.
- Assess the disinfecting effect of percolation under laboratory conditions.
- Produce a report, which describes the different methods and recommendations to future requirements taking into account the optimum effectivity, efficiency, reliability and economy.

This report deals with the objective described in the first bullet point and thus do not take solid waste into consideration. Perculation will be described in further details in a following report concerning the second bullet point.

#### **Conclusions**

Based on literature studies a number of methods for sanitazion of process wastewater from fish slaughterhouses/cutting plants are acceptable. Most of these methods are described in the historic Danish legislation "Bekendtgørelse nr. 755 af 28/07/2005 om bekæmpelse af visse smitsomme sygdomme hos fisk" (Ministerial orden no. 755 of 28/07/2005 regarding control of certain infectious diseases in fish) annex 1 and are approved according to the present Norwegian legislation "FOR 1997-02-20 nr 192: Forskrift om desinfeksjon av inntaksvann til og avløpsvann fra akvakulturrelatert virksomhet" (Regulation 1997-02-20 no 192: Regulation concerning disinfection of intake water and outlet water from aquaculture related enterprises). The methods are able to reduce the amount of virus 3 log for VHSV, IHNV, and ISAV. IPNV may not necessarily be reduced by 3 log using the recommended methods. For the pathogenic bacteria *Yersinia ruckeri*, *Aeromonas salmonicida* and *Vibrio anguillarum* the methods are also acceptable.

pH treatment:

- a) Mechanic separation (≤ 300  $\mu m$  filter) followed by acid treatment to pH  $\leq$
- 3.0 for ≥ 8 hours.
- b) Mechanic separation (≤ 300 µm filter) followed by basic treatment to pH ≥
- 12.0 for  $\geq$  24 hours.

**Chlorination:** 

- a) Mechanic separation ( $\leq$  300  $\mu m$  filter) or chemical precipitation (Fe- and/or
- Al-salts) followed by chlorination of the supernatant using an initial concentration of  $\geq$  50 mg/l residual chlorine and  $\geq$  10 mg/l residual chlorine
- after 15 minutes treatment.
- b) Mechanic separation (≤ 300 µm filter) or chemical precipitation (Fe- and/or
- Al-salts) followed by chlorination of the supernatant using an initial
- concentration of  $\geq$  50 mg/l residual chlorine and  $\geq$  2 mg/l residual chlorine
- after 25 minutes treatment.

Heat treatment:

- a) 65°C for 10 minutes.
- b) 70°C for 5 minutes.
- c) 75°C for 4 minutes.
- d) 80°C for 3 minutes.
- e) 85°C for 2 minutes.
- f) 90°C for 1 minute.
- g) 95°C for 45 seconds.
- h) 100°C for 30 seconds.

N.B. Proper stirring is necessary to make certain that no pockets with

inappropriate heating exist.

**UV-irradiation:** 

For wastewater treatment the method cannot at present be recommended as sanitizing method, as wastewater will be too organic polluted without a

significant clarification before irradiation.

Ozone:

Mechanic separation (≤ 300 μm filter) or chemical precipitation (Fe- and/or Al-

salts) followed by ozone treatment

a) fresh water: ≥ 0,15 mg/l residual ozone after 15 minutes treatment.

b) salt water: ≥ 0,2 mg/l TRO (total residual oxidants) after 15 minutes

treatment.

#### **Percolation**

Although generally considered a safe method for wastewater sanitation it has not been possible to find any references describing the decimating effect of percolation on fish pathogenic viruses. As a substitute for IPNV, it has not been possible to find publications describing the effect of percolation on other birnaviruses. The effect of percolation on other viruses has not been looked into. It is therefore not possible in this report to validate if the procedure is safe to use.

# Legislation

# **Danish legislation**

In the historic legislation "Bekendtgørelse nr. 755 af 28/07/2005 om bekæmpelse af visse smitsomme sygdomme hos fisk" (Ministerial orden no. 755 of 28/07/2005 regarding control of certain infectious diseases in fish) annex 1 describes different disinfection methods that, at that time, were allowed to use. These are:

Formic acid (HCOOH): Mechanic separation (≤ 300 μm filter) followed by treatment using formic acid

to a) pH  $\leq$  4.0 for  $\geq$  24 hours, or b) pH  $\leq$  3.5 in  $\geq$  8 hours.

NaOH: Mechanic separation (≤ 300 μm filter) followed by treatment using NaOH to

pH  $\geq$  12.0 for  $\geq$  24 hours.

**UV-irradiation:** a) chemical precipitation (Fe- and/or Al-salts) followed by UV irradiation of the

supernatant using an UV-dose  $\geq 25$  mWs/cm<sup>2</sup>.

b) Mechanic separation (≤ 40 µm filter) followed by UV irradiation of the

supernatant using an UV-dose  $\geq$  25 mWs/cm<sup>2</sup>.

Chlorination: a) mechanic separation (≤ 300 µm filter) or chemical precipitation (Fe- and/or

Al-salts) followed by chlorination of the supernatant using an initial

concentration of  $\geq$  50 mg/l residual chlorine and  $\geq$  10 mg/l residual chlorine

after 15 minutes treatment.

b) mechanic separation (≤ 300 µl filter) or chemical precipitation (Fe- and/or

Al-salts) followed by chlorination of the supernatant using an initial

concentration of  $\geq$  50 mg/l residual chlorine and  $\geq$  2 mg/l residual chlorine

after 25 minutes treatment.

a) 65°C for 10 minutes. Heat treatment:

b) 70°C for 5 minutes.

c) 75°C for 4 minutes.

d) 80°C for 3 minutes.

e) 85°C for 2 minutes. f) 90°C for 1 minute.

g) 95°C for 45 seconds.

h) 100°C for 30 seconds.

#### Percolation

In the present legislation "Bekendtgørelse nr. 755 af 08/07/2008 om autorisation og drift af akvakulturbrug og —virksomheder" (Ministerial order no. 755 of 08/07/2008 regarding authorisation and operation of aquaculture farms and — enterprises) fish cutting plants are according to § 14 obliged to percolate process wastewater. Wastewater may, after permission from the Danish Veterinary and Food Administration, also be discharged to seawater. Dispensation from percolation of wastewater can, according to § 15, be permitted if the wastewater is disinfected and the chosen method result in a complete inactivation of infectious matters.

# Legislation in the USA

Dr. P. Gary Egrie from the USDA APHIS Veterinary Services, informed that the Environmental Protection Agency (EPA) has regulatory authority for effluents into public waterways, however they have not developed regulations or recommended methods to address the discharge of aquatic animal pathogens from fish slaughterhouses.

# **Legislation in Norway**

In Norway the legislation "FOR 1997-02-20 nr 192: Forskrift om desinfeksjon av inntaksvann til og avløpsvann fra akvakulturrelatert virksomhet" (Regulation 1997-02-20 no 192: Regulation concerning disinfection of intake water and outlet water from aquaculture related enterprises) regulates the effluents from fish slaugtherhouses. In this legislation it is described in § 9 that outlet water from fish slaugtherhouses/fish cutting plants has to be filtered through a grating before further treatment. The size of the grating has be ≤ 1 mm. In § 10 hourshe demands for the methods for desinfection of the outlet water after filtration are described. The metods have to be documented through scientific documentation based on relevant experimental test designs (water quality, Temperature etc.) to induce at least 3 log (99,9%) inactivation of Aeromonas salmonicida subsp. salmonicida and ISAV, or based on a dose-repons curve for IPNV it is likely that ISAV is inactivated likewise. In § 11 on demands to the technical equipment it is described that approved technical equipment shall at least be equipped with security measures which guaranties that the disinfectant's/method's "concentration" (mg/l, mWs/cm2, °C etc.) and time are kept. Furthermore a safety device against malfunction and a recording unit has to be installed. According to § 8 the National Veterinary Institute (Veterinærinstituttet) is responsible for approval of methods. The approved methods can be found on the Norwegian Food Safety Authority (Mattilsynet) homepage and are as follows:

Formic acid (HCOOH): a) pH  $\leq$  4.0 for  $\geq$  24 hours,

or b) pH  $\leq$  3.5 in  $\geq$  8 hours.

NaOH:  $\geq$  12.0 for  $\geq$  24 hours.

UV-irradiation: Chemical precipitation followed by UV irradiation of the supernatant using an

UV-dose  $\geq$  25 mWs/cm<sup>2</sup>.

Chlorination: a) mechanic separation or chemical precipitation followed by chlorination of

the supernatant using an initial concentration of ≥ 50 mg/l residual chlorine

and ≥ 10 mg/l residual chlorine after 15 minutes treatment.

b) mechanic separation or chemical precipitation followed by chlorination of the supernatant using an initial concentration of  $\geq$  50 mg/l residual chlorine

and ≥ 2 mg/l residual chlorine after 25 minutes treatment.

Heat treatment: a) 65°C for 10 minutes.

b) 70°C for 5 minutes.

c) 75°C for 4 minutes.

d) 80°C for 3 minutes.

e) 85°C for 2 minutes.

f) 90°C for 1 minute.

g) 95°C for 45 seconds.

h) 100°C for 30 seconds.

These methods are basically the same as the methods in the former Danish legislation.

Ozone is not on the list for disinfection methods for wastewater from fish slaugherhourses or cutting plants. This method is approved for disinfection of wastewater from infection trial facilities handling A-, B- and C diseases, exotic and unknown pathogens. In facilities like this the following methods are approved:

Heat treatment: d) 80°C for 4 minutes.

e) 85°C for 3 minutes. f) 90°C for 2 minute. g) 95°C for 1 minut. h) 100°C for 30 seconds.

Chlorination: a) freshwater: ≥ 25 mg/l residual chlorine after 30 minutes treatment.

b) sea water: ≥ 35 mg/l residual chlorine after 30 minutes treatment.

Ozonation: a) freshwater: ≥ 0,15 mg/l residual ozone after 15 minutes treatment

(corresponds to a CT value of 135 mg\*s/l).

b) sea water: ≥ 0,2 mg/l TRO (total residual oxidants) after 15 minutes

treatment (corresponds to a CT value of 180 mg\*s/l).

Slaugther offals, which are not to be used as feed/food, shall be treated in accordance with the provisions laid down in regulation on animal by-products. This also applies to the organic sludge produced in conjunction with treatment of wastewater.

#### Legislation in UK

In England the solid waste from fish slaugtherhouses/cutting plants is regulated by the Animal By-products regulations. The Animal By-Products legislation dictates how different categories of solid waste are disposed of, including tissues from diseased animals. The latter would be Category 2 waste, and waste from apparently healthy animals would be Category 3 waste.

Assuming a fish processing plant is processing category 3 animal by-product/material then all waste/wash water is controlled under waste/environmental legislation and the operator needs to contact the Environment Agency (EA) regarding controls on this. If it is processing category 2 animal by-product/material then it needs to have a pre-treatment process - essentially a 6 mm mesh with all material caught in the drain trap disposed of as category 2. However again once the liquid has passed through the trap it is a matter for environmental regulation to control it.

The EA would expect that the wash water would go to a foul sewer under a Trade Effluent Agreement with the sewerage undertaker (local water company). The sewerage undertaking might require some filtering or

other treatment to be done before discharge to sewer. The wash water should not be sent to surface water drainage or be directly discharged to a river. However, if the premises were 'in the middle of no-where' with no sewerage links then the company would need a discharge consent from the Environment Agency; they would probably require some pre treatment dependent upon analysis of the proposed discharge. The alternative would be to tanker off site to a sewage works. There does not seem to be any requirements to treat the liquid on site regarding inactivation of pathogens (personal communication from Peter Dixon, CEFAS).

#### **Materials and Methods**

These procedures in the former Danish legislation will be evaluated, based on a literature review, together with potential other procedures for their capability to decimate fish pathogens. The underlying basis will be the non exotic fish pathogens listed in "Council Directive 2006/88/EC of 24 October 2006 on animal health requirements for aquaculture animals and products thereof, and on the prevention and control of certain diseases in aquatic animals". These diseases are viral haemorrhagic septicaemia (VHS) caused by VHS virus, infectious haematopoietic necrosis (IHN) caused by IHN virus, koi herpes virus (KHV) disease caused by KHV and infectious salmon anaemia (ISA) caused by ISA virus.

For the Danish aquaculture industry there are a number of other important pathogens such as e.g. infectious pancreatic necrosis virus (IPNV) causing IPN, *Renibacterium salmoninarum* causing bacterial kidney disease, *Aeromonas salmonicida* causing furunculosis, *Yersinia ruckeri* causing enteric redmouth disease and *Flavobacterium psychrophilum* causing rainbow trout fry syndrome.

Some of these diseases are furthermore listed in the Danish "Bekendtgørelse nr. 975 af 13/08/2010 om lister over smitsomme sygdomme til lov om hold af dyr" (Ministerial order no 975 of 13/08/2010 concerning listing of infectious diseases in relation to legislation regarding keeping of animals).

### Different kinds of methods for desinfection

#### Acid

Lowering of the pH to an unfavourable niveau for microorganisms can be done by using organic acids and inorganic acids. For the inorganic acid the effect is solely based on the pH denaturing the proteins. Beside the pH effect the organic acids will enter the fish cells more easily than inorganic acids and thereby enhance the speed of which autolysis of the cells occur, whereby e.g. viral particles within the cells will be reached.

An example of an often used organic acid is formic acid, HCOOH. Formic acid is the simplest carboxylic acid, named after the Latin word for ant, formica, as this is the acid produced by the ant. Formic acid has an acid dissociation constant at the logarithmic scale ( $pK_a$ ) of 3,7 and is a week acid.

An example of an inorganic acid that often is used to lower pH is hydrochloric acid, HCl. Depending on the source the  $pK_a$  for HCl is stated as -7 to -3 which means that in water the HCl will be completely dissociated into H<sup>+</sup> and Cl<sup>-</sup>.

If the wastewater is pretreated for example by filtration so lumps of waste (e.g. fish flesh) are not present, there will probably not be much difference in the effect whether an organic or inorganic acid is used to lower the pH.

#### **Base**

A high pH is unfavourable for microorganisms. To raise the pH sodium hydroxide, NaOH, can be used. NaOH is a strong base with a  $pK_a$  of approximately 13. NaOH is very soluble in water with liberation of heat. NaOH does not react with iron, but it will react with transition metals such as e.g. aluminium.

#### **UV** irradiation

UV light is divided into three ranges UV-A (320-400 nm), UV-B (280-320 nm) and UV-C (190-280 nm). The highest capacity to damage microorganisms is found in the UV-C band. The damaging effect of UV light is caused by the altering effect the UV-light has on nucleic acids. When the light is absorbed by the DNA/RNA molecule dimerization of two pyrimidine molecules can occur. This will lead to blocking of the replication (reviewed in 68).

Different kinds of microorganisms are more or less susceptible to UV light. Generally speaking, the susceptibility is higher for growing bacteria than for viruses and bacterial spores.

The intensity of the UV light and the time of irradiation are important factors for the ability of the light to inactivate the microorganism. The UV dose is the multiplication of the intensity and the time and is a measure for the amount of energy which reaches a surface. The dose is often expressed as mWs/cm<sup>2</sup>.

As UV light is not killing the microorganism there is a possibility for the microorganism to repair the damages of the DNA by photoreactivation. Light in the visible spectrum is able to activate enzymes which can repair the damages induced by the UV light (reviewed in 68). Keeping bacteria in the dark after UV irradiation for 15 hours will inhibit the photoractivation (72). There are, though, also different processes that may occur in the dark in bacterias that are able to repair damages. There is as such a possibility for bacterias to regain the ability to multiply after being exposed to UV light. Increasing the UV dose will decrease the ability for the bacteria to repair itself (72).

#### **Chlorination**

Chlorine is a well known disinfectant used for decades both in the industry as well as in the household. The effectivity of chlorine is dependent on factors such as pH, temperature, suspended solids, organic compounds and nitrogen containing compounds. Low pH, high temperature, and no suspended solids, organic compounds etc will enhance the disinfecting effect.

Chlorine kills pathogens such as bacteria and viruses by replacement of hydrogen atoms by chlorine breaking the chemical bonds in their molecules. The molecule will the change shape or fall apart. As the enzymes are destroyed the pathogen will eventually die.

When chlorine is added to water, underchloric acids form and depending on the pH value, underchloric acid

partly expires to hypochlorite ions:  $Cl_2 + H_2O \rightarrow HOCl + H^+ + Cl^ HOCl + H_2O \rightarrow H_3O^+ + OCl^-$ 

This falls apart to chlorine and oxygen atoms:  $OCI \rightarrow CI + O$ 

Underchloric acid (HOCl) is more reactive and is a stronger disinfectant than hypochlorite (OCl). HOCl is split into hydrochloric acid (HCl) and atomar oxygen (O). So the disinfecting properties of chlorine in water are based on the oxidising power of free oxygen atoms and on chlorine substitution reactions.

The cell wall of pathogenic microorganisms is negatively charged by nature. As such, it can be penetrated by the neutral underchloric acid, rather than by the negatively charged hypochlorite ion. Underchloric acid can penetrate slime layers, cell walls and protective layers of microorganisms and effectively kills pathogens as a result. The microorganisms will either die or suffer from reproductive failure.

HOCl reacts faster and is more effective than OCl<sup>-</sup>. The level of HOCl will decrease as the pH value goes up. The optimal pH for using chlorine with be at pH 5,5-7,5 (2).

#### Heat

Different combinations of temperture and time are able to inactivate microorganisms. When proteins are heated their three-dimensional structure will be destroyed as the weak hydrogen bonds dissociate due to the vibrations caused by heating. With destroyed proteins the microorganisms will not function properly.

#### **Iodine**

lodine comes from the Greek word iodes meaning violet or purple. Elemental iodine, iodide or iodine from iodophors (iodine complexed with a solubilising agent that releases free iodine when in solution) are used for disinfection. The disinfecting ability of iodine is less influenced by the pH than chlorine as long as the pH is below 8-9. Iodine is widely used in the aquaculture industry for disinfection of eggs.

#### **Ozonation**

Ozone is a potent oxidant with bactericidal and virucidal abilities. Ozone decomposes rapidly to oxygen limiting the ability to maintain a sufficient residual ozone concentration for the necessary time period. Compared to freshwater more longlived reaction products are formed when brackish and seawater are ozoneated. The damaging effect of ozone is probably caused by changes in the membrane structure causing leakage of proteins and nucleic acids, as well as lipid oxidation (reviewed in 68).

#### Percolation

Percolation concerns the movement of fluids through porous materials, here the movement of wastewater through underground earth. The hope is, that this transport will withheld pathogenic microorganisms and in this way "disinfect" the wastewater.

#### **Discussion**

In the present Danish legislation "Bekendtgørelse nr. 755 af 08/07/2008 om autorisation og drift af akvakulturbrug og —virksomheder" (Ministerial order no. 755 of 08/07/2008 regarding authorisation and operation of aquaculture farms and — enterprises) fish cutting plants are obliged to percolate process wastewater. Dispensation from percolation of wastewater can be permitted if the wastewater is disinfected and the chosen method results in complete inactivation of infectious matters. A complete inactivation of pathogens will not be a realistic goal as this will require sterilization of the wastewater, which is not economical feasible for the industry. In order to reduce the risk of transfer of diseases to a tolerable level a lower intensity of inactivation is acceptable. In Norway the requirement is a 3 log reduction for the pathogens *Aeromonas salmonicida* subsp. salmonicida and ISAV. Under Danish conditions a 3 log reduction will probably also be appropriate in order to reduce the risks to an acceptable level.

In the scientific literature numbers are available to give an indication of the amount of virus that may be present in process wastewater and in fish offals and how little virus that can initiate an outbreak.

In seawater from Pacific herring confined for the production of spawn-on-kelp 700 pfu/ml VHSV was observed (38). In infection trials using Pacific herring up to  $10^{7,7}$  pfu/g herring was detected at 6-8 days after infection. In the water in the flow-through aquarias at day 4-5,  $10^{2,5}$  pfu/ml was obtained. When the water flow was turned off for 3 hours the water reached  $10^{3,5}$  pfu/ml water. Virus shed by infected herring was on avereage >  $10^{6,5}$  pfu/h/fish (62).

When groups of wild herring were confined in the laboratory, they experienced severe mortality, occasionally exceeding 50%, with the prevalence of VHSV reaching 100% by 14 d postcapture. At 7-21 d postcapture, VHSV titers peaked in excess of 10<sup>8</sup> pfu/g of tissue (63).

In rainbow trout infected with the freshwater isolate DK-3592B, the fish were positive at a low titer (7.1 x  $10^2$  TCID<sub>50</sub>/g of tissue) by day 2 postchallenge, and the titer reached a peak (1.3 x  $10^8$  TCID<sub>50</sub>/g of tissue) by day 7 (13).

Another infection trial reported mean titres of 5,3 x  $10^6$  TCID<sub>50</sub>/g of tissue (29).

In experimentally infected rainbow trout challenged with VHSV by bath with  $10^2$ ,  $10^{3,7}$ , and  $10^5$  TCID<sub>50</sub>/ml of the cumulative mortality was 44, 64, and 96%, respectively, at 14 d post infection (26).

In an experimental infection trial using the isolate J167 from the English outbreak in 2006, an infection dose as low as  $10^1$  TCID<sub>50</sub>/ml water resulted in an accumulated mortality of 65% at day 21 in rainbow trout fry (19).

Vestergaard Jørgensen & Olesen reported that at the time when VHSV koncentration is highest in the fish  $(10^8 \, \text{pfu/g} \, \text{of tissue})$ , the amount of virus particles (pfu) in the water can be as high as  $10^3 \, \text{per ml}$  of water (50). In infection trials at  $10^{\circ}\text{C}$ , the incubation phase was  $1\frac{1}{2}$  week when using a viral dose of 50 pfu/ml water. When the dose was lowered to 25 pfu/ml of water the incubation phase was extended to 6 weeks and at a dose of 10 pfu/ml, VHS was not observed during the next 6 months. The authorms comments this results by noting that 10 pfu/ml may be to low a dose under the circumstances used in the infection trial or that the incubation period may be longer than the 6 months, but that under other circumstances such a low dose will be able to initiate an outbreak (50).

These results show that the amount of VHSV can be quite high in tissue from VHSV infected fish during an outbreak, and a dose of virus as low as  $10 \text{ TCID}_{50}/\text{ml}$  water may be able to initiate an outbreak. The amount

of virus that can be found in water during an outbreak is  $10^3$ - $10^4$  TCID<sub>50</sub>/ml water. A 3 log reduction of virus will reduce the amount of VHSV to 0-10 TCID<sub>50</sub>/ml water, a dose that probably only very seldom will be able to initiate a VHS outbreak.

In 11 of 15 wild-caught sockeye salmon in prespawning conditions IHNV was isolated from the following organs at a mean level among the positive organs (min – max) in pfu/g tissue of:

Gills 5,8 x  $10^3$  (3,0 x  $10^2$  - 5,5 x  $10^5$ , 10 positive), Kidney 5,8 x  $10^3$  (1 positive), spleen 5,2 x  $10^2$  (1,0 x  $10^2$  – 1,3 x  $10^3$ , 3 positive), pyloric caeca 5,1 x  $10^2$  (2,5 x  $10^{1-}$  1,4 x  $10^4$ , 4 positive), Brain 5,0 x  $10^1$  (1 positive) and eggs 4,0 x  $10^2$  (1 positive). From fish in spawning conditions IHNV was isolated from nil to 100% of the fish within 2 weeks and virus incidens was high in all organs and fluids except brain and serum (77).

In a study of the possible role of waterborne IHNV in transmission of the disease among spawning sockeye salmon both infection rates and virus titres were higher in fish held at high density in a side channel than in fish in the adjacent river. Virus was never isolated from river water, but was found in water from the side channel at levels ranging from 32.5 to 1600 pfu/ml (78).

In rainbow trout the amount of IHNV i ovarian fluid ranged from  $10^1 - 10^{6.5}$  TCID<sub>50</sub>/ml (7). In an infection trial in rainbow trout using IHNV mean titres of 5,1 x  $10^5$  TCID<sub>50</sub>/g of tissue was reported (29). As these values are correspondable to the VHSV values a 3 log reduction will also be acceptable for IHNV.

For IPNV Wolf & Quimby (unpublished, in 101) reported average IPNV titer in five adult carrier brook trout in  $TCID_{50}/g$  tissue ranging from  $10^{0,3}$  in muscle to  $10^{6,7}$  in kidney. In an IPNV infection trial in brook trout virus was shedded in the feces 8 weeks post infection at a mean titre of  $10^{3,5-4}$   $TCID_{50}/g$  (12). In another infection trial using IPNV Sp, moribund rainbow trout alevins kept at  $16^{\circ}$ C had a titer of  $10^{4} - 10^{6}$  pfu/g fish, whereas alevins kept at  $10^{\circ}$ C which had a titer up to  $10^{8}$  pfu/g (21).

In a hatchery outbreak, a level of  $10^{4.4}$  infective particles per ml in a tank supplied with 88 l/min of water was measured (101). Desautels & MacKelvie (17) titrated water from three troughs of trout fry during a serious IPN epizootic in a commercial rearing establishment at found an excess of  $10^5$  TCID<sub>50</sub>/ml. It is assumed that the amount of virus in process water will be less that the amount found in water during an IPN outbreak, and as such a 3 log reduction will also for this virus reduce the amount to an acceptable risk, regarded the water is not released to watersheds where IPNV free farms are situated downstream.

In Norway at present and in the historic Danish legislation a number of different methods are/were approved for sanitazion of wastewater. These included treatment with

#### pH (acidic):

Mechanic separation (≤ 300  $\mu$ m filter) followed by acid treatment to pH ≤ 3.0 for ≥ 8 hours.

The literature review showed that VHSV, IHNV and several other viruses are not inactivated by treatment at a pH of 4 for 24 hours. In order to decimate VHSV and IHNV to a non detectable level a treatment of pH 3 for 3-4 hours is needed. This will also inactivate *Aeromonas salmonicida* and probably also salmonid alphavirus and ISAV. *Yersinia ruckeri* will be decimated to some degree by this treatment, but not necessarily 3 log. Nodavirus is extremely acid stable and will not be inactivated by acid conditions. IPNV is also very stable at low pH, but pH < 2 should be able to inactivate IPNV (as well as *Yersinia* ruckeri) although survival for 35 days at pH 2 has been reported. Ranavirus has been recorded to both survive and be inactivated at pH 4.

#### pH (basic):

Mechanic separation ( $\leq$  300 µm filter) followed by basic treatment to pH  $\geq$  12.0 for  $\geq$  24 hours.

VHSV, IHNV, IPNV, SVCV, PFRV, SAV and *Aeromonas salmonicida* are inactivated at pH 12 for 24 hours. Nodavirus has been reported inactivated at that pH but also to survive. *Yersinia ruckeri* is also difficult to inactivate at pH 12, but will be decimated.

Ranavirus has been recorded to both survive and be inactivated at pH 12.

#### **UV-irradiation:**

- a) chemical precipitation (Fe- and/or Al-salts) followed by UV irradiation of the supernatant using an UVdose  $\geq$  25 mWs/cm<sup>2</sup>.
- b) Mechanic separation ( $\leq$  40  $\mu$ m filter) followed by UV irradiation of the supernatant using an UV-dose  $\geq$  25 mWs/cm<sup>2</sup>.

In laboratory trials a dose of 25 mWs/cm² (254 nm) induces satisfactory decimations of VHSV, IHNV and ISAV. In laboratory trial using wastewater from a fish cutting plant 3,1 mWs/cm² was needed to decimate VHSV 3 log. For IHNV 4 mWs/cm² was needed for a 3 log reduction in laboratory trials. For ISAV the needed dose for a 3 log reduction was 7,5 mWs/cm². In infection trial using tissue homogenate from ISA infected fish, a dose of 20 mWs/cm² was needed to decimate the virus so much that ISA was not induced in the IP injected fish. The bacteria *Aeromonas hydrophila*, *A. salmonicida*, *Vibrio anguillarum* and *Yersinia ruckeri* a 3 log decimation was obtained using a dose of 5-25 mWs/cm² in laboratory trials. For *Y. ruckeri*, in full scale trials using wastewater from fish slaugtherhouses a dose of 250 mWs/cm² gave a reduction of only 1 log despite precipitation with ferrichlorid;  $2\frac{1}{2}$  log was obtained using a dose of 1200 mWs/cm² and prefiltration with a 20 µm filter.

IPNV is far more resistant to UV light than VHSV and IHNV. In laboratory trials a dose of 200-250 mWs/cm² was required to obtain a 3 log reduction, and 800 mWs/cm² was needed for a 6 log reduction. In full scale trials using wastewater from fish slaugtherhouses a dose of 250 mWs/cm² produced only a ½ - 1 log reduction in virus titer. In order not to detect IPNV anymore a dose of 1500 mWs/cm² was needed. Nodavirus also seems to be quite resistant to UV irradiation. In laboratory trials a dose of 100-211 mWs/cm² has been reported to induce a 3 log reduction. In an infection trial a dose of 100 mWs/cm² of the virus was reported to inhibit disease in the fish.

The results from the full scale trials suggest that even though UV irradiation in laboratory trials is effective it may not be possible to use this method on process wastewater in fish slaugtherhouses/cutting plants, despite pretreatment of the water by filtration or chemical precipitation. The authors conclude though that the ineffectual pretreatment probably was due to operating problems and inadequate optimisation of the process. Further full-scale tests showed that the quality of the wastewater was improved by chemical precipitation, and the best result was obtained by first adding ferrichlorid to pH 3,9 followed by addition of NaOH to pH 6,4, polymerisation and flotation. This treatment reduced the amount of organic matter with 65% measured as COD (chemical oxygen demand) and reduced the amount of total nitrogen with 73%. Furthermore was fat and floating material separated in the flotation tank (28). Whether UV irradiation after this treatment whould provide an acceptable reduction of the pathogens is unknown but probable.

#### **Chlorination:**

- a) mechanic separation ( $\leq$  300  $\mu$ m filter) or chemical precipitation (Fe- and/or Al-salts) followed by chlorination of the supernatant using an initial concentration of  $\geq$  50 mg/l residual chlorine and  $\geq$  10 mg/l residual chlorine after 15 minutes treatment.
- b) mechanic separation ( $\leq$  300  $\mu$ m filter) or chemical precipitation (Fe- and/or Al-salts) followed by chlorination of the supernatant using an initial concentration of  $\geq$  50 mg/l residual chlorine and  $\geq$  2 mg/l residual chlorine after 25 minutes treatment.

Generally speaking the amount of chlorine needed depends on the temperature, the pH, the degree of organic contamination and the titer of the pathogen. The necessary amount will rise if the temperature fall, the pH rise, more dirty conditions prevail and the titer of the pathogen goes up. The dose mentioned in the Danish ministerial order no 755 of 28/07/2005 and in the Norwegian list of approved methods (50 mg/l free chlorine (10 mg/l residual chlorine efter 15 min or 2 mg/l residual chlorine after 25 min) is probably acceptable for a 3 log reduction under clean conditions for VHSV, IHNV and bacterial pathogens, and also for the more resistant viruses such as IPNV and nodavirus. But for the conditions that prevail in wastewater from fish cutting plants this dose will not be acceptable without a proper pretreatment of the water. For *Yersinia ruckeri* the dose to induce a 3 log reduction is 250 mg/l for more than 2 hours in full-scale tests using wastewater from fish cutting plants. In a full-scale test using NaOCI IPNV was stable at the same dose administered for 1 hour, whereas when the dose was administered as chloramine-T a 4 log reduction was obtained.

In a full-scale trial where the wastewater was pretreated by adding NaOH to pH 12 followed by addition of ferrichlorid to pH 6,5-7,5 *Y. ruckeri* was inactivated using 48 mg/l chlorine for half an hour. This result shows that chemical precipitation of the wastewater will reduce the amount of chlorine needed to disinfect the wastewater. Mechanical separation was not tested.

Pretreatment of the wastewater was obligatory in the historic Danish ministerial order either as mechanic separation using a filter or as chemical precipitation.

A few papers have reported on the use of chlorine produced by electrolyzation of the water, which seems a usable method.

**Heat treatment:** a) 65°C for 10 minutes.

b) 70°C for 5 minutes.

c) 75°C for 4 minutes.

d) 80°C for 3 minutes.

e) 85°C for 2 minutes.

f) 90°C for 1 minute.

g) 95°C for 45 seconds.

h) 100°C for 30 seconds.

Despite the conflicting results reported by different authors or obtained from different experiments the combinations of time and temperature stated in the Danish ministerial order no 755 of 28/07/2005 and in the Norwegian list of approved methods will probably be acceptable for fish pathogenic bacteria and viruses for at least a 3 log reduction if a proper stirring of the wastewater is secured to avoid pockets of water not reaching the desired temperature for the stated amount of time.

#### **Percolation**

It has not been possible to find any references describing the decimating effect of percolating of fish pathogenic viruses. Furthermore it has not been possible to find publications describing the effect of percolating other birnaviruses. It is therefore not possible to validate if this procedure is safe to use.

#### **lodine products**

lodine based disinfection products are useful for disinfection of virus and bacterias but less suitable for use when parasites and fungi are the microorganism in question. IPNV and nodavirus seems to be a bit more resistant that VHSV and IHNV. It has not been possible to find published tests on the efficiency of iodine using wastewater from fish slaugtherhourse or cutting plants. Iodine is sensitive towards titer of pathogen, Temperature, pH and organic contamination, with more iodine needed as the titer of the pathogen goes up, temperature goes down, pH  $\geq$  8 and organic contamination goes up. Recommeded dose:  $\geq$  150 ppm for 10 min at pH < 8.

#### Ozone

Ozone seems to be an effective product to decimate the concentration of fish viral and bacterial pathogens. None of the papers papers covered in this report has tested ozone under circumstances comparable to the conditions prevailing in wastewater from fish cutting plants and it is as such unknown how usable the method will be for this specific purpose. In Norway ozone is not listed as an approved method for disinfection of wastewater from fish slaugtherhouses/cutting plants, but the method is approved for use in infection trial facilities with the following doses:

- a) freshwater: ≥ 15 mg/l residual ozone after 15 minutes treatment (corresponds to a C T value of 135 mg\*s/l).
- b) sea water: ≥ 0,2 mg/l TRO (total residual oxidants) after 15 minutes treatment (corresponds to a C T value of 180 mg\*s/l).

If these doses are adopted, and the water is pretreated, all fish pathogenic bacteria and viruses should be inactivated.

#### **Pretreatment of water**

Regardless of the method chosen for disinfection of the wastewater from fish processing plants the effect will be better the cleaner the water is. It is therefore very important the the water is treated to reduce the amount of organic matter in the water. The results from the full scale trials in Norway referred to under the headline "Chlorination" in this paragraph shows the importancy of pretreatment of the water before disinfection. There are several ways of pretreating the water of which mechanical separation and chemical precipitation was accepted in the historical Danish legislation.

# **Tables**

# pН

# Virus

# **VHSV**

| Concentration pathogen                   | Disinfectant | Contact time | Concentration disinfectant | рН              | Temp. | Result                 | Method                             | Reference                 | Comments   |
|--|--------------|--------------|----------------------------|-----------------|-------|------------------------|------------------------------------|---------------------------|--|
| 4,0 x 10 <sup>7</sup> pfu/ml             | HCI          | 10 min       |                            | 2,5             | 8-10  | Survival               |                                    | 52                        | Final concentration 1,5 x 10 <sup>4</sup>                    |
| 4,0 x 10 <sup>7</sup> pfu/ml             | HCI          | 60 min       |                            | 2,5             | 8-10  | "inactivated"          |                                    | 52                        | Final concentration < 10                                     |
| 10 <sup>5,2</sup> TCID <sub>50</sub> /ml | рН           | 60 min       |                            | 3               |       | Survival               |                                    | 4                         | 10 <sup>2,2</sup> TCID <sub>50</sub> /ml after 60 min        |
| 10 <sup>5,2</sup> TCID <sub>50</sub> /ml | рН           | 180 min      |                            | 3               |       | "inactivated"          |                                    | 4                         |  |
| 10 <sup>5,8</sup> TCID <sub>50</sub> /ml | рН           | 60 min       |                            | 3               |       | 99,9 % reduction       | 5 % calf serum added               | 4                         | <1   |
| 10 <sup>5,8</sup> TCID <sub>50</sub> /ml | рН           | 180 min      |                            | 3               |       | "inactivated"          | 5 % calf serum added               | 4                         |  |
| 10 <sup>5,1</sup> TCID <sub>50</sub> /ml | HCI          | 24 hours     |                            | 4               | 4     | Not detectable         | Pathogen mixed into minced herring | 47                        | Detection limit:<br>10 <sup>2,2</sup> TCID <sub>50</sub> /ml |
|  | рН           | 7 days       |                            | 4               |       | Survival               |                                    | 20, Dixon                 |  |
| 1062-015 / 1                             | ·            | ,            |                            |                 | - 10  |                        |                                    | (pers. com.)              |  |
| 10 <sup>6,2</sup> TCID <sub>50</sub> /ml | рН           | 60 min       |                            | 9               | 10    | Stable                 |                                    | 4                         |  |
| 10 <sup>5,7</sup> TCID <sub>50</sub> /ml | кон          | 48 hours     |                            | 11              | 4     | Stable                 | Pathogen mixed into minced herring | 47                        |  |
| 10 <sup>5,7</sup> TCID <sub>50</sub> /ml | кон          | 1 t          |                            | 12              | 4     | Not detectable         | Pathogen mixed into minced herring | 47                        | Detection limit:<br>10 <sup>2,2</sup> TCID <sub>50</sub> /ml |
|  | pН           | 6 hours      |                            | 12              |       | inaktiveret            |                                    | 20, Dixon<br>(pers. com.) |  |
| 1,5 x 10 <sup>7</sup> pfu/ml             | NaOH         | 120 min      |                            | 12,2            | 8-10  | Survival               |                                    | 52                        | Final concentration 1,8 x 10 <sup>4</sup>                    |
| 10 <sup>6,5</sup> TCID <sub>50</sub> /ml | NaOH         | 5 min        | 2%                         | 11,85-<br>11,90 |       | > 99,99 %<br>reduction | 10 % calf serum added              | 5, 4                      | Survival   |
| 10 <sup>5,8</sup> TCID <sub>50</sub> /ml | NaOH         | 5 min        | 2%                         | 11,85-<br>11,90 |       | "inactivated"          |                                    | 4                         |  |
| 10 <sup>5,8</sup> TCID <sub>50</sub> /ml | NaOH         | 10 min       | 2%                         | 11,85-<br>11,90 |       | "inactivated"          | 10 % calf serum added              | 4                         |  |
| 10 <sup>6,5</sup> TCID <sub>50</sub> /ml | NaOH         | 10 min       | 2%                         | 11,85-<br>11,90 |       | "inactivated"          |                                    | 4                         |  |

Conclusion: VHSV is inactivated at pH 3 and pH 12 after 3 hours contact time. For 3 log inactivation pH 3 or pH 12 for 10 minuts is suitable.

# *IHNV*

| Concentration pathogen                 | Disinfectant                  | Contact time | Concentration disinfectant | рН  | Temp. | Result         | Method       | Reference                 | Comments |
|--|-------------------------------|--------------|----------------------------|-----|-------|----------------|--------------|---------------------------|----------|
| 10 <sup>6</sup> TCID <sub>50</sub> /ml | рН                            | 4 hours      |                            | 3   | 4     | Not detectable | Virus in MEM | 79                        |          |
|  | citrate/phos-<br>phate buffer | 7 hours      |                            | 4,0 | 22    | Survival       |              | 100                       |          |
|  | рН                            | 7 days       |                            | 4   |       | Survival       |              | 20, Dixon<br>(pers. com.) |          |
|  | pН                            | 6 hours      |                            | 12  |       | "inactivated"  |              | 20, Dixon<br>(pers. com.) |          |

Conclusion: IHNV is inactivated at pH 3 for 4 hours, but not at pH 4. At pH 12, tested after 6 hours contact time, IHNV is inactivated.

# **ISAV**

| Concentration pathogen | Disinfectant | Contact time | Concentration disinfectant | рН   | Temp. | Result          | Method  | Reference                 | Comments  |
|------------------------|--------------|--------------|----------------------------|------|-------|-----------------|---|---------------------------|---|
|                        | Formic acid  | 8 hours      | -                          | 4    | -     | "inactivated"   | Tissue homogenate of liver,<br>kidney and spleen from<br>moribund ISA-fish, treated<br>and IP-injected in fish. | 94                        | Testet at pH 3,5, 4,0 and 4,5 at 8 hours and 24 hours.      |
|                        | рН           | 7 days       |                            | 4    |       | "inactivated"   |   | 20, Dixon<br>(pers. com.) |   |
|                        | рН           | 30 min       |                            | 4    |       | Not detectable  | Addition of HCl or NaOH to virus in L15 medium to pH 3, 4, 5, 7, 9 or 11.                                       | 27                        |   |
|                        | рН           | 30 min       |                            | 5-9  |       | Stable          | Addition of HCl or NaOH to virus in L15 medium to pH 3, 4, 5, 7, 9 or 11.                                       | 27                        |   |
|                        | рН           | 30 min       |                            | 11   |       | > 90% reduction | Addition of HCl or NaOH to virus in L15 medium to pH 3, 4, 5, 7, 9 or 11.                                       | 27                        |   |
|                        | NaOH         | 48 hours     | -                          | 11,5 | -     | "inactivated"   | Tissue homogenate of liver,<br>kidney and spleen from<br>moribund ISA-fish, treated<br>and IP-injected in fish. | 94                        | Testet at pH 11,0, 11,5 and 12,0 at 8, 12, 24 and 48 hours. |
|                        | NaOH         | 24 hours     | -                          | 12   | -     | "inactivated"   | Tissue homogenate of liver,<br>kidney and spleen from<br>moribund ISA-fish, treated<br>and IP-injected in fish. | 94                        | Testet at pH 11,0, 11,5 and 12,0 at 8, 12, 24 and 48 hours. |
|                        | рН           | 24 hours     |                            | 12   |       | Survival        |   | 20, Dixon<br>(pers. com.) |   |

Conclusion: ISAV is inactivated at pH 4, but the contact time has to be relative long. There is disagreement among the references regarding inactivation at pH 12.

# *IPNV*

| Concentration pathogen                   | Disinfectant                  | Contact time | Concentration disinfectant | рН  | Temp. | Result                                | Method   | Reference                 | Comments  |
|--|-------------------------------|--------------|----------------------------|-----|-------|---------------------------------------|--|---------------------------|---|
| 10 <sup>6,6</sup> TCID <sub>50</sub> /ml | Formic acid<br>(HCOOH)        | 6 min        |                            | 1,5 | 7     | Not detectable<br>(> 5 log reduction) | Laboratory trial, 1 part<br>process water + 2 parts<br>"bløggevand" from fish<br>slaugtherhouse. | 28                        |   |
| 10 <sup>5,5</sup> TCID <sub>50</sub> /ml | Formic acid<br>(HCOOH)        | 1t           |                            | 2,0 |       | Not detectable<br>(> 4 log reduction) | Full-scale trial (Vikan<br>Akvavet), wastewater from<br>fish slaugtherhouse.                     | 28                        | Salinity 20 ‰, "bløggevand"<br>(blood, fish slime and<br>epithelial cells in salt water)<br>diluted with fresh water. |
| 10 <sup>7,0</sup> TCID <sub>50</sub> /ml | рН                            | 35 days      |                            | 2   | 4     | 5 log reduction                       |  | 74                        | Isolate VR-299  |
| 10 <sup>7,0</sup> TCID <sub>50</sub> /ml | рН                            | 20 days      |                            | 2   | 4     | 3 log reduction                       |  | 74                        | Isolate VR-299. Result read on a graph.   |
| 6,7 x 10 <sup>6</sup> pfu/ml             | HCI                           | 60 min       |                            | 2,5 | 8-10  | Stable                                |  | 52                        | Type Sp. Final concentration 5,3 x 10 <sup>6</sup>  |
| 10 <sup>5,5</sup> TCID <sub>50</sub> /ml | Formic acid<br>(HCOOH)        | 24 hours     |                            | 2,5 |       | 1-2 log reduction                     | Full-scale trial (Vikan<br>Akvavet), wastewater from<br>fish slaugtherhouse.                     | 28                        | Salinity 20 ‰, "bløggevand"<br>(blood, fish slime and<br>epithelial cells in salt water)<br>diluted with fresh water. |
| 10 <sup>6,6</sup> TCID <sub>50</sub> /ml | Formic acid<br>(HCOOH)        | 10 hours     |                            | 2,5 | 7     | 2 log reduction                       | Laboratory trial, 1 part<br>process water + 2 parts<br>"bløggevand" from fish<br>slaugtherhouse. | 28                        |   |
| 10 <sup>5,5</sup> TCID <sub>50</sub> /ml | Formic acid<br>(HCOOH)        | 24 hours     |                            | 3   |       | Stable                                | Full-scale trial (Vikan<br>Akvavet), wastewater from<br>fish slaugtherhouse.                     | 28                        | Salinity 20 ‰, "bløggevand"<br>(blood, fish slime and<br>epithelial cells in salt water)<br>diluted with fresh water. |
| 10 <sup>7,5</sup> TCID <sub>50</sub> /ml | рН                            | 4 hours      |                            | 3   | 4     | Stable                                | Virus in MEM   | 171                       |   |
| 10 <sup>6,5</sup> TCID <sub>50</sub> /ml | рН                            | 360 min      |                            | 3   |       | Stable                                |  | 4                         |   |
| 10 <sup>6,8</sup> TCID <sub>50</sub> /ml | рН                            | 360 min      |                            | 3   |       | Stable                                | 5 % calf serum added   | 4                         |   |
| 10 <sup>6,6</sup> TCID <sub>50</sub> /ml | Formic acid<br>(HCOOH)        | 10 hours     |                            | 3,5 | 7     | 3 log reduction                       | Laboratory trial, 1 part<br>process water + 2 parts<br>"bløggevand" from fish<br>slaugtherhouse. | 28                        |   |
|  | Citrate/phos-<br>phate buffer | 14 days      |                            | 4,0 | 22    | Survival                              |  | 100                       |   |
|  | рН                            | 28 days      |                            | 4   |       | Survival                              |  | 20, Dixon<br>(pers. com.) |   |

| 10 <sup>7,0</sup> TCID <sub>50</sub> /ml     | рН   | 35 days  |    | 7               | 4    | Stable                             |  | 74                        | Isolate VR-299  |
|--|------|----------|----|-----------------|------|------------------------------------|--|---------------------------|---|
| 10 <sup>6,5-7,0</sup> TCID <sub>50</sub> /ml | PBS  | 109 uger |    |                 | 4    | Survival                           |  | 74                        | Isolate VR-299  |
| 10 <sup>5,8</sup> TCID <sub>50</sub> /ml     | рН   | 60 min   |    | 9               | 10   | Stable                             |  | 4                         |   |
| 10 <sup>7,0</sup> TCID <sub>50</sub> /ml     | рН   | 35 days  |    | 9               | 4    | 5 log reduction                    |  | 74                        | Isolate VR-299  |
| 10 <sup>7,0</sup> TCID <sub>50</sub> /ml     | рН   | 26 days  |    | 9               | 4    | 3 log reduction                    |  | 74                        | Isolate VR-299. Result Read off a graph.  |
| 10 <sup>5,3</sup> TCID <sub>50</sub> /ml     | кон  | 48 hours |    | 10              | 4    | Stable                             | Pathogen mixed into minced herring   | 47                        |   |
| 10 <sup>5,3</sup> TCID <sub>50</sub> /ml     | кон  | 24 hours |    | 11              | 4    | Survival                           | Pathogen mixed into minced herring   | 47                        |   |
| 10 <sup>5,3</sup> TCID <sub>50</sub> /ml     | кон  | 48 hours |    | 11              | 4    | Not detectable                     | Pathogen mixed into minced herring   | 47                        | Detection limit: 10 <sup>2</sup> TCID <sub>50</sub> /ml   |
| 10 <sup>5,5</sup> TCID <sub>50</sub> /ml     | NaOH | 24 hours |    | 11,6            |      | 3½ log reduction                   | Full-scale trial (Vikan<br>Akvavet), wastewater from<br>fish slaugtherhouse.                     | 28                        | Salinity 20 ‰, "bløggevand"<br>(blood, fish slime and<br>epithelial cells in salt water)<br>diluted with fresh water. |
| 10 <sup>4,5</sup> TCID <sub>50</sub> /ml     | NaOH | 5 min    | 2% | 11,85-<br>11,90 |      | "inactivated"                      | 10 % calf serum added  | 4                         |   |
| 10 <sup>4,8</sup> TCID <sub>50</sub> /ml     | NaOH | 5 min    | 2% | 11,85-<br>11,90 |      | "inactivated"                      |  | 4, 5                      |   |
| 10 <sup>4,5</sup> TCID <sub>50</sub> /ml     | NaOH | 10 min   | 2% | 11,85-<br>11,90 |      | "inactivated"                      | 10 % calf serum added  | 4                         |   |
| 10 <sup>4,8</sup> TCID <sub>50</sub> /ml     | NaOH | 10 min   | 2% | 11,85-<br>11,90 |      | "inactivated"                      |  | 4                         |   |
| 10 <sup>5,5</sup> TCID <sub>50</sub> /ml     | NaOH | 24 hours |    | 12.0            |      | 3½ log reduction                   | Full-scale trial (Vikan<br>Akvavet), wastewater from<br>fish slaugtherhouse.                     | 28                        | Salinity 20 ‰, "bløggevand"<br>(blood, fish slime and<br>epithelial cells in salt water)<br>diluted with fresh water. |
| 10 <sup>5,3</sup> TCID <sub>50</sub> /ml     | кон  | 16 hours |    | 12              | 4    | Not detectable                     | Pathogen mixed into minced herring   | 47                        | Detection limit: 10 <sup>2</sup> TCID <sub>50</sub> /ml   |
| 10 <sup>6,6</sup> TCID <sub>50</sub> /ml     | NaOH | 6 min    |    | 12,0            | 7    | Not detectable (> 5 log reduction) | Laboratory trial, 1 part<br>process water + 2 parts<br>"bløggevand" from fish<br>slaugtherhouse. | 28                        |   |
|  | рН   | 20 min   |    | 12              |      | "inactivated"                      |  | 20, Dixon<br>(pers. com.) |   |
| 2,2 x 10 <sup>4</sup> pfu/ml                 | NaOH | 10 min   |    | 12,2            | 8-10 | "inactivated"                      |  | 52                        | Final concentration < 10  |
| 10 <sup>5,5</sup> TCID <sub>50</sub> /ml     | NaOH | 1 hour   |    | 12,4            |      | Not detectable (> 4 log reduction) | Full-scale trial (Vikan<br>Akvavet), wastewater from<br>fish slaugtherhouse.                     | 28                        | Salinity 20 ‰, "bløggevand"<br>(blood, fish slime and<br>epithelial cells in salt water)<br>diluted with fresh water. |

Conslution: In order to secure inactivation of IPNV, the pH has to as low as 2 or high as 12 with a contact time for at least 1 hour. There are reports stating surval time of several weeks at pH 2, but in full scale trials using wastewater containing blood, slime and skin scrapings in saltwater 1 hour contact time was sufficient to inactivate IPNV.

# **Nodavirus**

| Concentration pathogen                   | Disinfectant | Contact time | Concentration disinfectant | рН | Temp. | Result   | Method   | Reference                 | Comments                    |
|--|--------------|--------------|----------------------------|----|-------|--|--|---------------------------|-----------------------------|
| 10 <sup>6,5</sup> TCID <sub>50</sub> /ml | HCI          | 7 days       |                            | 2  | 15    | Stable   | Grown virus diluted 1:100 in distilled water. Tested after 1 hour and 1, 3, 7, 15, 21 and 42 days. | 30                        | Isolate: sea bass nodavirus |
| 10 <sup>6,5</sup> TCID <sub>50</sub> /ml | HCI          | 21 days      |                            | 2  | 15    | Survival<br>(5 log reduction)                                    | Grown virus diluted 1:100 in distilled water. Tested after 1 hour and 1, 3, 7, 15, 21 and 42 days. | 30                        | Isolate: sea bass nodavirus |
| 10 <sup>6,5</sup> TCID <sub>50</sub> /ml | HCI          | 42 days      |                            | 2  | 15    | Not detectable   | Grown virus diluted 1:100 in distilled water. Tested after 1 hour and 1, 3, 7, 15, 21 and 42 days. | 30                        | Isolate: sea bass nodavirus |
| 10 μg purified<br>virus                  | HCI          | 10 min       |                            | 3  | 20    | Not<br>inactivated(0/800<br>larvae survived,<br>control 472/800) | Diluted in 1 ml PBS. Used for infection trial in day old striped jack larvae.                      | 9                         | Isolate: SJNNV              |
| 10 <sup>6,5</sup> TCID <sub>50</sub> /ml | HCI          | 42 days      |                            | 3  | 15    | Stable   | Grown virus diluted 1:100 in distilled water. Tested after 1 hour and 1, 3, 7, 15, 21 and 42 days. | 30                        | Isolate: sea bass nodavirus |
|  | рН           | 7 days       |                            | 4  |       | Survival   |  | 20, Dixon<br>(pers. com.) |                             |
| 10 μg purified<br>virus                  | PBS          | 10 min       |                            | 7  | 20    | Not<br>inactivated(0/800<br>larvae survived,<br>control 472/800) | Diluted in 1 ml PBS. Used for infection trial in day old striped jack larvae.                      | 9                         | Isolate: SJNNV              |
| 10 <sup>6,5</sup> TCID <sub>50</sub> /ml | NaOH         | 42 days      |                            | 9  | 15    | Stable   | Grown virus diluted 1:100 in distilled water. Tested after 1 hour and 1, 3, 7, 15, 21 and 42 days. | 30                        | Isolate: sea bass nodavirus |
| 10 <sup>6,0</sup> TCID <sub>50</sub> /ml | КОН          | 48 hours     |                            | 10 | 4     | Stable   | Pathogen mixed into minced herring   | 47                        |                             |
| 10 <sup>6,0</sup> TCID <sub>50</sub> /ml | кон          | 24 hours     |                            | 11 | 4     | Survival   | Pathogen mixed into minced herring   | 47                        |                             |

| 10 <sup>6,0</sup> TCID <sub>50</sub> /ml | кон  | 48 hours | 11 | 4  | Not detectable   | Pathogen mixed into minced herring   | 47                        | Detection limit:<br>10 <sup>2</sup> TCID <sub>50</sub> /ml |
|--|------|----------|----|----|--|--|---------------------------|--|
| 10 <sup>6,5</sup> TCID <sub>50</sub> /ml | NaOH | 7 days   | 11 | 15 | Survival (2-3 log<br>reduction)  | Grown virus diluted 1:100 in distilled water. Tested after 1 hour and 1, 3, 7, 15, 21 and 42 days. | 30                        | Isolate: sea bass nodavirus                                |
| 10 <sup>6,5</sup> TCID <sub>50</sub> /ml | NaOH | 15 days  | 11 | 15 | Not detectable   | Grown virus diluted 1:100 in distilled water. Tested after 1 hour and 1, 3, 7, 15, 21 and 42 days. | 30                        | Isolate: sea bass nodavirus                                |
| 10 <sup>6,5</sup> TCID <sub>50</sub> /ml | NaOH | 15 days  | 11 | 15 | Not detectable   | Grown virus diluted 1:100 in distilled water. Tested after 1 hour and 1, 3, 7, 15, 21 and 42 days. | 30                        | Isolate: sea bass nodavirus                                |
| 10 <sup>6,0</sup> TCID <sub>50</sub> /ml | кон  | 12 hours | 12 | 4  | Not detectable   | Pathogen mixed into minced herring   | 47                        | Detection limit: 10 <sup>2</sup><br>TCID <sub>50</sub> /ml |
| 10 µg purified<br>virus                  | NaOH | 10 min   | 12 | 20 | "Effective" (238/800 larvae survived, antigen ELISA negativ - control 472/800) | Diluted in 1 ml PBS. Used for infection trial in day old striped jack larvae.                      | 9                         | Isolate: SJNNV   |
|  | рН   | 24 hours | 12 |    | Survival   |  | 20, Dixon<br>(pers. com.) |  |

Conclusion: Nodavirus seemingly is able to withstand low pH. There are disagreements conserning the ability of the virus to withstand pH 12.

# **PFRV**

| Concentration pathogen              | Disinfectant | Contact time | Concentration disinfectant | рН              | Temp. | Result        | Method                | Reference | Comments |
|-------------------------------------|--------------|--------------|----------------------------|-----------------|-------|---------------|-----------------------|-----------|----------|
| 6,5 log10<br>TCID <sub>50</sub> /ml | рН           | 60 min       |                            | 9               | 10    | Stable        |                       | 4         |          |
| 6,5 log10<br>TCID <sub>50</sub> /ml | NaOH         | 5 min        | 2%                         | 11,85-<br>11,90 |       | "inactivated" | 10 % calf serum added | 4         |          |
| 6,8 log10<br>TCID <sub>50</sub> /ml | NaOH         | 5 min        | 2%                         | 11,85-<br>11,90 |       | "inactivated" |                       | 4, 5      |          |
| 6,8 log10<br>TCID <sub>50</sub> /ml | NaOH         | 10 min       | 2%                         | 11,85-<br>11,90 |       | "inactivated" | 10 % calf serum added | 4         |          |
| 6,2 log10<br>TCID <sub>50</sub> /ml | NaOH         | 10 min       | 2%                         | 11,85-<br>11,90 |       | "inactivated" |                       | 4         |          |

Conclusion: PFRV is inactivated at pH 12 for 5 minuts.

# Ranavirus

| Concentration pathogen | Disinfectant | Contact time | Concentration disinfectant | рН | Temp. | Result         | Method                        | Reference                 | Comments     |
|------------------------|--------------|--------------|----------------------------|----|-------|----------------|-------------------------------|---------------------------|--------------|
|                        | рН           | 28 days      |                            | 4  |       | Survival       |                               | 20, Dixon<br>(pers. com.) |              |
|                        | рН           | 1 hour       |                            | 4  |       | Not detectable | Virus in cell culture medium. | 67                        | Isolat: EHNV |
|                        | рН           | 6 hours      |                            | 12 |       | Survival       |                               | 20, Dixon<br>(pers. com.) |              |
|                        | рН           | 1 hour       |                            | 12 |       | Not detectable | Virus in cell culture medium. | 67                        | Isolat: EHNV |

Conclusion: the literature does not agree on the inactivation of ranavirus at pH 4 and 12.

# SAV

| Concentration pathogen                 | Disinfectant | Contact time | Concentration disinfectant | рН  | Temp. | Result                         | Method                                | Reference | Comments                             |
|--|--------------|--------------|----------------------------|-----|-------|--------------------------------|---------------------------------------|-----------|--------------------------------------|
| 10 <sup>7</sup> TCID <sub>50</sub> /ml | рН           | 4 hours      |                            | 3   | 4     | Not detectable                 | Virus in MEM                          | 79        | Salmon pancreas disease virus (SPDV) |
|  | HCl          | mixing       |                            | 4   | 4     | "inactivated"                  |                                       | 33        | SAV1                                 |
|  | Formic acid  | 5 min        |                            | 4   | 4     | Survival.<br>99,99 % reduction |                                       | 33        | SAV1                                 |
|  | Formic acid  | 1 dag        |                            | 4   | 4     | "inactivated"                  | Tested after 5 min, 1 day and 7 days. | 33        | SAV1                                 |
|  | Formic acid  | 7 days       |                            | 5   | 4     | "inactivated"                  | Tested after 5 min, 1 day and 7 days. | 33        | SAV1                                 |
|  | Formic acid  | 7 days       |                            | 6   | 4     | Stable                         | Tested after 5 min, 1 day and 7 days. | 33        | SAV1                                 |
|  | Formic acid  | 7 days       |                            | 7,2 | 4     | Stable                         | Tested after 5 min, 1 day and 7 days. | 33        | SAV1                                 |
|  | NaOH         | mixing       |                            | 12  | 4     | "inactivated"                  |                                       | 33        | SAV1                                 |

Conclusion: SAV can be inactivated at pH 4 using a contact time of 24 hours or > 3 log reduction after a contact time of 5 min. At pH 3 or 12 hourshe virus is inactivated immediately.

#### **SVCV**

| Concentration pathogen                   | Disinfectant | Contact time | Concentration disinfectant | рН | Temp.         | Result            | Method                                | Reference                 | Comments |
|--|--------------|--------------|----------------------------|----|---------------|-------------------|---------------------------------------|---------------------------|----------|
| 10 <sup>7,2</sup> TCID <sub>50</sub> /ml | рН           | 30 min       |                            | 3  | room<br>temp. | > 3 log reduction | Titrated after 30, 60 and 120 minuts. | 3                         |          |
| 10 <sup>7,2</sup> TCID <sub>50</sub> /ml | рН           | 120 min      |                            | 3  | room<br>temp. | 6 log reduction   | Titrated after 30, 60 and 120 minuts. | 3                         |          |
|  | рН           | 28 days      |                            | 4  |               | Survival          |                                       | 20, Dixon<br>(pers. com.) |          |
| 10 <sup>6,5</sup> TCID <sub>50</sub> /ml | pН           | 60 min       |                            | 9  | 10            | Stable            |                                       | 4                         |          |

|  | рН   | 6 days  |    |                 | Survival                |                       | 64                        |          |
|--|------|---------|----|-----------------|-------------------------|-----------------------|---------------------------|----------|
| 10 <sup>7,2</sup> TCID <sub>50</sub> /ml | NaOH | 5 min   | 2% | 11,85-<br>11,90 | "inactivated"           | 10 % calf serum added | 4                         |          |
| 10 <sup>6,8</sup> TCID <sub>50</sub> /ml | NaOH | 5 min   | 2% | 11,85-<br>11,90 | > 99,99 %<br>reduction  |                       | 4, 5                      | Survival |
| 10 <sup>7,5</sup> TCID <sub>50</sub> /ml | NaOH | 10 min  | 2% | 11,85-<br>11,90 | "inactivated"           | 10 % calf serum added | 4                         |          |
| 10 <sup>7,5</sup> TCID <sub>50</sub> /ml | NaOH | 10 min  | 2% | 11,85-<br>11,90 | "inactivated"           |                       | 4                         |          |
|  | рН   | 6 hours |    | 12              | Survival/"inactivat ed" |                       | 20, Dixon<br>(pers. com.) |          |

Conclusion: SVCV kan inaktiveres ved pH 3 (3 log, 30 min) og pH 12.

# Bacteria

#### Aeromonas salmonicida

| Concentration pathogen       | Disinfectant                  | Contact time | Concentration disinfectant | рН  | Temp. | Result        | Method                             | Reference                 | Comments                                       |
|------------------------------|-------------------------------|--------------|----------------------------|-----|-------|---------------|------------------------------------|---------------------------|--|
| 1,4 x 10 <sup>7</sup> cfu/ml | citrate/phos-<br>phate buffer | 90 min       |                            | 4,0 | 22    | "inactivated" |                                    | 100                       |  |
|                              | рН                            | 2 hours      |                            | 4   |       | "inactivated" |                                    | 20, Dixon<br>(pers. com.) |  |
| 5 x 10 <sup>8</sup> cfu/ml   | кон                           | 12 hours     |                            | 10  | 22    | "inactivated" | Pathogen mixed into minced herring | 47                        | Testet efter 12 hours, 24<br>hours og 48 hours |
|                              | рН                            | 10 min       |                            | 12  |       | "inactivated" |                                    | 20, Dixon<br>(pers. com.) |  |

Conclusion: A. salmonicida is inactivated at pH 4 (testet at 90 min contact time) and pH 12 (10 min contact time).

# Lactococcus garviae

| Concentration pathogen | Disinfectant | Contact time | Concentration disinfectant | рН | Temp. | Result   | Method | Reference                 | Comments |
|------------------------|--------------|--------------|----------------------------|----|-------|----------|--------|---------------------------|----------|
|                        | рН           | 7 days       |                            | 4  |       | Survival |        | 20, Dixon<br>(pers. com.) |          |
|                        | рН           | 14 days      |                            | 12 |       | Survival |        | 20, Dixon<br>(pers. com.) |          |

Conclusion: According to this experiment *L. garviae* can withstand both pH 4 and 12.

# Listonella (Vibrio) anguillarum

| Concentration pathogen | Disinfectant | Contact time | Concentration disinfectant | рН | Temp. | Result        | Method | Reference                 | Comments |
|------------------------|--------------|--------------|----------------------------|----|-------|---------------|--------|---------------------------|----------|
|                        | рН           | 24 hours     |                            | 4  |       | Survival      |        | 20, Dixon<br>(pers. com.) |          |
|                        | рН           | 30 min       |                            | 12 |       | "inactivated" |        | 20, Dixon<br>(pers. com.) |          |

Conclusion: V. anguillarum can withstand pH 4, but is inactivated at pH 12 using a contact time of 30 min.

# Mycobacterium chelonae

| Concentration pathogen       | Disinfectant                  | Contact time | Concentration disinfectant | рН  | Temp. | Result        | Method | Reference                 | Comments |
|------------------------------|-------------------------------|--------------|----------------------------|-----|-------|---------------|--------|---------------------------|----------|
| 1,4 x 10 <sup>7</sup> cfu/ml | Citrate/phos-<br>phate buffer | > 14 days    |                            | 4,0 | 22    | "inactivated" |        | 100                       |          |
|                              | рН                            | 2 days       |                            | 4   |       | Survival      |        | 20, Dixon<br>(pers. com.) |          |
|                              | рН                            | 48 hours     |                            | 12  |       | Survival      |        | 20, Dixon<br>(pers. com.) |          |

Conclusion: M. chelonae can withstand both pH 4 and 12.

# Photobacterium damselae

| Concentration pathogen | Disinfectant | Contact time | Concentration disinfectant | рН | Temp. | Result        | Method | Reference                 | Comments |
|------------------------|--------------|--------------|----------------------------|----|-------|---------------|--------|---------------------------|----------|
|                        | рН           | 24 hours     |                            | 4  |       | Survival      |        | 20, Dixon<br>(pers. com.) |          |
|                        | рН           | 10 min       |                            | 12 |       | "inactivated" |        | 20, Dixon<br>(pers. com.) |          |

Conclusion: P. damselae survives pH 4, but is inactivated at pH 12 for 10 min.

#### Renibacterium salmoninarum

| Concentration pathogen       | Disinfectant                  | Contact time | Concentration disinfectant | рН  | Temp. | Result        | Method | Reference                 | Comments |
|------------------------------|-------------------------------|--------------|----------------------------|-----|-------|---------------|--------|---------------------------|----------|
| 1,4 x 10 <sup>7</sup> cfu/ml | Citrate/phos-<br>phate buffer | 4 hours      |                            | 4,0 | 22    | Survival      |        | 100                       |          |
|                              | рН                            | 24 hours     |                            | 4   |       | "inactivated" |        | 20, Dixon<br>(pers. com.) |          |
|                              | рН                            | 6 hours      |                            | 12  |       | "inactivated" |        | 20, Dixon<br>(pers. com.) |          |

Conclusion: *R. salmoninarum* survives pH 4 for at least 4 hours, but is inactivated after 24 hours. Can be inactivated at pH 12 using a contact time of 6 hours.

# Streptococcus iniae

| Concentration pathogen | Disinfectant | Contact time | Concentration disinfectant | рН | Temp. | Result        | Method | Reference                 | Comments |
|------------------------|--------------|--------------|----------------------------|----|-------|---------------|--------|---------------------------|----------|
|                        | рН           | 24 hours     |                            | 4  |       | "inactivated" |        | 20, Dixon<br>(pers. com.) |          |
|                        | рН           | 30 min       |                            | 12 |       | "inactivated" |        | 20, Dixon<br>(pers. com.) |          |

Conclusion: S. iniae can be inactivated at pH 4 (24 hours contact time) og pH 12 (30 min contact time).

# Yersinia ruckeri

| Concentration pathogen    | Disinfectant           | Contact time | Concentration disinfectant | рН   | Temp. | Result                             | Method   | Reference | Comments  |
|---------------------------|------------------------|--------------|----------------------------|------|-------|------------------------------------|--|-----------|---|
| 10 <sup>8,28</sup> cfu/ml | Formic acid<br>(HCOOH) | 1 hour       |                            | 1,5  | 7     | Not detectable (> 8 log reduction) | Laboratory trial, 1 part<br>process water + 2 parts<br>"bløggevand" from fish<br>slaugtherhouse. | 28        |   |
| 10 <sup>8,28</sup> cfu/ml | Formic acid<br>(HCOOH) | 6 min        |                            | 1,5  | 7     | 7 log reduction                    | Laboratory trial, 1 part<br>process water + 2 parts<br>"bløggevand" from fish<br>slaugtherhouse. | 28        |   |
| 10 <sup>4,69</sup> cfu/ml | Formic acid<br>(HCOOH) | 10 hours     |                            | 1,98 | 7     | Not detectable (≥ 4 log reduction) | Full-scale trial (Norskagfisk),<br>blood water from fish<br>slaugtherhouse.                      | 28        | Salinity 14-15 ‰  |
| 10 <sup>6,77</sup> cfu/ml | Formic acid<br>(HCOOH) | 0,1 hour     |                            | 2    |       | Not detectable (> 6 log reduction) | Full-scale trial, wastewater from fish slaugtherhouses.  | 28        | Salinity 20 ‰, "bløggevand"<br>(blood, fish slime and<br>epithelial cells in salt water)<br>diluted with fresh water. |
| 10 <sup>6,77</sup> cfu/ml | Formic acid<br>(HCOOH) | 1 hour       |                            | 2,5  |       | Not detectable (> 6 log reduction) | Full-scale trial, wastewater from fish slaugtherhouses.  | 28        | Salinity 20 ‰, "bløggevand"<br>(blood, fish slime and<br>epithelial cells in salt water)<br>diluted with fresh water. |
| 10 <sup>4,69</sup> cfu/ml | Formic acid<br>(HCOOH) | 10 hours     |                            | 2,5  | 7     | Not detectable (≥ 4 log reduction) | Full-scale trial (Norskagfisk),<br>blood water from fish<br>slaugtherhouse.                      | 28        | Salinity 14-15 ‰  |
| 10 <sup>8,28</sup> cfu/ml | Formic acid<br>(HCOOH) | 5 hours      |                            | 2,5  | 7     | Not detectable (> 8 log reduction) | Laboratory trial, 1 part<br>process water + 2 parts<br>"bløggevand" from fish<br>slaugtherhouse. | 28        |   |

| 10 <sup>6,77</sup> cfu/ml | Formic acid<br>(HCOOH) | 24 hours |    | 3    |   | 4 log reduction                       | Full-scale trial, wastewater from fish slaugtherhouses.  | 28                        | Salinity 20 ‰, "bløggevand"<br>(blood, fish slime and<br>epithelial cells in salt water)<br>diluted with fresh water. |
|---------------------------|------------------------|----------|----|------|---|---------------------------------------|--|---------------------------|---|
| 10 <sup>8,28</sup> cfu/ml | Formic acid<br>(HCOOH) | 10 hours | 3  | 3,5  | 7 | 3 log reduction                       | Laboratory trial, 1 part<br>process water + 2 parts<br>"bløggevand" from fish<br>slaugtherhouse. | 28                        |   |
| 10 <sup>4,69</sup> cfu/ml | Formic acid<br>(HCOOH) | 10 hours | 3, | ,98  | 7 | Not detectable<br>(≥ 4 log reduction) | Full-scale trial (Norskagfisk),<br>blood water from fish<br>slaugtherhouse.                      | 28                        | Salinity 14-15 ‰  |
|                           | рН                     | 24 hours |    | 4    |   | Survival                              |  | 20, Dixon (pers. com.)    |   |
| 10 <sup>6,77</sup> cfu/ml | NaOH                   | 24 hours | 1  | 1,6  |   | 2-3 log reduction                     | Full-scale trial (Vikan<br>Akvavet), wastewater from<br>fish slaugtherhouse.                     | 28                        | Salinity 20 ‰, "bløggevand"<br>(blood, fish slime and<br>epithelial cells in salt water)<br>diluted with fresh water. |
| 10 <sup>6,77</sup> cfu/ml | NaOH                   | 5 hours  | 1: | 2,0  |   | Not detectable<br>(> 6 log reduction) | Full-scale trial (Vikan<br>Akvavet), wastewater from<br>fish slaugtherhouse.                     | 28                        | Salinity 20 ‰, "bløggevand"<br>(blood, fish slime and<br>epithelial cells in salt water)<br>diluted with fresh water. |
| 10 <sup>8,28</sup> cfu/ml | NaOH                   | 10 hours | 1: | 2,0  | 7 | Stable? Survival.                     | Laboratory trial, 1 part<br>process water + 2 parts<br>"bløggevand" from fish<br>slaugtherhouse. | 28                        |   |
|                           | рН                     | 10 min   | 1  | 12   |   | "inactivated"                         |  | 20, Dixon<br>(pers. com.) |   |
| 10 <sup>6,77</sup> cfu/ml | NaOH                   | 1 hour   | 1. | 2,4  |   | Not detectable<br>(> 6 log reduction) | Full-scale trial (Vikan<br>Akvavet), wastewater from<br>fish slaugtherhouse.                     | 28                        | Salinity 20 ‰, "bløggevand"<br>(blood, fish slime and<br>epithelial cells in salt water)<br>diluted with fresh water. |
| 10 <sup>4,69</sup> cfu/ml | NaOH                   | 24 hours | 12 | 2,44 | 7 | 2 log reduction                       | Full-scale trial (Norskagfisk),<br>blood water from fish<br>slaugtherhouse.                      | 28                        | Salinity 14-15 ‰  |
| 10 <sup>8,28</sup> cfu/ml | NaOH                   | 10 hours | 1: | 2,5  | 7 | 2-3 log reduction                     | Laboratory trial, 1 part<br>process water + 2 parts<br>"bløggevand" from fish<br>slaugtherhouse. | 28                        |   |
| 10 <sup>4,69</sup> cfu/ml | NaOH                   | 5 hours  | 12 | 2,70 | 7 | Not detectable<br>(≥ 4 log reduction) | Full-scale trial (Norskagfisk),<br>blood water from fish<br>slaugtherhouse.                      | 28                        | Salinity 14-15 ‰  |
| 10 <sup>4,69</sup> cfu/ml | NaOH                   | 10 hours | 12 | 2,81 | 7 | Not detectable (≥ 4 log reduction)    | Full-scale trial (Norskagfisk),<br>blood water from fish   | 28                        | Salinity 14-15 ‰  |

|                           |      |          |      |   |                   | slaugtherhouse.  |    |  |
|---------------------------|------|----------|------|---|-------------------|--|----|--|
| 10 <sup>8,28</sup> cfu/ml | NaOH | 10 hours | 13,0 | 7 | 3-4 log reduction | Laboratory trial, 1 part<br>process water + 2 parts<br>"bløggevand" from fish<br>slaugtherhouse. | 28 |  |

Conclusion: *Y. ruckeri* can be inactivated at pH 2.5 (contact time 1-10 hours). There are reports of 3 log inactivation at pH 3.5 and a contact time of 10 hours. There are conflicting data with regard to high alkaline pH, in full scale trials using wastewater from slaughterhouses complete inactivation at pH> 12 as well as only 2-4 log reduction is shown. This may be due to lacking proper stirring in the full-scale experiments so pockets where the bacteria have not been treated have occurred.

#### **Parasites**

| Pathogen                | Concentration pathogen | Disinfectant | Contact time | Concentration disinfectant | рН  | Temp. | Result       | Method                   | Reference | Comments  |
|-------------------------|------------------------|--------------|--------------|----------------------------|-----|-------|--------------|--------------------------|-----------|---|
| Trichodina<br>jadranica | 2,1                    | HCl          | 24<br>hours  |                            | 5   | 25    | Stable (2,4) | <i>In vivo,</i> eel      | 75        | Categorisation<br>(category/number of<br>parasites): 0/0, 1/1-10, 2/11-<br>100, 3/100-1000, 4/>1000 |
| Gyrodactylus<br>salaris |                        | рН           | Few<br>days  |                            | < 5 |       | Dies         |                          | 1         | No reference stated!  |
| Myxosoma cerebralis     |                        | КОН          | 2 days       | 0,5%                       |     | 22    | All dead     | <i>In vitro</i> - spores | 42        | Tested at 0,01, 0,1 and 1%  |

# UV

#### Virus

#### **VHSV**

| Concentration pathogen                   | Disinfectant | Contact time | Concentration disinfectant       | рН | Temp. | Result          | Method                                       | Reference | Comments          |
|--|--------------|--------------|----------------------------------|----|-------|-----------------|--|-----------|-------------------|
| 10 <sup>4-5</sup> TCID <sub>50</sub> /ml | UV           |              | $0,79 \pm 0,15 \text{ mWs/cm}^2$ |    |       | 99,9% reduction | Virus in fresh water.                        | 81        | UV-C              |
| 10 <sup>4-5</sup> TCID <sub>50</sub> /ml | UV           |              | app. 1,5 mWs/cm <sup>2</sup>     |    |       | "inactivated"   | Virus in fresh water.                        | 81        | Read off a graph. |
| app. 6 log TCID                          | UV           |              | 1,8 mWs/cm <sup>2</sup>          |    |       | 99,9% reduction |  | 46        | UV-C              |
| 10 <sup>7,2</sup> TCID <sub>50</sub> /ml | UV           | 10 min       | 254 nm, 5 cm afstand             |    | 20    | "inactivated"   |  | 4         |                   |
| 10 <sup>4-5</sup> TCID <sub>50</sub> /ml | UV           |              | $3,1 \pm 0,18 \text{ mWs/cm}^2$  |    |       | 99,9% reduction | Virus in wastewater from fish cutting plant. | 81        | UV-C              |
| app. 6 log TCID                          | UV           |              | 4,0 mWs/cm <sup>2</sup>          |    |       | "inactivated"   |  | 46        | UV-C              |
| 10 <sup>4-5</sup> TCID <sub>50</sub> /ml | UV           |              | app. 4 mWs/cm <sup>2</sup>       |    |       | "inactivated"   | Virus in wastewater from                     | 81        | Read off a graph. |

|   |    |                        |  |      | fish cutting plant. |    |  |
|---|----|------------------------|--|------|---------------------|----|--|
| I | UV | 10 mWs/cm <sup>2</sup> |  | LD90 |                     | 76 |  |

Conclusion: VHSV is susceptible to UV irradiation with  $\geq$  3 log reduction when exposed to 4 mWs/cm<sup>2</sup>. It has been reported though, that 10 mWs/cm<sup>2</sup> is needed for a 2 log reduction.

#### **IHNV**

| Concentration pathogen                       | Disinfectant | Contact<br>time | Concentration disinfectant    | рН | Temp. | Result                    | Method                               | Reference | Comments    |
|--|--------------|-----------------|-------------------------------|----|-------|---------------------------|--------------------------------------|-----------|-------------|
|  | UV           |                 | 1 mWs/cm <sup>2</sup>         |    |       | 99% infectivity reduction |                                      | 102       |             |
| 10 <sup>4,8-6,3</sup> TCID <sub>50</sub> /ml | UV           |                 | 1,0 - 2,0 mWs/cm <sup>2</sup> |    |       | ID <sub>99</sub>          | UV intensity: 100 μW/cm <sup>2</sup> | 103       | strain CHAB |
| 10 <sup>6,8-7,8</sup> TCID <sub>50</sub> /ml | UV           |                 | 1,5 - 3,0 mWs/cm <sup>2</sup> |    |       | ID <sub>99</sub>          | UV intensity: 200 μW/cm <sup>2</sup> | 103       | strain RTTO |
|  | UV           |                 | 2 - 3 mWs/cm <sup>2</sup>     |    |       | 99% reduction             |                                      | 104       |             |
|  | UV           |                 | 2 mWs/cm <sup>2</sup>         |    |       | 3 log reduction           |                                      | 86        |             |
| 10 <sup>4,8-6,3</sup> TCID <sub>50</sub> /ml | UV           | 30 sec          | 3 mWs/cm <sup>2</sup>         |    |       | >2-4 log reduction        | UV intensity: 100 μW/cm <sup>2</sup> | 103       | strain CHAB |
| 10 <sup>6,8-7,8</sup> TCID <sub>50</sub> /ml | UV           | 30 sec          | 4 mWs/cm <sup>2</sup>         |    |       | >3 log reduction          | UV intensity: 200 μW/cm <sup>2</sup> | 103       | strain RTTO |

Conclusion: IHNV is susceptible to UV irradiation with 3 log reduction when exposed to 4 mWs/cm<sup>2</sup>.

#### **ISAV**

| Concentration pathogen                   | Disinfectant | Contact<br>time | Concentration disinfectant     | рН  | Temp. | Result          | Method  | Reference | Comments  |
|--|--------------|-----------------|--------------------------------|-----|-------|-----------------|---|-----------|---|
| 10 <sup>4-5</sup> TCID <sub>50</sub> /ml | UV           |                 | 3,3 ± 0,35 mWs/cm <sup>2</sup> |     |       | 99,9% reduction | Virus in fresh water.   | 81        | UV-C  |
| 10 <sup>4-5</sup> TCID <sub>50</sub> /ml | UV           |                 | 5,1 ± 1,3 mWs/cm <sup>2</sup>  |     |       | 99,9% reduction | virus i havvand   | 81        | UV-C  |
| 10 <sup>4-5</sup> TCID <sub>50</sub> /ml | UV           |                 | app. 5,5 mWs/cm <sup>2</sup>   |     |       | "inactivated"   | Virus in fresh water.   | 81        | Read off a graph.                                 |
| 10 <sup>4-5</sup> TCID <sub>50</sub> /ml | UV           |                 | app. 6,5 mWs/cm <sup>2</sup>   |     |       | "inactivated"   | virus i havvand   | 81        | Read off a graph.                                 |
| 10 <sup>4-5</sup> TCID <sub>50</sub> /ml | UV           |                 | 7,2 ± 1,6 mWs/cm <sup>2</sup>  |     |       | 99,9% reduction | Virus in wastewater from fish cutting plant.  | 81        | UV-C  |
|  | UV           |                 | 7,5 mWs/cm <sup>2</sup>        | 7,9 | 5     | 99,9% reduction | Sea water, sterile filtered.  | 73        | UV-C  |
| 10 <sup>4-5</sup> TCID <sub>50</sub> /ml | UV           |                 | app. 8 mWs/cm <sup>2</sup>     |     |       | "inactivated"   | Virus in wastewater from fish cutting plant.  | 81        | Read off a graph.                                 |
|  | UV           | -               | 20 mWs/cm <sup>2</sup>         | -   | -     | "inactivated"   | Tissue homogenate of liver,<br>kidney and spleen from<br>moribund ISA-fish, treated<br>and IP-injected in fish. | 94        | Tested at UV-doses 0,5 - 50 mWs/cm <sup>2</sup> . |

Conclusion: ISAV is susceptible to UV irradiation with  $\geq$  3 log reduction when exposed to 8 mWs/cm<sup>2</sup>.

# **IPNV**

| Concentration pathogen                       | Disinfectant                                | Contact<br>time | Concentration disinfectant          | рН  | Temp. | Result                    | Method   | Reference | Comments  |
|--|---|-----------------|-------------------------------------|-----|-------|---------------------------|--|-----------|---|
| 10 <sup>7,3-8,3</sup> TCID <sub>50</sub> /ml | UV  |                 | 100 - 150 mWs/cm <sup>2</sup>       |     |       | ID <sub>99</sub>          | UV intensity: 1000 μW/cm <sup>2</sup>  | 103       | serotype Buhl   |
|  | UV  |                 | 100 mWs/cm <sup>2</sup>             |     |       | 99% infectivity reduction |  | 102       |   |
| 10 <sup>6,7</sup> TCID <sub>50</sub> /ml     | UV  |                 | 118,8 ± 5,7 mWs/cm <sup>2</sup>     |     |       | 99,9% reduction           | Virus in fresh water.  | 81        | UV-C  |
|  | UV  |                 | 150 mWs/cm <sup>2</sup>             |     |       | 99% reduction             |  | 104       | Type buhl   |
| 10 <sup>6,7</sup> TCID <sub>50</sub> /ml     | UV  |                 | app. 170 mWs/cm <sup>2</sup>        |     |       | "inactivated"             | Virus in fresh water.  | 81        | Read off a graph.   |
| 10 <sup>7,3-8,3</sup> TCID <sub>50</sub> /ml | UV  | 3 min 20<br>sec | 200 mWs/cm <sup>2</sup>             |     |       | 3 log reduction           | UV intensity: 1000 μW/cm <sup>2</sup>  | 103       | serotype Buhl   |
|  | UV  |                 | 200 mWs/cm <sup>2</sup>             |     |       | 3 log reduction           |  | 86        |   |
|  | UV  |                 | 246 mWs/cm <sup>2</sup>             | 7,9 | 5     | 99,9% reduction           | Sea water, sterile filtered.   | 73        | UV-C  |
| 10 <sup>4,5</sup> TCID <sub>50</sub> /ml     | UV, after<br>ferrichlorid-<br>precipitation |                 | 250 mWs/cm <sup>2</sup>             |     | 75    | ½-1 log reduction         | Full-scale trial (Vikan<br>Akvavet), wastewater from<br>fish slaugtherhouse. | 28        | Salinity 20 ‰, "bløggevand"<br>(blood, fish slime and<br>epithelial cells in salt water)<br>diluted with fresh water. |
| 10 <sup>8,2</sup> TCID <sub>50</sub> /ml     | UV  | 60 min          | 254 nm, 5 cm afstand                |     | 20    | "inactivated"             |  | 4         |   |
|  | UV  |                 | 330 mWs/cm <sup>2</sup>             |     |       | LD80                      |  | 76        |   |
| 10 <sup>3-4</sup> TCID <sub>50</sub> /ml     | UV  |                 | 336,7 ± 27,5<br>mWs/cm <sup>2</sup> |     |       | 99,9% reduction           | Virus in wastewater from fish cutting plant.                                 | 81        | UV-C  |
| 10 <sup>7,0</sup> TCID <sub>50</sub> /ml     | UV  | 6-15 min        | 720 – 1800 mWs/cm <sup>2</sup>      |     |       | 6 log reduction           | 2000 μW/cm²  | 74        | Isolate VR-299. Result read off a graph.  |
| 10 <sup>7,0</sup> TCID <sub>50</sub> /ml     | UV  | 30 min          | 792 mWs/cm <sup>2</sup>             |     |       | 5,8 log reduction         | 440 μW/cm²   | 74        | Isolate VR-299. Result read off a graph.  |
| 10 <sup>3-4</sup> TCID <sub>50</sub> /ml     | UV  |                 | app. 1500 mWs/cm <sup>2</sup>       |     |       | "inactivated"             | Virus in wastewater from fish cutting plant.                                 | 81        | Read off a graph.   |

Conclusion: IPNV is susceptible to UV irradiation with 3 log reduction when exposed to 250-350 mWs/cm<sup>2</sup>.

# Nodavirus

| Concentration pathogen  | Disinfectant | Contact<br>time | Concentration disinfectant | рН  | Temp. | Result  | Method  | Reference | Comments                             |
|-------------------------|--------------|-----------------|----------------------------|-----|-------|---|---|-----------|--------------------------------------|
| 10 μg purified<br>virus | UV           |                 | 10 mWs/cm <sup>2</sup>     | 7   | 20    | Not<br>inactivated(0/800<br>larvae survived,<br>control 326/800)                | Diluted in 1 ml PBS. Used for infection trial in day old striped jack larvae. | 9         | Isolate: SJNNV                       |
|                         | UV           |                 | 100 mWs/cm <sup>2</sup>    |     |       | 99% infectivity reduction   |   | 102       | Tvivl om referencens<br>pålidelighed |
| 10 μg purified<br>virus | UV           |                 | 100 mWs/cm <sup>2</sup>    | 7   | 20    | "Effective" (222/800 larvae survived, antigen ELISA negative - control 326/800) | Diluted in 1 ml PBS. Used for infection trial in day old striped jack larvae. | 9         | Isolate: SJNNV                       |
|                         | UV           |                 | 104 mWs/cm <sup>2</sup>    | 7,9 | 5     | 99,9% reduction   | Sea water, sterile filtered.  | 73        | UV-C                                 |
|                         | UV           | 8 min           | 211,2 mWs/cm <sup>2</sup>  |     |       | 3 log reduction   | 440 μW/cm2  | 30        | Isolate: sea bass nodavirus          |
|                         | UV           | 10 min          | 264 mWs/cm <sup>2</sup>    |     |       | Not detectable  | 440 μW/cm2  | 30        | Isolate: sea bass nodavirus          |

Conclusion: Nodavirus is susceptible to UV irradiation with 3 log reduction when exposed to 100-200 mWs/cm<sup>2</sup>.

# **PFRV**

| Concentration pathogen                   | Disinfectant | Contact<br>time | Concentration disinfectant | рН | Temp. | Result                    | Method | Reference | Comments |
|--|--------------|-----------------|----------------------------|----|-------|---------------------------|--------|-----------|----------|
|  | UV           |                 | 1 mWs/cm <sup>2</sup>      |    |       | 99% infectivity reduction |        | 102       |          |
| 10 <sup>5,3</sup> TCID <sub>50</sub> /ml | UV           | 10 min          | 254 nm, 5 cm afstand       |    | 20    | "inactivated"             |        | 4         |          |

# **SVCV**

| Concentration pathogen                   | Disinfectant | Contact<br>time | Concentration disinfectant | рН | Temp. | Result                    | Method | Reference | Comments                             |
|--|--------------|-----------------|----------------------------|----|-------|---------------------------|--------|-----------|--------------------------------------|
|  | UV           |                 | 1 mWs/cm <sup>2</sup>      |    |       | 99% infectivity reduction |        | 102       | Tvivl om referencens<br>pålidelighed |
| 10 <sup>5,2</sup> TCID <sub>50</sub> /ml | UV           | 10 min          | 254 nm, 5 cm afstand       |    | 20    | "inactivated"             |        | 4         |                                      |

# Viruses from eel

| Pathogen | Concentration pathogen | Disinfectant | Contact time | Concentration disinfectant | рН | Temp. | Result                    | Method | Reference | Comments |
|----------|------------------------|--------------|--------------|----------------------------|----|-------|---------------------------|--------|-----------|----------|
| EVA      |                        | UV           |              | 1 mWs/cm <sup>2</sup>      |    |       | 99% infectivity reduction |        | 102       |          |
| EVEX     |                        | UV           |              | 1 mWs/cm <sup>2</sup>      |    |       | 99% infectivity reduction |        | 102       |          |

Conclusion: EVA and EVEX are susceptible to UV irradiation with 2 log reduction when exposed to 1 mWs/cm<sup>2</sup>.

# Channel catfish virus

| Concentration pathogen                         | Disinfectant | Contact<br>time | Concentration disinfectant    | рН | Temp. | Result           | Method                               | Reference | Comments |
|--|--------------|-----------------|-------------------------------|----|-------|------------------|--------------------------------------|-----------|----------|
|  | UV           |                 | 2 mWs/cm <sup>2</sup>         |    |       | 99% reduction    |                                      | 104       |          |
| 10 <sup>6,55-7,05</sup> TCID <sub>50</sub> /ml | UV           |                 | 1,8 - 2,0 mWs/cm <sup>2</sup> |    |       | ID <sub>99</sub> | UV intensity: 100 μW/cm <sup>2</sup> | 103       |          |

Conclusion: CCV is susceptible to UV irradiation with 2 log reduction when exposed to 2 mWs/cm<sup>2</sup>.

# **OMV**

| Concentration pathogen  | Disinfectant | Contact<br>time | Concentration disinfectant  | рН | Temp. | Result           | Method                               | Reference | Comments |
|---|--------------|-----------------|-----------------------------|----|-------|------------------|--------------------------------------|-----------|----------|
|   | UV           |                 | 2 mWs/cm <sup>2</sup>       |    |       | 99% reduction    |                                      | 104       |          |
| 10 <sup>3,05</sup> -10 <sup>6,55</sup> TCID <sub>50</sub> /ml | UV           |                 | 1,0-2,0 mWs/cm <sup>2</sup> |    |       | ID <sub>99</sub> | UV intensity: 100 μW/cm <sup>2</sup> | 103       |          |
|   | UV           |                 | 1,4 mWs/cm <sup>2</sup>     |    |       | 3 log reduction  |                                      | 86        |          |

Conclusion: OMV is susceptible to UV irradiation with 2 log reduction when exposed to 2 mWs/cm<sup>2</sup>.

# Chum salmon virus

| Concentration pathogen                          | Disinfectant | Contact<br>time | Concentration disinfectant | рН | Temp. | Result        | Method                                | Reference | Comments |
|---|--------------|-----------------|----------------------------|----|-------|---------------|---------------------------------------|-----------|----------|
| $10^{4,3}$ - $10^{5,05}$ TCID <sub>50</sub> /ml | UV           |                 | 100 mWs/cm <sup>2</sup>    |    |       | 99% reduction | UV intensity: 1000 μW/cm <sup>2</sup> | 103, 104  |          |

# Herpesvirus salmonis

| Ī | Concentration   | Disinfectant | Contact | Concentration         | На  | Temp. | Result        | Method                               | Reference | Comments |
|---|---|--------------|---------|-----------------------|-----|-------|---------------|--------------------------------------|-----------|----------|
|   | pathogen  | Districctant | time    | disinfectant          | Pii | remp. | resure        | Weenoa                               | Reference | Comments |
|   | 10 <sup>4,05</sup> -10 <sup>4,30</sup> TCID <sub>50</sub> /ml | UV           |         | 2 mWs/cm <sup>2</sup> |     |       | 99% reduction | UV intensity: 100 μW/cm <sup>2</sup> | 104, 103  |          |

# Bacteria

# Aeromonas hydrophila

| Concentration pathogen                      | Disinfectant | Contact<br>time | Concentration disinfectant     | рН | Temp. | Result            | Method  | Reference | Comments |
|---|--------------|-----------------|--------------------------------|----|-------|-------------------|---|-----------|----------|
| 10 <sup>4</sup> -10 <sup>5</sup> cfu/ml     | UV           | 3,2 sec         | 3,3 – 5,3 mWs/cm <sup>2</sup>  |    | 12,5  | > 99.0% reduction | Water with dissolved organic matter without filtration. | 15        |          |
| 10 <sup>4</sup> -10 <sup>5</sup> cfu/ml     | UV           | 3,2 sec         | 4,0 – 4,75 mWs/cm <sup>2</sup> |    | 12,5  | > 99.3% reduction | Water with dissolved organic matter with filtration.    | 15        |          |
| 10 <sup>4</sup> -10 <sup>5</sup> cfu/ml     | UV           | 3,2 sec         | 4,5 mWs/cm <sup>2</sup>        |    | 12,5  | > 99.8% reduction | Spring water, 25 nm filtration before UV irradiation.   | 15        |          |
| 10 <sup>3</sup> cfu                         | UV           |                 | 5 mWs/cm <sup>2</sup>          |    |       | ≥ 99,9% reduction | Spreadning on agar plate followed by UV irradiation.    | 104, 59   |          |
| 10 <sup>4</sup> -10 <sup>5</sup> cfu/ml     | UV           | 3,2 sec         | 12 - 30 mWs/cm <sup>2</sup>    |    | 12,5  | > 99.9% reduction | Water with dissolved organic matter without filtration. | 15        |          |
| 10 <sup>4</sup> -10 <sup>5</sup> cfu/ml     | UV           | 3,2 sec         | 13 - 29 mWs/cm <sup>2</sup>    |    | 12,5  | > 99.9% reduction | Water with dissolved organic matter with filtration.    | 15        |          |
| 10 <sup>4</sup> -10 <sup>5</sup> cfu/ml     | UV           | 3,2 sec         | 21 - 24 mWs/cm <sup>2</sup>    |    | 12,5  | > 99.9% reduction | Spring water, 25 nm filtration before UV irradiation.   | 15        |          |
| 1,3 x10 <sup>7</sup> TCID <sub>50</sub> /ml | UV           |                 | 23,1 mWs/cm <sup>2</sup>       |    |       | > 4 log reduction |   | 60        |          |

Conclusion: A. hydrophila is susceptible to UV irradiation with 3 log reduction when exposed to 5-25 mWs/cm<sup>2</sup>.

# Aeromonas punctata

| Concentration pathogen                      | Disinfectant | Contact<br>time | Concentration disinfectant | рН | Temp. | Result            | Method   | Reference | Comments |
|---|--------------|-----------------|----------------------------|----|-------|-------------------|--|-----------|----------|
| 10 <sup>3</sup> cfu                         | UV           |                 | 4 mWs/cm <sup>2</sup>      |    |       | ≥ 99,9% reduction | Spreadning on agar plate followed by UV irradiation. | 104, 59   |          |
| 2,2 x10 <sup>5</sup> TCID <sub>50</sub> /ml | UV           |                 | 23,1 mWs/cm <sup>2</sup>   |    |       | 99,97% reduction  |  | 60        |          |

# Aeromonas salmonicida

| Concentration pathogen | Disinfectant | Contact<br>time | Concentration disinfectant | рН  | Temp. | Result          | Method   | Reference | Comments                 |
|------------------------|--------------|-----------------|----------------------------|-----|-------|-----------------|--|-----------|--------------------------|
| 10 <sup>7</sup> CFU/ml | UV           |                 | 0,05 mW/cm <sup>2</sup>    | 7,2 | 7     | 99,9% reduction | PBS  | 71        | Reduction rate: 0,15/sec |
| 10 <sup>7</sup> CFU/ml | UV           |                 | 0,05 mW/cm <sup>2</sup>    | 7,8 | 7     | 99,9% reduction | Wastewater from aquaculture (15,7 ‰ salinity). | 71        | Reduction rate: 0,14/sec |

| 10 <sup>7</sup> CFU/ml                      | UV | 48 sec  | 2,34 mWs/cm <sup>2</sup>       | 7,2 | 7    | 99,9 % reduction  | PBS   | 71      |                                   |
|---|----|---------|--------------------------------|-----|------|-------------------|---|---------|-----------------------------------|
| 10 <sup>7</sup> CFU/ml                      | UV | 50 sec  | 2,38 mWs/cm <sup>2</sup>       | 7,8 | 7    | 99,9 % reduction  | Wastewater from aquaculture (15,7 ‰ salinity).                                      | 71      |                                   |
| 10 <sup>4</sup> -10 <sup>5</sup> cfu/ml     | UV | 3,2 sec | 3,3 – 5,3 mWs/cm <sup>2</sup>  |     | 12,5 | > 99.0% reduction | Water with dissolved organic matter without filtration.                             | 15      |                                   |
|   | UV |         | 3,4 mWs/cm <sup>2</sup>        |     |      | 3 log reduction   |   | 86      |                                   |
| 10 <sup>3</sup> cfu                         | UV |         | 4 mWs/cm <sup>2</sup>          |     |      | ≥ 99,9% reduction | Spreadning on agar plate followed by UV irradiation.                                | 104, 59 |                                   |
| 10 <sup>4</sup> -10 <sup>5</sup> cfu/ml     | UV | 3,2 sec | 4,0 – 4,75 mWs/cm <sup>2</sup> |     | 12,5 | > 99.3% reduction | Water with dissolved organic matter with filtration.                                | 15      |                                   |
| 10 <sup>4</sup> -10 <sup>5</sup> cfu/ml     | UV | 3,2 sec | 4,5 mWs/cm <sup>2</sup>        |     | 12,5 | > 99.8% reduction | Spring water, 25 nm filtration before UV irradiation.                               | 15      |                                   |
| 10 <sup>4</sup> -10 <sup>5</sup> cfu/ml     | UV | 3,2 sec | 12 - 30 mWs/cm <sup>2</sup>    |     | 12,5 | > 99.9% reduction | Water with dissolved organic matter without filtration.                             | 15      |                                   |
| 10 <sup>4</sup> -10 <sup>5</sup> cfu/ml     | UV | 3,2 sec | 13 - 29 mWs/cm <sup>2</sup>    |     | 12,5 | > 99.9% reduction | Water with dissolved organic matter with filtration.                                | 15      |                                   |
|   | UV |         | 13 mWs/cm <sup>2</sup>         |     | 12,5 | "inactivated"     | Infection trial in water filtratrated with 25 nm filter followed by UV irradiation. | 15      | 98,5% mortality in control group. |
| 10 <sup>4</sup> -10 <sup>5</sup> cfu/ml     | UV | 3,2 sec | 21 - 24 mWs/cm <sup>2</sup>    |     | 12,5 | > 99.9% reduction | Spring water, 25 nm filtration before UV irradiation.                               | 15      |                                   |
| 5,6 x10 <sup>6</sup> TCID <sub>50</sub> /ml | UV |         | 23,1 mWs/cm <sup>2</sup>       |     | 18,3 | > 4 log reduction |   | 60      |                                   |

Conclusion: A. salmonicida is susceptible to UV irradiation with  $\geq$  3 log reduction when exposed to 5-25 mWs/cm<sup>2</sup>.

# Escherichia coli

| Concentration pathogen | Disinfectant | Contact<br>time | Concentration disinfectant | рН | Temp. | Result            | Method   | Reference | Comments |
|------------------------|--------------|-----------------|----------------------------|----|-------|-------------------|--|-----------|----------|
| 10 <sup>3</sup> cfu    | UV           |                 | 4 mWs/cm <sup>2</sup>      |    |       | ≥ 99,9% reduction | Spreadning on agar plate followed by UV irradiation. | 104, 59   |          |

# Natural flora (heterothrophic bacteria)

| Concentration pathogen       | Disinfectant         | Contact<br>time | Concentration disinfectant    | рН  | Temp. | Result            | Method  | Reference | Comments   |
|------------------------------|----------------------|-----------------|-------------------------------|-----|-------|-------------------|---|-----------|--|
| 10 <sup>3,5</sup> cfu/ml     | UV                   |                 |                               |     |       | 93,6% reduction   | flowrate 4 l/m  | 53        | Sea water, filtrated (10 μm)                             |
| 7,4 x 10 <sup>5</sup> cfu/ml | UV                   |                 | 10 mWs/cm <sup>2</sup>        |     | 5     | 2,5 log reduction | Sea water, Artemia added.                             | 69        |  |
| 7,4 x 10 <sup>5</sup> cfu/ml | UV                   |                 | 13 mWs/cm <sup>2</sup>        |     | 5     | 2,5 log reduction | Sea water, Artemia added.                             | 69        | The artemia protects the bacteria.                       |
| 7,4 x 10 <sup>5</sup> cfu/ml | UV                   |                 | 22 mWs/cm <sup>2</sup>        |     | 5     | 2,5 log reduction | Sea water, Artemia added.                             | 69        | The artemia protects the bacteria.                       |
| 7,4 x 10 <sup>5</sup> cfu/ml | UV med præfiltrering |                 | 22 mWS/cm <sup>2</sup>        |     | 5     | > 5 log reduction | Sea water, Artemia added,<br>filtration through 50 μm | 69        | cfu after filtration app. The same as before filtration. |
| 4,7 x 10 <sup>4</sup> cfu/ml | UV                   |                 | 150 mWs/cm <sup>2</sup>       |     |       | 4 log reduction   | flowrate 2,0 m <sup>3</sup>                           | 56        | Natural flora in wastewater from hathing facility.       |
| app. 9000 cfu/ml             | UV                   |                 | app. 1800 mWs/cm <sup>2</sup> | 7,5 |       | 1,7 log reduction | Fish farm, recirculation.                             | 88        |  |

Conclusion: there are conflicting results concerning the resistance of the natural flora towards UV irradiation. The composition of the natural flora will depend on a lot of different variables, which will influence the effect of UV irradiation.

# Pseudomonas flourescens

| Concentration pathogen                      | Disinfectant | Contact<br>time | Concentration disinfectant     | pН | Temp. | Result            | Method  | Reference | Comments |
|---|--------------|-----------------|--------------------------------|----|-------|-------------------|---|-----------|----------|
| 10 <sup>4</sup> -10 <sup>5</sup> cfu/ml     | UV           | 3,2 sec         | 3,3 – 5,3 mWs/cm <sup>2</sup>  |    | 12,5  | > 99.0% reduction | Water with dissolved organic matter without filtration. | 15        |          |
| 10 <sup>4</sup> -10 <sup>5</sup> cfu/ml     | UV           | 3,2 sec         | 4,0 – 4,75 mWs/cm <sup>2</sup> |    | 12,5  | > 99.3% reduction | Water with dissolved organic matter with filtration.    | 15        |          |
| 10 <sup>4</sup> -10 <sup>5</sup> cfu/ml     | UV           | 3,2 sec         | 4,5 mWs/cm <sup>2</sup>        |    | 12,5  | > 99.8% reduction | Spring water, 25 nm filtration before UV irradiation.   | 15        |          |
| 10³ cfu                                     | UV           |                 | 5 mWs/cm <sup>2</sup>          |    |       | ≥ 99,9% reduction | Spreadning on agar plate followed by UV irradiation.    | 104, 59   |          |
| 10 <sup>4</sup> -10 <sup>5</sup> cfu/ml     | UV           | 3,2 sec         | 12 - 30 mWs/cm <sup>2</sup>    |    | 12,5  | > 99.9% reduction | Water with dissolved organic matter without filtration. | 15        |          |
| 10 <sup>4</sup> -10 <sup>5</sup> cfu/ml     | UV           | 3,2 sec         | 13 - 29 mWs/cm <sup>2</sup>    |    | 12,5  | > 99.9% reduction | Water with dissolved organic matter with filtration.    | 15        |          |
| 10 <sup>4</sup> -10 <sup>5</sup> cfu/ml     | UV           | 3,2 sec         | 21 - 24 mWs/cm <sup>2</sup>    |    | 12,5  | > 99.9% reduction | Spring water, 25 nm filtration before UV irradiation.   | 15        |          |
| 1,5 x10 <sup>7</sup> TCID <sub>50</sub> /ml | UV           |                 | 23,1 mWs/cm <sup>2</sup>       |    | 20,4  | 4 log reduction   |   | 60        |          |

Conclusion: *P. flourescens* is susceptible to UV irradiation with  $\geq$  3 log reduction when exposed to 5-25 mWs/cm<sup>2</sup>.

# Vibrio anguillarum

| Concentration pathogen                      | Disinfectant | Contact<br>time | Concentration disinfectant     | рН | Temp. | Result            | Method  | Reference | Comments |
|---|--------------|-----------------|--------------------------------|----|-------|-------------------|---|-----------|----------|
|   | UV           |                 | 2,9 mWs/cm <sup>2</sup>        |    |       | 3 log reduction   |   | 86        |          |
| 10 <sup>4</sup> -10 <sup>5</sup> cfu/ml     | UV           | 3,2 sec         | 3,3 – 5,3 mWs/cm <sup>2</sup>  |    | 12,5  | > 99.0% reduction | Water with dissolved organic matter without filtration. | 15        |          |
| 10 <sup>3</sup> cfu                         | UV           |                 | 4 mWs/cm <sup>2</sup>          |    |       | ≥ 99,9% reduction | Spreadning on agar plate followed by UV irradiation.    | 104, 59   |          |
| 10 <sup>4</sup> -10 <sup>5</sup> cfu/ml     | UV           | 3,2 sec         | 4,0 – 4,75 mWs/cm <sup>2</sup> |    | 12,5  | > 99.3% reduction | Water with dissolved organic matter with filtration.    | 15        |          |
| 10 <sup>4</sup> -10 <sup>5</sup> cfu/ml     | UV           | 3,2 sec         | 4,5 mWs/cm <sup>2</sup>        |    | 12,5  | > 99.8% reduction | Spring water, 25 nm filtration before UV irradiation.   | 15        |          |
| 10 <sup>4</sup> -10 <sup>5</sup> cfu/ml     | UV           | 3,2 sec         | 12 – 30 mWs/cm <sup>2</sup>    |    | 12,5  | > 99.9% reduction | Water with dissolved organic matter without filtration. | 15        |          |
| 10 <sup>4</sup> -10 <sup>5</sup> cfu/ml     | UV           | 3,2 sec         | 13 - 29 mWs/cm <sup>2</sup>    |    | 12,5  | > 99.9% reduction | Water with dissolved organic matter with filtration.    | 15        |          |
| 10 <sup>4</sup> -10 <sup>5</sup> cfu/ml     | UV           | 3,2 sec         | 21 – 24 mWs/cm <sup>2</sup>    |    | 12,5  | > 99.9% reduction | Spring water, 25 nm filtration before UV irradiation.   | 15        |          |
| 1,9 x10 <sup>6</sup> TCID <sub>50</sub> /ml | UV           |                 | 23,1 mWs/cm <sup>2</sup>       |    | 20,3  | > 5 log reduction |   | 60        |          |

Conclusion: V. anguillarum is susceptible to UV irradiation with  $\geq 3 \log reduction$  when exposed to 5-25 mWs/cm<sup>2</sup>.

# Vibrio ordalii

| Concentration pathogen | Disinfectant | Contact<br>time | Concentration disinfectant | рН | Temp. | Result          | Method | Reference | Comments |
|------------------------|--------------|-----------------|----------------------------|----|-------|-----------------|--------|-----------|----------|
|                        | UV           |                 | 5,5 mWs/cm <sup>2</sup>    |    |       | 3 log reduction |        | 86        |          |

# Yersinia ruckeri

| Concentration pathogen                  | Disinfectant | Contact<br>time | Concentration disinfectant    | рН | Temp. | Result            | Method  | Reference | Comments |
|---|--------------|-----------------|-------------------------------|----|-------|-------------------|---|-----------|----------|
| 10 <sup>4</sup> -10 <sup>5</sup> cfu/ml | UV           | 3,2 sec         | 3,3 – 5,3 mWs/cm <sup>2</sup> |    | 12,5  | > 99.0% reduction | Water with dissolved organic matter without filtration. | 15        |          |
| 10 <sup>4</sup> -10 <sup>5</sup> cfu/ml | UV           | 3,2 sec         | 4 – 4,75 mWs/cm <sup>2</sup>  |    | 12,5  | > 99.3% reduction | Water with dissolved organic matter with filtration.    | 15        |          |

| 10 <sup>4</sup> -10 <sup>5</sup> cfu/ml | UV   | 3,2 sec | 4,5 mWs/cm <sup>2</sup>     | 12,5 | > 99.8% reduction | Spring water, 25 nm<br>filtration before UV<br>irradiation.                  | 15 |   |
|---|--|---------|-----------------------------|------|-------------------|--|----|---|
| 10 <sup>4</sup> -10 <sup>5</sup> cfu/ml | UV   | 3,2 sec | 12 - 30 mWs/cm <sup>2</sup> | 12,5 | > 99.9% reduction | Water with dissolved organic matter without filtration.                      | 15 |   |
| 10 <sup>4</sup> -10 <sup>5</sup> cfu/ml | UV   | 3,2 sec | 13 – 29 mWs/cm <sup>2</sup> | 12,5 | > 99.9% reduction | Water with dissolved organic matter with filtration.                         | 15 |   |
| 10 <sup>4</sup> -10 <sup>5</sup> cfu/ml | UV   | 3,2 sec | 21 – 24 mWs/cm <sup>2</sup> | 12,5 | > 99.9% reduction | Spring water, 25 nm filtration before UV irradiation.                        | 15 |   |
| 10 <sup>5,21</sup> cfu/ml               | UV, after<br>ferrichlorid<br>precipitation |         | 250 mWs/cm <sup>2</sup>     | 65   | 1 log reduction   | Full-scale trial (Vikan<br>Akvavet), wastewater from<br>fish slaugtherhouse. | 28 | Salinity 20 ‰, "bløggevand"<br>(blood, fish slime and<br>epithelial cells in salt water)<br>diluted with fresh water. |
|   | UV   |         | 1200 mWs/cm <sup>2</sup>    |      | 2½ log reduction  | Full-scale trial (Norskagfisk),<br>blood water from fish<br>slaugtherhouse.  | 28 | forfilter 20 μm   |

Conclusion: In laboratory trials *Y. ruckeri* is susceptible to UV irradiation with  $\geq 3$  log reduction when exposed to 5-25 mWs/cm<sup>2</sup>. In full scale trials, after precipitation using ferrichlorid only 1 log reduction was obtained after a dose of 250 mWs/cm<sup>2</sup>, and it was not possible to obtain a 3 log reduction using a dose of 1200 mWs/cm<sup>2</sup> despite prefiltration through a 20  $\mu$ m filter.

#### **Parasites**

#### Ichthyophthirius multifiliis

| Concentration pathogen | Disinfectant | Contact<br>time | Concentration disinfectant | рН      | Temp. | Result                 | Method  | Reference | Comments   |
|------------------------|--------------|-----------------|----------------------------|---------|-------|------------------------|---|-----------|------------|
|                        | UV           |                 | 92 mWs/cm <sup>2</sup>     | 6,1-7,3 |       | Transmission prevented | Transmission of Ich from infected to free fish. | 34        | 1 UV lamp. |

#### Myxosoma cerebralis

| Concentration pathogen | Disinfectant                      | Contact<br>time | Concentration disinfectant | рН | Temp. | Result                               | Method  | Reference | Comments  |
|------------------------|-----------------------------------|-----------------|----------------------------|----|-------|--------------------------------------|---|-----------|---|
|                        | UV                                |                 | 4 mWs/cm <sup>2</sup>      |    |       | > 5 log reduction                    | Infectivity of myxospores in tubifex.   | 37        | Myxospore suspension  |
|                        | UV, pre<br>filtration of<br>water |                 | 28 mWs/cm <sup>2</sup>     |    |       | 86-100% reduction<br>af infektivitet | 25 µm filter, UV irradiation of contaminated water, fish added, clinic and % spores registered. | 41        | Ingen klinik, ingen spores i<br>det ene forsøg, 14% spores i<br>det andet forsøg. Kontrolfisk<br>100% spores og klinik. |

|                         | UV | 35 mWs/cm <sup>2</sup>   | "inactivated"      | Infection trial wiht UV irradiated spores (UV-doses 35000, 43000 and 112000 μWs/cm2) | 40 |                      |
|-------------------------|----|--------------------------|--------------------|--|----|----------------------|
| 2 x 10 <sup>4</sup> TAM | UV | 40 mWS/cm <sup>2</sup>   | "inactivated"      | Smitteforsøg med UV-<br>behandlede<br>triactinomyxoner<br>(1000/fisk)                | 36 |                      |
|                         | UV | 48 mWs/cm <sup>2</sup>   | 4,75 log reduction | Infektivitet af myxospores i tubifex   | 37 | Myxospore suspension |
|                         | UV | 1700 mWs/cm <sup>2</sup> | alle døde          | myxospores i suspension  | 44 |                      |

## **Fungae and oomycetes**

# Achlya flagellata

| Concentration pathogen | Disinfectant | Contact<br>time | Concentration disinfectant | рН | Temp. | Result        | Method | Reference | Comments |
|------------------------|--------------|-----------------|----------------------------|----|-------|---------------|--------|-----------|----------|
|                        | UV           |                 | 220 mWs/cm <sup>2</sup>    |    |       | "inactivated" |        | 59        |          |

## Aphanomyces laevis

| Concentration pathogen | Disinfectant | Contact<br>time | Concentration disinfectant | рН | Temp. | Result        | Method | Reference | Comments |
|------------------------|--------------|-----------------|----------------------------|----|-------|---------------|--------|-----------|----------|
|                        | UV           |                 | 210 mWs/cm <sup>2</sup>    |    |       | "inactivated" |        | 59        |          |

## Saprolegnia

| Pathogen                  | Concentration pathogen | Disin-<br>fectant | Contact<br>time | Concentration disinfectant    | рН | Temp. | Result        | Method                                    | Reference | Comments |
|---------------------------|------------------------|-------------------|-----------------|-------------------------------|----|-------|---------------|---|-----------|----------|
| Saprolegnia<br>anispora   |                        | UV                |                 | 150 mWs/cm <sup>2</sup>       |    |       | "inactivated" | Punched agar disk with hyphae irradiated. | 104, 59   |          |
| Saprolegnia<br>parasitica |                        | UV                |                 | 200 – 230 mWs/cm <sup>2</sup> |    |       | "inactivated" | Punched agar disk with hyphae irradiated. | 104, 59   |          |
| Saproglegnia<br>sp.       |                        | UV                |                 | 210 – 250 mWs/cm <sup>2</sup> |    |       | "inactivated" | Punched agar disk with hyphae irradiated. | 104, 59   |          |

# UV in combination with other treatments

#### **IPNV**

| Concentration pathogen | Disinfectant | Contact time | Concentration disinfectant         | рН | Temp. | Result   | Method | Reference | Comments |
|------------------------|--------------|--------------|------------------------------------|----|-------|----------|--------|-----------|----------|
|                        | UV/ozone     |              | 161 mWs/cm <sup>2</sup> +<br>ozone |    |       | Survival |        | 86        |          |

### Aeromonas salmonicida

| Concentration pathogen | Disinfectant      | Contact<br>time | Concentration disinfectant                  | рН  | Temp. | Result           | Method   | Reference | Comments                 |
|------------------------|-------------------|-----------------|---|-----|-------|------------------|--|-----------|--------------------------|
| 10 <sup>7</sup> CFU/ml | UV/NaOCI          |                 | 0,05 mW/cm <sup>2</sup> /<br>0,2 mg/l added | 7,2 | 7     | 99,9% reduction  | PBS  | 71        | Reduction rate: 0,32/sec |
| 10 <sup>7</sup> CFU/ml | UV/NaOCI          |                 | 0,05 mW/cm <sup>2</sup> /<br>2,0 mg/l added | 7,8 | 7     | 99,9% reduction  | Wastewater from aquaculture (15,7 % salinity). | 71        | Reduction rate: 0,26/sec |
| 10 <sup>7</sup> CFU/ml | UV/I <sub>2</sub> |                 | 0,05 mW/cm <sup>2</sup> /<br>1,0 mg/l added | 7,2 | 7     | 99,9 % reduction | PBS  | 71        | Reduction rate: 0,42/sec |
| 10 <sup>7</sup> CFU/ml | UV/I <sub>2</sub> |                 | 0,05 mW/cm <sup>2</sup> /<br>1,3 mg/l added | 7,8 | 7     | 99,9 % reduction | Wastewater from aquaculture (15,7 % salinity). | 71        | Reduction rate: 0,28/sec |
| 10 <sup>7</sup> CFU/ml | UV/ozone          |                 | 0,05 mW/cm <sup>2</sup> /<br>0,1 mg/l       | 7,2 | 7     | 99,9 % reduction | PBS  | 71        | Reduction rate: 0,32/sec |

## Natural flora (heterothrophic bacteria)

| Concentration pathogen | Disinfectant | Contact time | Concentration disinfectant            | рН  | Temp. | Result             | Method                   | Reference | Comments |
|------------------------|--------------|--------------|---------------------------------------|-----|-------|--------------------|--------------------------|-----------|----------|
| 116 ± 25 cfu/ml        | ozone/UV     |              | 0,21 mg/l, 54,7<br>mJ/cm <sup>2</sup> | 7,5 | 14,3  | 1,81 log reduction | fish farm, recirculation | 87        |          |

# Miscellaneous chlorine compounds

NB: It it not always stated if the concentration of the disinfectant is as free chlorine or as the disinfectant itself.

#### Virus

#### **VHSV**

| Concentration pathogen                   | Disinfectant            | Contact<br>time | Concentration disinfectant | рН        | Temp. | Result           | Method   | Reference | Comments  |
|--|-------------------------|-----------------|----------------------------|-----------|-------|------------------|--|-----------|---|
| 10 <sup>7,8</sup> TCID <sub>50</sub> /ml | Benzalkonium chlorid    | 6 hours         | 1%                         |           |       | Stable           | 10 % calf serum added  | 4, 5      |   |
| 10 <sup>8</sup> TCID <sub>50</sub> /ml   | Benzalkonium<br>chlorid | 30 min          | 1:1000                     |           | 15    | Not detectable   | 10% (w/v) dilution in PBS.<br>1% fetal calf serum.               | 65        | Strain JF00Ehi1. Benzalkoniumklorid 10% (w/v). Dilution scale 1:1000. |
| 10 <sup>8</sup> TCID <sub>50</sub> /ml   | Benzalkonium<br>chlorid | 5 min           | 1:1000                     |           | 15    | Not detectable   | 10% (w/v) dilution in artificail sea water. 1% fetal calf serum. | 65        | Strain JF00Ehi1. Benzalkoniumklorid 10% (w/v). Dilution scale 1:1000. |
| 10 <sup>7,2</sup> TCID <sub>50</sub> /ml | NaOCl                   | 10 min          | 7,6 mg/ml Cl <sub>2</sub>  | 7,07-7,49 | 10    | ≥99 % reduction  |  | 4         |   |
| 10 <sup>6,8</sup> TCID <sub>50</sub> /ml | NaOCl                   | 60 min          | 7,6 mg/ml Cl <sub>2</sub>  | 7,07-7,49 | 10    | Stable           | 2,5 % calf serum added   | 4         |   |
| 10 <sup>6,2</sup> TCID <sub>50</sub> /ml | NaOCl                   | 5 min           | 25 mg/ml Cl <sub>2</sub>   | 7,07-7,49 | 10    | ≥ 99 % reduction |  | 4         |   |
| 10 <sup>6,5</sup> TCID <sub>50</sub> /ml | NaOCl                   | 10 min          | 25 mg/ml Cl <sub>2</sub>   | 7,07-7,49 | 10    | ≥ 99 % reduction | 2,5 % calf serum added   | 4         |   |
| 10 <sup>6,5</sup> TCID <sub>50</sub> /ml | NaOCl                   | 2 min           | 36 mg/ml Cl <sub>2</sub>   | 7,07-7,49 | 10    | ≥ 99 % reduction |  | 4         |   |
| 10 <sup>6,5</sup> TCID <sub>50</sub> /ml | NaOCl                   | 10 min          | 36 mg/ml Cl <sub>2</sub>   | 7,07-7,49 | 10    | ≥ 99 % reduction | 2,5 % calf serum added   | 4         |   |
| 10 <sup>6,5</sup> TCID <sub>50</sub> /ml | NaOCl                   | 5 min           | 54 mg/ml Cl <sub>2</sub>   | 7,07-7,49 | 10    | ≥ 99 % reduction |  | 4         |   |
| 10 <sup>6,8</sup> TCID <sub>50</sub> /ml | NaOCl                   | 5 min           | 54 mg/ml Cl <sub>2</sub>   | 7,07-7,49 | 10    | ≥ 99 % reduction | 2,5 % calf serum added   | 4         |   |
| 10 <sup>7,8</sup> TCID <sub>50</sub> /ml | NaOCl                   | 2 min           | 98 mg/ml Cl <sub>2</sub>   | 7,07-7,49 | 10    | ≥ 99 % reduction |  | 4         |   |
| 10 <sup>7,2</sup> TCID <sub>50</sub> /ml | NaOCl                   | 10 min          | 98 mg/ml Cl <sub>2</sub>   | 7,07-7,49 | 10    | ≥ 99 % reduction | 2,5 % calf serum added   | 4         |   |
| 10 <sup>6,5</sup> TCID <sub>50</sub> /ml | NaOCl                   | < 2 min         | 515 mg/ml Cl <sub>2</sub>  | 7,07-7,49 | 10    | ≥ 99 % reduction |  | 4         |   |
| 10 <sup>6,8</sup> TCID <sub>50</sub> /ml | NaOCl                   | < 2 min         | 515 mg/ml Cl <sub>2</sub>  | 7,07-7,49 | 10    | ≥99 % reduction  | 2,5 % calf serum added   | 4         |   |
| 10 <sup>7</sup> TCIID <sub>50</sub> /ml  | NaOCl                   | 1 min           | 50 ppm                     |           | 15    | Not detectable   | Diluted in PBS. 1% fetal calf serum.                             | 65        | Isolat JF00EHi1. Dilution scale 1:50.                                 |
| 10 <sup>7</sup> TCID <sub>50</sub> /ml   | NaOCl                   | 1 min           | 50 ppm                     |           | 15    | Ineffective.     | Diluted in artifical sea water. 1% fetal calf serum.             | 65        | Isolat JF00EHi1. Dilution scale 1:50.                                 |
| 10 <sup>7</sup> TCID <sub>50</sub> /ml   | NaOCl                   | 5 min           | 100 ppm                    |           | 15    | Not detectable   | Diluted in artifical sea water. 1% fetal calf serum.             | 65        | Isolat JF00EHi1. Dilution scale 1:50                                  |
| 10 <sup>7</sup> TCID <sub>50</sub> /ml   | NaOCl                   | 1 min           | 200 ppm                    |           | 15    | Not detectable   | Diluted in artifical sea water. 1% fetal calf serum.             | 65        | Isolat JF00EHi1. Dilution scale 1:50                                  |

Conclusion: In laboratory experiements under dirty conditions (addition of 1% calf serum) it was possible to decimate VHSV to non detectable by use of 100 mg/l chlorine for 5 min. Another experiment showed that using the same dose it takes 2 min to decimate VHSV  $\geq$  2 bg under clean conditions but 10 min under dirty conditions (2,5% calf serum). It is not possible to validate the dose to decimate VHSV 3 log based on these figures.

#### **IHNV**

| Concentration pathogen                                   | Disinfectant | Contact<br>time | Concentration disinfectant | рН  | Temp. | Result        | Method                                       | Reference | Comments |
|--|--------------|-----------------|----------------------------|-----|-------|---------------|--|-----------|----------|
| 10 <sup>4</sup> - 10 <sup>5</sup> TCID <sub>50</sub> /ml | NaOCl        | 30 sec          | 0,1 mg/l residual          | 6,9 | 10    | "inactivated" | Distilled water.                             | 99, 98    |          |
| 10 <sup>4</sup> - 10 <sup>5</sup> TCID <sub>50</sub> /ml | NaOCl        | 5 min           | 0,5 mg/l residual          | 6,9 | 10    | "inactivated" | Soft lake water., 30 mg/l CaCO <sub>3</sub>  | 99, 98    |          |
| 10 <sup>4</sup> - 10 <sup>5</sup> TCID <sub>50</sub> /ml | NaOCl        | 10 min          | 0,5 mg/l residual          | 8,2 | 10    | "inactivated" | Hard lake water., 120 mg/l CaCO <sub>3</sub> | 99, 98    |          |
| 10 <sup>4</sup> - 10 <sup>5</sup> TCID <sub>50</sub> /ml | NaOCl        | 30 sec          | 1,0 mg/l residual          | 8,2 | 10    | "inactivated" | Hard lake water., 120 mg/l CaCO <sub>3</sub> | 99, 98    |          |
|  | NaOCI        | 30 min          | 10 ppm Cl <sub>2</sub>     |     |       | "inactivated" |  | 8         |          |

Conclusion: Chlorine is effective to inactive IHNV at low cencentrations of short contact time. The tests are performed under fairly clean conditions and not under conditions comparable to wastewater from fish cutting plants.

#### **ISAV**

| Concentration pathogen | Disinfectant     | Contact<br>time | Concentration disinfectant | рН | Temp. | Result              | Method  | Reference | Comments    |
|------------------------|------------------|-----------------|----------------------------|----|-------|---------------------|---|-----------|-------------|
| 6,8 log10 ffu/ml       | chloramine-T     | 5 min           | 0,25%                      |    | 4     | > 6,5 log reduction | Hard water, 342 ppm total hardness.   | 89        | Buffodine   |
| 6,2 log10 ffu/ml       | CIO <sub>2</sub> | 5 min           | 25 ppm                     |    | 4     | 1,2 log reduction   | Hard water, 342 ppm total hardness, no addition of serum.                     | 89        | Zydox AD-05 |
| 5,1 log10 ffu/ml       | CIO <sub>2</sub> | 5 min           | 50 ppm                     |    | 4     | 1,4 log reduction   | Hard water, 342 ppm total hardness, addition of serum.                        | 89        | Zydox AD-05 |
| 5,1 log10 ffu/ml       | CIO <sub>2</sub> | 5 min           | 50 ppm                     |    | 4     | > 4,8 log reduction | Hard water, 342 ppm total hardness, addition of serum.                        | 89        | Zydox AD-05 |
| 6,2 log10 ffu/ml       | CIO <sub>2</sub> | 5 min           | 50 ppm                     |    | 4     | 5,3 log reduction   | Hard water, 342 ppm total hardness, no addition of serum.                     | 89        | Zydox AD-05 |
| 5,5 log10 ffu/ml       | OCI <sup>-</sup> | 5 min           | 100 ppm                    |    | 4     | > 5,2 log reduction | Hard water, 342 ppm total<br>hardness, with and<br>without addition of serum. | 89        |             |

|  | NaOCI | 15 min | 100 mg/l | - | - | "inactivated" | Tissue homogenate of liver, kidney and spleen from moribund ISA-fish, treated and IP-injected in fish. | 94 | Testet ved konc. 5, 10, 20,<br>50 og 100 mg/l med<br>kontakttid 15 min og 30<br>min. |
|--|-------|--------|----------|---|---|---------------|--|----|--|
|--|-------|--------|----------|---|---|---------------|--|----|--|

Conclusion: Under laboratory conditions it is possible to obtain 5 log reduction of ISAV using 100 mg/l chlorine for 5 minuts.

#### KHV

| Concentration pathogen                              | Disinfectant             | Contact time | Concentration disinfectant | рН | Temp. | Result          | Method  | Reference | Comments  |
|---|--------------------------|--------------|----------------------------|----|-------|-----------------|---|-----------|---|
| 1 - 1,5 x 10 <sup>4</sup><br>TCID <sub>50</sub> /ml | Benzalkonium<br>chloride | 30 sec       | 60 mg/l                    |    | 0     | Not detectable  | Virus and disinfectant<br>mixed 1:1, tested after 30<br>sek and 20 min. Diluted<br>1:10 using L15 medium og<br>inoculated 200 μl. | 55        | Strain KHV-I. The method cannot detect a 3 log reduction.       |
| 1 - 1,5 x 10 <sup>4</sup><br>TCID <sub>50</sub> /ml | Benzalkonium<br>chloride | 30 sec       | 30 mg/l                    |    | 25    | Not detectable  | Virus and disinfectant<br>mixed 1:1, tested after 30<br>sek and 20 min. Diluted<br>1:10 using L15 medium og<br>inoculated 200 μl. | 55        | Strain KHV-I. The method cannot detect a 3 log reduction.       |
|   | NaOCl                    | 20 min       | 0,3 mg/l residual          |    |       | 98,5% reduction |   | 55        | Strain KHV-I.   |
| 1 - 1,5 x 10 <sup>4</sup><br>TCID <sub>50</sub> /ml | NaOCI                    | 20 min       | 200 mg/l                   |    | 0     | "inactivated"   | Virus and disinfectant<br>mixed 1:1, tested after 30<br>sec and 20 min. Diluted<br>1:10 using L15 medium og<br>inoculated 200 μl. | 99, 98    | Strain KHV-I. The method cannot detect a 3 log reduction.       |
| 1 - 1,5 x 10 <sup>4</sup><br>TCID <sub>50</sub> /ml | NaOCI                    | 20 min       | 250 mg/l                   |    | 25    | "inactivated"   | Virus and disinfectant<br>mixed 1:1, tested after 30<br>sec and 20 min. Diluted<br>1:10 using L15 medium og<br>inoculated 200 μl. | 99, 98    | Strain KHV-I. The method<br>cannot detect a 3 log<br>reduction. |

Conclusion: It does seem as if KHV is susceptible to chlorine. It is difficult to assess the dose to reduce the titer 3 log for KHV, as the methods used are not able to detect such a reduction.

#### *IPNV*

| Concentration pathogen                   | Disinfectant            | Contact<br>time | Concentration disinfectant | рН | Temp. | Result            | Method                | Reference | Comments            |
|--|-------------------------|-----------------|----------------------------|----|-------|-------------------|-----------------------|-----------|---------------------|
| 10 <sup>7,5</sup> TCID <sub>50</sub> /ml | Benzalkonium<br>chlorid | 6 hours         | 1%                         |    |       | Stable            | 10 % calf serum added | 4, 5      | Trade name Mefarol. |
|  | chloramine-T            | 30 min          | 3,2%                       |    | 4     | > 4 log reduction | Clean conditions.     | 49        |                     |

| 10 <sup>5,5</sup> TCID <sub>50</sub> /ml | chloramine-T<br>(SETAX) | 24 hours | 50 mg/l                   | 7,5       |               | Stable           | Full-scale trial (Vikan<br>Akvavet), wastewater from<br>fish slaugtherhouse. | 28 | Salinity 20 ‰,<br>"bløggevand" (blood, fish<br>slime and epithelial cells in<br>salt water) diluted with<br>fresh water. |
|--|-------------------------|----------|---------------------------|-----------|---------------|------------------|--|----|--|
| 10 <sup>5,5</sup> TCID <sub>50</sub> /ml | chloramine-T<br>(SETAX) | 24 hours | 100 mg/l                  | 7,5       |               | 2 log reduction  | Full-scale trial (Vikan<br>Akvavet), wastewater from<br>fish slaugtherhouse. | 28 | Salinity 20 ‰, "bløggevand" (blood, fish slime and epithelial cells in salt water) diluted with fresh water.             |
| 10 <sup>5,5</sup> TCID <sub>50</sub> /ml | chloramine-T<br>(SETAX) | 1 hour   | 250 mg/l                  | 7,5       |               | 4 log reduction  | Full-scale trial (Vikan<br>Akvavet), wastewater from<br>fish slaugtherhouse. | 28 | Salinity 20 ‰, "bløggevand" (blood, fish slime and epithelial cells in salt water) diluted with fresh water.             |
| 10 <sup>6</sup> infectious<br>units/ml   | chloramine-T<br>(SETAX) | 30 min   | 3 g/l                     |           | 4             | 1 log reduction  | Sea water added 10% salmon blood.  | 22 |  |
| 10 <sup>6</sup> infectious<br>units/ml   | chloramine-T<br>(SETAX) | 30 min   | 10 g/l                    |           | 4             | 2 log reduction  | Sea water added 10% salmon blood.  | 22 |  |
| 10 <sup>5</sup> TCID <sub>50</sub> /ml   | Cl <sub>2</sub>         | 30 min   | 25 ppm (=25<br>mg/l)      |           | room<br>temp. | "inactivated"    | Tap water.   | 17 | Chlorine concentration after correction for medium and diluent addition.   |
| 10 <sup>5</sup> TCID <sub>50</sub> /ml   | Cl <sub>2</sub>         | 30 min   | 25 ppm                    |           | room<br>temp. | "inactivated"    | PBS  | 17 | Chlorine concentration after correction for medium and diluent addition.   |
| 10 <sup>7,5</sup> TCID <sub>50</sub> /ml | Cl <sub>2</sub>         | 30 min   | 40 ppm                    |           | room<br>temp. | "inactivated"    | Salt water.  | 17 | Chlorine concentration<br>after correction for<br>medium and diluent<br>addition.  |
| 10 <sup>5</sup> TCID <sub>50</sub> /ml   | NaOCI                   | 1 min    | 0,1 mg/l clorine residual | 6,9       | 10            | "inactivated"    | Distilled water.   | 99 |  |
| 10 <sup>5</sup> TCID <sub>50</sub> /ml   | NaOCl                   | 10 min   | 0,2 mg/l clorine residual | 8,2       | 10            | Stable           | Hard lake water.   | 99 |  |
| 10 <sup>5</sup> TCID <sub>50</sub> /ml   | NaOCl                   | 2 min    | 0,7 mg/l clorine residual | 8,2       | 10            | "inactivated"    | Hard lake water.   | 99 |  |
| 10 <sup>5</sup> TCID <sub>50</sub> /ml   | NaOCl                   | 10 min   | 0,2 mg/l clorine residual | 8,2       | 10            | "inactivated"    | Soft lake water.   | 99 |  |
| 10 <sup>3,8</sup> TCID <sub>50</sub> /ml | NaOCl                   | 60 min   | 7,5 mg/ml Cl <sub>2</sub> | 7,07-7,49 | 10            | Stable           |  | 4  |  |
| 10 <sup>4,2</sup> TCID <sub>50</sub> /ml | NaOCl                   | 60 min   | 7,5 mg/ml Cl <sub>2</sub> | 7,07-7,49 | 10            | Stable           | 2,5 % calf serum added   | 4  |  |
| 10 <sup>5,5</sup> TCID <sub>50</sub> /ml | NaOCl                   | 2 min    | 30 mg/ml Cl <sub>2</sub>  | 7,07-7,49 | 10            | ≥ 99 % reduction |  | 4  |  |

| 6.2  |       | 1        | î .                         | 1         |    |                  | 1   |    |  |
|--|-------|----------|-----------------------------|-----------|----|------------------|---|----|--|
| 10 <sup>6,2</sup> TCID <sub>50</sub> /ml                 | NaOCl | 30 min   | 30 mg/ml Cl <sub>2</sub>    | 7,07-7,49 | 10 | ≥ 99 % reduction | 2,5 % calf serum added  | 4  |  |
| 10 <sup>6,2</sup> TCID <sub>50</sub> /ml                 | NaOCl | 2 min    | 36 mg/ml Cl <sub>2</sub>    | 7,07-7,49 | 10 | ≥ 99 % reduction |   | 4  |  |
| 10 <sup>6,2</sup> TCID <sub>50</sub> /ml                 | NaOCl | 20 min   | 36 mg/ml Cl <sub>2</sub>    | 7,07-7,49 | 10 | ≥ 99 % reduction | 2,5 % calf serum added  | 4  |  |
| 10 <sup>5,5</sup> TCID <sub>50</sub> /ml                 | NaOCl | 2 min    | 56 mg/ml Cl <sub>2</sub>    | 7,07-7,49 | 10 | ≥ 99 % reduction |   | 4  |  |
| 10 <sup>5,2</sup> TCID <sub>50</sub> /ml                 | NaOCl | 20 min   | 56 mg/ml Cl <sub>2</sub>    | 7,07-7,49 | 10 | ≥ 99 % reduction | 2,5 % calf serum added  | 4  |  |
| 10 <sup>6,5</sup> TCID <sub>50</sub> /ml                 | NaOCl | 2 min    | 106 mg/ml Cl <sub>2</sub>   | 7,07-7,49 | 10 | ≥ 99 % reduction |   | 4  |  |
| 10 <sup>6,2</sup> TCID <sub>50</sub> /ml                 | NaOCl | 20 min   | 106 mg/ml Cl <sub>2</sub>   | 7,07-7,49 | 10 | ≥ 99 % reduction | 2,5 % calf serum added  | 4  |  |
| 10 <sup>5,2</sup> TCID <sub>50</sub> /ml                 | NaOCl | < 2 min  | 520 mg/ml Cl <sub>2</sub>   | 7,07-7,49 | 10 | ≥ 99 % reduction |   | 4  |  |
| 10 <sup>5,5</sup> TCID <sub>50</sub> /ml                 | NaOCl | < 2 min  | 520 mg/ml Cl <sub>2</sub>   | 7,07-7,49 | 10 | ≥ 99 % reduction |   | 4  |  |
| 10 <sup>4</sup> - 10 <sup>5</sup> TCID <sub>50</sub> /ml | NaOCl | 60 sec   | 0,1 mg/l residual           | 6,9       | 10 | "inactivated"    | Distilled water.  | 98 |  |
| 10 <sup>4</sup> - 10 <sup>5</sup> TCID <sub>50</sub> /ml | NaOCl | 10 min   | 0,2 mg/l residual           | 6,9       | 10 | "inactivated"    | Soft lake water., 30 mg/l CaCO <sub>3</sub>                                     | 98 |  |
| 10 <sup>4</sup> - 10 <sup>5</sup> TCID <sub>50</sub> /ml | NaOCl | 2 min    | 0,7 mg/l residual           | 8,2       | 10 | "inactivated"    | Hard lake water., 120 mg/l CaCO <sub>3</sub>                                    | 98 |  |
| 10 <sup>4</sup> - 10 <sup>5</sup> TCID <sub>50</sub> /ml | NaOCl | 10 min   | 0,2 mg/l residual           | 8,2       | 10 | Stable           | Hard lake water., 120 mg/l CaCO <sub>3</sub>                                    | 98 |  |
| 10 <sup>3,9</sup> TCID <sub>50</sub> /ml                 | NaOCl | 5 min    | 1 mg/l clorine<br>residual  |           | 21 | "inactivated"    | Tested using 0,13, 0,25, 0,5, 1, 2, 4, 8 and 16 mg/l residual. Distilled water  | 23 | IPNV: Serotype Buhl.   |
| 10 <sup>4,5</sup> TCID <sub>50</sub> /ml                 | NaOCl | 5 min    | 4 mg/l clorine<br>residual  | 6,6-8,9   | 21 | "inactivated"    | Tested using 0,13, 0,25, 0,5, 1, 2, 4, 8 and 16 mg/l residual. Distilled water  | 23 | IPNV: Serotype Buhl.   |
| 10 <sup>4,3</sup> TCID <sub>50</sub> /ml                 | NaOCl | 15 sec   | 5 mg/l clorine<br>residual  |           | 21 | "inactivated"    | Ttested at time 0, 15, 30, 60, 120 s. Distilled water.                          | 23 | IPNV: Serotype Buhl.   |
| 10 <sup>4,5</sup> TCID <sub>50</sub> /ml                 | NaOCl | 5 min    | 16 mg/l clorine<br>residual | 9,0-10,0  | 21 | "inactivated"    | Tested using 0,25, 0,5, 1, 2,<br>4, 8 and 16 mg/l residual.<br>Distilled water. | 23 | IPNV: Serotype Buhl.   |
| 10 <sup>5,5</sup> TCID <sub>50</sub> /ml                 | NaOCl | 24 hours | 50 mg/l                     | 7,5       |    | Stable           | Full-scale trial (Vikan<br>Akvavet), wastewater from<br>fish slaugtherhouse.    | 28 | Salinity 20 ‰, "bløggevand" (blood, fish slime and epithelial cells in salt water) diluted with fresh water. |
| 10 <sup>5,5</sup> TCID <sub>50</sub> /ml                 | NaOCl | 24 hours | 100 mg/l                    | 7,5       |    | Stable           | Full-scale trial (Vikan<br>Akvavet), wastewater from<br>fish slaugtherhouse.    | 28 | Salinity 20 ‰, "bløggevand" (blood, fish slime and epithelial cells in salt water) diluted with fresh water. |
| 10 <sup>5,5</sup> TCID <sub>50</sub> /ml                 | NaOCl | 1 hour   | 250 mg/l                    | 7,5       |    | Stable           | Full-scale trial (Vikan<br>Akvavet), wastewater from<br>fish slaugtherhouse.    | 28 | Salinity 20 ‰, "bløggevand" (blood, fish slime and epithelial cells in salt water) diluted with fresh water. |

| 10 <sup>6,6</sup> TCID <sub>50</sub> /ml | NaOCl | 10 hours | 43 mg/l  | 7 | 1 log reduction  | Laboratory trial, 1 part<br>process water + 2 parts<br>"bløggevand" from fish<br>slaugtherhouse. | 28 |  |
|--|-------|----------|----------|---|------------------|--|----|--|
| 10 <sup>6,6</sup> TCID <sub>50</sub> /ml | NaOCl | 10 hours | 130 mg/l | 7 | 5 log reduction  | Laboratory trial, 1 part process water + 2 parts "bløggevand" from fish slaugtherhouse.          | 28 |  |
| 10 <sup>6,6</sup> TCID <sub>50</sub> /ml | NaOCl | 10 hours | 260 mg/l | 7 | 4 log reduction  | Laboratory trial, 1 part process water + 2 parts "bløggevand" from fish slaugtherhouse.          | 28 |  |
| 10 <sup>6,6</sup> TCID <sub>50</sub> /ml | NaOCl | 5 hours  | 130 mg/l | 7 | 3 log reduction  | Laboratory trial, 1 part process water + 2 parts "bløggevand" from fish slaugtherhouse.          | 28 |  |
| 10 <sup>6,6</sup> TCID <sub>50</sub> /ml | NaOCl | 5 hours  | 260 mg/l | 7 | 3½ log reduction | Laboratory trial, 1 part process water + 2 parts "bløggevand" from fish slaugtherhouse.          | 28 |  |

Conclusion: In laboratory experiments under highly contaminated conditions that exist in process and wastewater from fish slaughterhouses chlorine in a dose of 130 mg/l with a contact time of 10 hours gave 5 log reduction in titer. During full-scale trials it has not been possible to achieve these results. It was, for example, not possible in a full-scale test to inactivate IPNV using NaOCl at a concentration of 250 mg/l chlorine and a contact time of 1 hour. Using Chloramine-T has it been possible with a contact time of 1 hour and concentration of 250 mg/l chlorine to achieve a 4 log inactivation in a full-scale trials. In the full-scale trials no continous stirring was performed and pockets of non /low treated areas may have occurred and/or the virus may have been protected by different aggregations.

#### **Nodavirus**

| Concentration pathogen | Disinfectant            | Contact<br>time | Concentration disinfectant | рН | Temp. | Result      | Method   | Reference | Comments       |
|------------------------|-------------------------|-----------------|----------------------------|----|-------|-------------|--|-----------|----------------|
| 10 μg purified virus   | Benzalkonium<br>chlorid | 10 min          | 50 μg/ml                   |    | 20    | "Effective" | Diluted in 1 ml PBS. Used<br>for infection trial in day old<br>striped jack larvae.<br>Concentration testet: 2,5 -<br>100 µg/ml. | 9         | Isolate: SJNNV |
| 10 μg purified virus   | CaOCl                   | 10 min          | 50 μg/ml                   |    | 20    | "Effective" | Diluted in 1 ml PBS. Used<br>for infection trial in day old<br>striped jack larvae.<br>Concentration testet: 2,5 -<br>100 µg/ml. | 9         | Isolate: SJNNV |

| 10 <sup>7,25 TCID</sup> <sub>50</sub> /ml | Cl <sub>2</sub> | 5 min  | 50 ppm   | 15 | Not detectable  | Distilled water Tested after 5, 15 and 30 min.   | 30 | Isolate: sea bass nodavirus |
|---|-----------------|--------|----------|----|-----------------|--|----|-----------------------------|
| 10 <sup>7,25 TCID</sup> <sub>50</sub> /ml | Cl <sub>2</sub> | 30 min | 25 ppm   | 15 | Not detectable  | Distilled water Tested after 5, 15 and 30 min.   | 30 | Isolate: sea bass nodavirus |
| 10 <sup>7,25 TCID</sup> <sub>50</sub> /ml | Cl <sub>2</sub> | 30 min | 100 ppm  | 15 | 2 log reduction | HBSS + calf serum. Tested after 5, 15 and 30 min.  | 30 | Isolate: sea bass nodavirus |
| 10 μg purified virus                      | NaOCl           | 10 min | 50 μg/ml | 20 | "Effective"     | Diluted in 1 ml PBS. Used for infection trial in day old striped jack larvae. Concentration testet: 2,5 - 100 µg/ml. | 9  | Isolate: SJNNV              |

Conclusion: Under laboratory settings and clean conditions nodavirus is easily incativated using a dose of 50 mg/l for 5 minuts. When adding calf serum it was only possible to obtain a 2 log reduction using a concentration of 100 mg/l for 30 minuts.

#### Hirame rhabdovirus

| Concentration pathogen                   | Disinfectant     | Contact<br>time | Concentration<br>disinfectant | рН | Temp. | Result            | Method                                    | Reference | Comments  |
|--|------------------|-----------------|-------------------------------|----|-------|-------------------|---|-----------|---|
|  | OCI <sup>-</sup> | 1 min           | 0,42 mg/l                     |    |       | >99% reduction    |   | 57        | Hypoclorite produced by use of batch electrolytic system. |
| 10 <sup>4,5 TCID</sup> <sub>50</sub> /ml | OCI <sup>-</sup> | 1 min           | 0,34 mg/l                     |    |       | 3 log reduction   | flowrate 3,5 m <sup>3</sup> /t, el. 1,5 A | 54        | Electrolyzed salt water.                                  |
| 10 <sup>4,5 TCID</sup> <sub>50</sub> /ml | OCI <sup>-</sup> | 2,5 min         | 0,49 mg/l                     |    |       | > 4 log reduction | flowrate 3,5 m <sup>3</sup> /t, el. 2 A   | 54        | Electrolyzed salt water.                                  |

Conclusion: Electrolysation of saltwater to produce chlorine seem to be an effective method to inactivate hirame rhabovirus using a dose of 0,5 mg/l and a contact time of 2,5 minuts. There has not been tested under dirty conditions, which may reduce the effect of chlorine.

#### oncorhynchus masou virus

| Concentration pathogen | Disinfectant         | Contact<br>time | Concentration disinfectant | рН | Temp. | Result        | Method | Reference | Comments |
|------------------------|----------------------|-----------------|----------------------------|----|-------|---------------|--------|-----------|----------|
|                        | Benzalkonium chlorid | 30 sec          | 100 ppm                    |    | 0     | "inactivated" |        | 35        |          |
|                        | Benzalkonium chlorid | 30 sec          | 100 ppm                    |    | 25    | "inactivated" |        | 35        |          |
|                        | Benzalkonium chlorid | 20 min          | 100 ppm                    |    | 0     | "inactivated" |        | 35        |          |
|                        | Benzalkonium chlorid | 20 min          | 100 ppm                    |    | 15    | "inactivated" |        | 35        |          |

| NaOCl | 30 sec | 100 ppm | 0  | "inactivated" | 35 |  |
|-------|--------|---------|----|---------------|----|--|
| NaOCl | 30 sec | 100 ppm | 25 | "inactivated" | 35 |  |
| NaOCl | 20 min | 50 ppm  | 0  | "inactivated" | 35 |  |
| NaOCl | 20 min | 50 ppm  | 15 | "inactivated" | 35 |  |

Conclusion: Under laboratory conditions *O. masou* virus is inactivated using a dose of 100 mg/l for 30 sec, or 50 mg/l for 20 min.

#### **PFRV**

| Concentration pathogen           | Disinfectant            | Contact<br>time | Concentration disinfectant | рН            | Temp. | Result           | Method                 | Reference | Comments            |
|----------------------------------|-------------------------|-----------------|----------------------------|---------------|-------|------------------|------------------------|-----------|---------------------|
| 6,2 log10 TCID <sub>50</sub> /ml | Benzalkonium<br>chlorid | 6 hours         | 1%                         |               |       | Stable           | 10 % calf serum added  | 4         | Trade name Mefarol. |
| 6,5 log10 TCID <sub>50</sub> /ml | NaOCl                   | 20 min          | 7,6 mg/ml Cl₂              | 7,07-<br>7,49 | 10    | ≥ 99 % reduction |                        | 4         |                     |
| 5,8 log10 TCID <sub>50</sub> /ml | NaOCl                   | 60 min          | 7,6 mg/ml Cl <sub>2</sub>  | 7,07-<br>7,49 | 10    | Stable           | 2,5 % calf serum added | 4         |                     |
| 6,5 log10 TCID <sub>50</sub> /ml | NaOCl                   | 20 min          | 25 mg/ml Cl₂               | 7,07-<br>7,49 | 10    | ≥ 99 % reduction |                        | 4         |                     |
| 6,2 log10 TCID <sub>50</sub> /ml | NaOCl                   | 20 min          | 25 mg/ml Cl₂               | 7,07-<br>7,49 | 10    | ≥ 99 % reduction | 2,5 % calf serum added | 4         |                     |
| 6,8 log10 TCID <sub>50</sub> /ml | NaOCl                   | 20 min          | 36 mg/ml Cl₂               | 7,07-<br>7,49 | 10    | ≥ 99 % reduction |                        | 4         |                     |
| 6,8 log10 TCID <sub>50</sub> /ml | NaOCl                   | 20 min          | 36 mg/ml Cl₂               | 7,07-<br>7,49 | 10    | ≥ 99 % reduction | 2,5 % calf serum added | 4         |                     |
| 6,8 log10 TCID <sub>50</sub> /ml | NaOCl                   | 5 min           | 54 mg/ml Cl₂               | 7,07-<br>7,49 | 10    | ≥ 99 % reduction |                        | 4         |                     |
| 6,5 log10 TCID <sub>50</sub> /ml | NaOCl                   | 10 min          | 54 mg/ml Cl₂               | 7,07-<br>7,49 | 10    | ≥ 99 % reduction | 2,5 % calf serum added | 4         |                     |
| 6,8 log10 TCID <sub>50</sub> /ml | NaOCl                   | 5 min           | 101 mg/ml Cl <sub>2</sub>  | 7,07-<br>7,49 | 10    | ≥ 99 % reduction |                        | 4         |                     |
| 7,2 log10 TCID <sub>50</sub> /ml | NaOCl                   | 10 min          | 101 mg/ml Cl <sub>2</sub>  | 7,07-<br>7,49 | 10    | ≥ 99 % reduction | 2,5 % calf serum added | 4         |                     |
| 6,5 log10 TCID <sub>50</sub> /ml | NaOCl                   | < 2 min         | 540 mg/ml Cl₂              | 7,07-<br>7,49 | 10    | ≥ 99 % reduction |                        | 4         |                     |
| 6,5 log10 TCID <sub>50</sub> /ml | NaOCI                   | 2 min           | 540 mg/ml Cl₂              | 7,07-<br>7,49 | 10    | ≥ 99 % reduction | 2,5 % calf serum added | 4         |                     |

Conclusion: As the author has not noted the precise reduction it is difficult to evalute which dose to use to inactivate PFRV. It does seem, though, as PFRV is susceptible to chlorine when using the right dose for the right time.

### Ranavirus

| Concentration pathogen     | Disinfectant  | Contact<br>time | Concentration disinfectant | рН | Temp. | Result             | Method  | Reference | Comments                          |
|----------------------------|---------------|-----------------|----------------------------|----|-------|--------------------|---|-----------|-----------------------------------|
| 1 x 10 <sup>7</sup> PFU/ml | Chlorhexidine | 1 min           | 0,005%                     |    | 22    | 1,7 log reduction  | Novalsan 0,25%  | 14        | Isolate from American<br>bullfrog |
| 1 x 10 <sup>7</sup> PFU/ml | Chlorhexidine | 5 min           | 0,005%                     |    | 22    | 2,6 log reduction  | Novalsan 0,25%  | 14        | Isolate from American<br>bullfrog |
| 1 x 10 <sup>7</sup> PFU/ml | Chlorhexidine | 1 min           | 0,015%                     |    | 22    | 3,25 log reduction | Novalsan 0,75%  | 14        | Isolate from American<br>bullfrog |
| 1 x 10 <sup>7</sup> PFU/ml | Chlorhexidine | 1 min           | 0,040%                     |    | 22    | 3,75 log reduction | Novalsan 2,0%   | 14        | Isolate from American<br>bullfrog |
| 1 x 10 <sup>7</sup> PFU/ml | NaOCl         | 1 min           | 0,012%                     |    | 22    | 0,5 log reduction  |   | 14        | Isolate from American bullfrog    |
| 1 x 10 <sup>7</sup> PFU/ml | NaOCl         | 5 min           | 0,012%                     |    | 22    | 0,5 log reduction  |   | 14        | Isolate from American bullfrog    |
| 1 x 10 <sup>7</sup> PFU/ml | NaOCI         | 1 min           | 0,060%                     |    | 22    | 0,9 log reduction  |   | 14        | Isolate from American bullfrog    |
| 1 x 10 <sup>7</sup> PFU/ml | NaOCI         | 5 min           | 0,060%                     |    | 22    | 1,8 log reduction  |   | 14        | Isolate from American bullfrog    |
| 1 x 10 <sup>7</sup> PFU/ml | NaOCI         | 1 min           | 0,18%<br>(1,8 g/l)         |    | 22    | "inactivated"      |   | 14        | Isolate from American bullfrog    |
|                            | NaOCI         | 5 hours         | 400 mg/l                   |    |       | Survival           | Virus i udtørret<br>cellekulturmedium<br>overhældt med NaOCl.<br>Testet efter 2 og 5 hours. | 67        | ENV                               |
|                            | NaOCI         | 2 hours         | 200 mg/l                   |    |       | "inactivated"      | Virus in cell culture<br>medium Kun testet efter 2<br>hours og ved denne dosis.             | 67        | EHNV                              |

Conclusion: It seems as if ranavirus is quite resistant to chlorine.

### **SVCV**

| Concentration pathogen                   | Disinfectant            | Contact time | Concentration disinfectant | рН | Temp. | Result            | Method  | Reference | Comments    |
|--|-------------------------|--------------|----------------------------|----|-------|-------------------|---|-----------|-------------|
| 6,5 log10 TCID <sub>50</sub> /ml         | Benzalkonium chlorid    | 6 hours      | 1%                         |    |       | Stable            | 10 % calf serum added                             | 4, 5      |             |
| 10 <sup>7,1</sup> TCID <sub>50</sub> /ml | Benzalkonium<br>chlorid | 20 min       | 100 ppm                    |    | 22    | > 4 log reduction | Diluted in PBS. Contact time 30 sec or 20 min. 1% | 61        | Isolate S30 |

|                                  |       |         |                           |               |    |                  | calf serum.            |   |  |
|----------------------------------|-------|---------|---------------------------|---------------|----|------------------|------------------------|---|--|
| 6,2 log10 TCID <sub>50</sub> /ml | NaOCl | 20 min  | 7,6 mg/ml Cl <sub>2</sub> | 7,07-<br>7,49 | 10 | ≥ 99 % reduction |                        | 4 |  |
| 6,8 log10 TCID <sub>50</sub> /ml | NaOCl | 60 min  | 7,6 mg/ml Cl <sub>2</sub> | 7,07-<br>7,49 | 10 | Stable           | 2,5 % calf serum added | 4 |  |
| 6,5 log10 TCID <sub>50</sub> /ml | NaOCl | 10 min  | 27 mg/ml Cl <sub>2</sub>  | 7,07-<br>7,49 | 10 | ≥ 99 % reduction |                        | 4 |  |
| 6,5 log10 TCID <sub>50</sub> /ml | NaOCl | 10 min  | 27 mg/ml Cl <sub>2</sub>  | 7,07-<br>7,49 | 10 | ≥ 99 % reduction | 2,5 % calf serum added | 4 |  |
| 7,5 log10 TCID <sub>50</sub> /ml | NaOCl | 2 min   | 36 mg/ml Cl <sub>2</sub>  | 7,07-<br>7,49 | 10 | ≥ 99 % reduction |                        | 4 |  |
| 7,2 log10 TCID <sub>50</sub> /ml | NaOCl | 10 min  | 36 mg/ml Cl <sub>2</sub>  | 7,07-<br>7,49 | 10 | ≥ 99 % reduction | 2,5 % calf serum added | 4 |  |
| 5,8 log10 TCID <sub>50</sub> /ml | NaOCl | 2 min   | 55 mg/ml Cl <sub>2</sub>  | 7,07-<br>7,49 | 10 | ≥ 99 % reduction |                        | 4 |  |
| 5,5 log10 TCID <sub>50</sub> /ml | NaOCl | 5 min   | 55 mg/ml Cl <sub>2</sub>  | 7,07-<br>7,49 | 10 | ≥ 99 % reduction | 2,5 % calf serum added | 4 |  |
| 5,5 log10 TCID <sub>50</sub> /ml | NaOCl | < 2 min | 101 mg/ml Cl <sub>2</sub> | 7,07-<br>7,49 | 10 | ≥ 99 % reduction |                        | 4 |  |
| 5,8 log10 TCID <sub>50</sub> /ml | NaOCl | 2 min   | 101 mg/ml Cl <sub>2</sub> | 7,07-<br>7,49 | 10 | ≥ 99 % reduction | 2,5 % calf serum added | 4 |  |
| 6,2 log10 TCID <sub>50</sub> /ml | NaOCl | < 2 min | 506 mg/ml Cl <sub>2</sub> | 7,07-<br>7,49 | 10 | ≥ 99 % reduction |                        | 4 |  |
| 6,5 log10 TCID <sub>50</sub> /ml | NaOCl | 2 min   | 506 mg/ml Cl <sub>2</sub> | 7,07-<br>7,49 | 10 | ≥ 99 % reduction | 2,5 % calf serum added | 4 |  |

Conclusion: As the author has not noted the precise reduction it is difficult to evalute which dose to use to inactivate PFRV. It does seem, though, as PFRV is susceptible to chlorine when using the right dose for the right time.

#### Yellowtail ascites virus

| Concentration pathogen                   | Disinfectant     | Contact<br>time | Concentration disinfectant | рН | Temp. | Result               | Method                                    | Reference | Comments  |
|--|------------------|-----------------|----------------------------|----|-------|----------------------|---|-----------|---|
|  | OCI <sup>-</sup> | 1 min           | 0,42 mg/l                  |    |       | >99% reduction       |   | 57        | Hypoclorite produced by use of batch electrolytic system. |
| 10 <sup>4,5</sup> TCID <sub>50</sub> /ml | OCI <sup>-</sup> | 1 min           | 0,58 mg/l                  |    |       | > 4 logi naktivering | flowrate 3,5 m <sup>3</sup> /t, el. 2.5 A | 54        | Electrolyzed salt water.                                  |
| 10 <sup>4,5</sup> TCID <sub>50</sub> /ml | OCI <sup>-</sup> | 1 min           | 0,45 mg/l                  |    |       | 3 log reduction      | flowrate 3,5 m <sup>3</sup> /t, el. 2 A   | 97        | Electrolyzed salt water.                                  |

#### Bacteria

#### Aeromonas salmonicida

| Concentration pathogen                  | Disinfectant            | Contact<br>time | Concentration disinfectant | рН  | Temp. | Result             | Method   | Reference | Comments  |
|---|-------------------------|-----------------|----------------------------|-----|-------|--------------------|--|-----------|---|
| 10 <sup>5,5</sup> cfu/ml                | Benzalkonium chlorid    | 3 min           | 0,02%                      |     | 5     | Stable             | Test of effect of<br>Temperature.  | 85        |   |
| 10 <sup>5,5</sup> cfu/ml                | Benzalkonium<br>chlorid | 3 min           | 0,02%                      |     | 15    | 3 log reduction    | Test of effect of<br>Temperature.  | 85        |   |
| 10 <sup>5,5</sup> cfu/ml                | Benzalkonium<br>chlorid | 3 min           | 0,02%                      |     | 25    | > 4½ log reduction | Test of effect of<br>Temperature.  | 85        |   |
| 10 <sup>5,5</sup> cfu/ml                | Benzalkonium<br>chlorid | 3 min           | 0,02%                      |     | 15    | 2 log reduction    | Artificial sea water.  | 85        |   |
| 10 <sup>5,5</sup> cfu/ml                | Benzalkonium<br>chlorid | 3 min           | 0,02%                      |     | 15    | 3 log reduction    | Hard water (300 ppm<br>CaCO₃)  | 85        |   |
| 10 <sup>5,5</sup> cfu/ml                | Benzalkonium<br>chlorid | 3 min           | 0,02%                      |     | 15    | 4½ log reduction   | Distilled water.   | 85        |   |
| 10 <sup>5</sup> cfu/ml                  | Benzalkonium chlorid    | 5 min           | 0,03%                      |     | 20    | > 4 log reduction  |  | 85        | Concentration of commercial product.                |
| 10 <sup>8</sup> cfu/ml                  | Benzalkonium chlorid    | 4 min           | 0,03%                      |     | 20    | > 7 log reduction  | Test of effect of bacteria titer.  | 85        |   |
| 10 <sup>6,5</sup> cfu/ml                | Benzalkonium chlorid    | 2 min           | 0,03%                      |     | 20    | > 5½ log reduction | Test of effect of bacteria titer.  | 85        |   |
| 10 <sup>5</sup> cfu/ml                  | Benzalkonium chlorid    | 1 min           | 0,03%                      |     | 20    | > 4 log reduction  | Test of effect of bacteria titer.  | 85        |   |
| 10 <sup>5,5</sup> cfu/ml                | Benzalkonium chlorid    | 1 min           | 0,03%                      |     | 20    | 3½ log reduction   | 300 ppm calf serum added.  | 85        |   |
| 10 <sup>5,5</sup> cfu/ml                | Benzalkonium chlorid    | 1 min           | 0,03%                      |     | 20    | 4½ log reduction   | 0 ppm calf serum added.  | 85        |   |
| 10 <sup>5</sup> cfu/ml                  | Benzalkonium chlorid    | 1 min           | 0,1%                       |     | 20    | > 4 log reduction  |  | 85        | Concentration of commercial product.                |
|   | chloramine-T            | 30 min          | 0,08 - 0,5 % (v/v)         |     | 4     | > 5 log reduction  | Tested at 0,001, 0,01, 0,05, 0,08, 0,1, 0,5, 0,7 and 1%. Hard water, organic loaded. | 49        |   |
| 10 <sup>8</sup> -10 <sup>9</sup> CFU/ml | chloramine-T<br>(SETAX) | 1 min           | 1 g/l                      |     | 4     | ≥ 6 log reduction  | Sea water added 10% salmon blood.  | 22        |   |
| 10 <sup>3</sup> cells/ml                | NaOCl                   | 10 min          | 0,01 mg/l<br>residual      | 6,9 | 20    | Stable             | Distilled water.   | 97, 98    | Samples tested after ½, 1, 2, 5, 10, 20 and 30 min. |
| 10 <sup>3</sup> cells/ml                | NaOCI                   | 1 min           | 0,01 mg/l<br>residual      | 6,9 | 20    | 2 log reduction    | Distilled water.   | 97, 98    | Samples tested after ½, 1, 2, 5, 10, 20 and 30 min. |
| 10 <sup>3</sup> cells/ml                | NaOCl                   | 10 min          | 0,05 mg/l<br>residual      | 6,9 | 20    | Stable             | Soft lake water., 30 mg/l<br>CaCO <sub>3</sub>                                       | 97, 98    |   |

| 10 <sup>3</sup> cells/ml | NaOCl | 10 min | 0,05 mg/l<br>residual | 8,2 | 20 | Stable             | Hard lake water., 120 mg/l<br>CaCO <sub>3</sub>      | 97, 98 |   |
|--------------------------|-------|--------|-----------------------|-----|----|--------------------|--|--------|---|
| 10 <sup>3</sup> cells/ml | NaOCl | 30 sec | 0,1 mg/l residual     | 6,9 | 20 | "inactivated"      | Distilled water.                                     | 97, 98 | Samples tested after ½, 1, 2, 5, 10, 20 and 30 min. |
| 10 <sup>3</sup> cells/ml | NaOCl | 30 sec | 0,1 mg/l residual     | 6,9 | 20 | "inactivated"      | Soft lake water., 30 mg/l<br>CaCO <sub>3</sub>       | 97, 98 |   |
| 10 <sup>3</sup> cells/ml | NaOCl | 30 sec | 0,2 mg/l residual     | 8,2 | 20 | "inactivated"      | Hard lake water., 120 mg/l<br>CaCO <sub>3</sub>      | 97, 98 |   |
| 10 <sup>7</sup> CFU/ml   | NaOCl | 1 min  | 0,2 mg/l added        | 7,2 | 7  | 4 log reduction    | PBS  | 71     | Read off a graph.                                   |
| 10 <sup>7</sup> CFU/ml   | NaOCl | 36 sec | 0,2 mg/l added        | 7,2 | 7  | 99,9 % reduction   | PBS  | 71     |   |
| 10 <sup>7</sup> CFU/ml   | NaOCl |        | 0,2 mg/l added        | 7,2 | 7  | 99,9% reduction    | PBS  | 71     | Reduction rate: 0,20/sec                            |
| 10 <sup>7</sup> CFU/ml   | NaOCl | 1 min  | 2 mg/l added          | 7,8 | 7  | 4 log reduction    | Wastewater from aquaculture (15,7 ‰ salinity).       | 71     | Read off a graph.                                   |
| 10 <sup>7</sup> CFU/ml   | NaOCl |        | 2,0 mg/l added        | 7,8 | 7  | 99,9% reduction    | Wastewater from<br>aquaculture (15,7 ‰<br>salinity). | 71     | Reduction rate: 0,19/sec                            |
| 10 <sup>7</sup> CFU/ml   | NaOCl |        | 4,0 mg/l added        | 7,8 | 7  | 99,9% reduction    | Wastewater from<br>aquaculture (15,7 ‰<br>salinity). | 71     | Reduction rate: 0,30/sec                            |
| 10 <sup>5,5</sup> cfu/ml | NaOCl | 5 min  | 4 ppm                 |     | 15 | > 4½ log reduction | Artificial sea water.                                | 85     |   |
| 10 <sup>5,5</sup> cfu/ml | NaOCl | 1 min  | 4 ppm                 |     | 15 | 4½ log reduction   | Hard water (300 ppm<br>CaCO <sub>3</sub> )           | 85     |   |
| 10 <sup>5,5</sup> cfu/ml | NaOCl | 1 min  | 4 ppm                 |     | 15 | > 4½ log reduction | Distilled water.                                     | 85     |   |
| 10 <sup>8</sup> cfu/ml   | NaOCl | 5 min  | 5 ppm                 |     | 20 | Stable             | Test of effect of bacteria titer.                    | 85     | Concentration of commercial product. (Purelox)      |
| 10 <sup>6</sup> cfu/ml   | NaOCl | 5 min  | 5 ppm                 |     | 20 | > 5 log reduction  | Test of effect of bacteria titer.                    | 85     | Concentration of commercial product. (Purelox)      |
| 10 <sup>4</sup> cfu/ml   | NaOCl | 3 min  | 5 ppm                 |     | 20 | > 3 log reduction  | Test of effect of bacteria titer.                    | 85     | Concentration of commercial product. (Purelox)      |
| 10 <sup>5,5</sup> cfu/ml | NaOCl | 1 min  | 5 ppm                 |     | 20 | Stable             | 300 ppm calf serum added.                            | 85     |   |
| 10 <sup>5,5</sup> cfu/ml | NaOCl | 1 min  | 5 ppm                 |     | 20 | 3 log reduction    | 10 ppm calf serum added.                             | 85     |   |
| 10 <sup>5,5</sup> cfu/ml | NaOCl | 1 min  | 5 ppm                 |     | 20 | 4½ log reduction   | 0 ppm calf serum added.                              | 85     |   |
| 10 <sup>5,5</sup> cfu/ml | NaOCl | 1 min  | 5 ppm                 |     | 5  | 4 log reduction    | Test of effect of<br>Temperature.                    | 85     |   |
| 10 <sup>5,5</sup> cfu/ml | NaOCl | 1 min  | 5 ppm                 |     | 15 | 4½ log reduction   | Test of effect of<br>Temperature.                    | 85     |   |
| 10 <sup>5,5</sup> cfu/ml | NaOCl | 1 min  | 5 ppm                 |     | 25 | > 4½ log reduction | Test of effect of<br>Temperature.                    | 85     |   |

| 10 <sup>5</sup> cfu/ml       | NaOCl            | 1 min | 10 ppm    | 20 | > 4 log reduction |   | 85 | Concentration of commercial product. (Purelox) |
|------------------------------|------------------|-------|-----------|----|-------------------|---|----|--|
| 3,8 x 10 <sup>6</sup> cfu/ml | OCI <sup>-</sup> | 1 min | 0,11 mg/l |    | 4 log reduction   | flowrate 3,0 m <sup>3</sup> /t, el. 0,5 A | 54 | Electrolyzed salt water.                       |
| 3,8 x 10 <sup>6</sup> cfu/ml | OCI <sup>-</sup> | 1 min | 0.06 mg/l |    | 3 log reduction   | flowrate 3,5 m <sup>3</sup> /t, el. 0,5 A | 54 | Electrolyzed salt water.                       |

Conclusion: Under laboratory condtions *Aeromonas salmonicida* is sensitive to chlorine.

## Carnobacterium piscicola

| Concentration pathogen | Disinfectant | Contact<br>time | Concentration disinfectant | рН | Temp. | Result            | Method   | Reference | Comments |
|------------------------|--------------|-----------------|----------------------------|----|-------|-------------------|--|-----------|----------|
|                        | chloramine-T | 30 min          | 0,1 - 0,5 % (v/v)          |    | 4     | > 5 log reduction | Tested at 0,001, 0,01, 0,05, 0,08, 0,1, 0,5, 0,7 and 1%. Hard water, organic loaded. | 49        |          |

### Edwardsiella tarda

| Concentration pathogen | Disinfectant     | Contact<br>time | Concentration disinfectant | рН | Temp. | Result          | Method  | Reference | Comments  |
|------------------------|------------------|-----------------|----------------------------|----|-------|-----------------|---|-----------|---|
| 10 <sup>7</sup> CFU/ml | NaOCl            | 20 min          | 400 ppm                    |    | 20    | 4 log reduction | Dilution 1:1 of bacteria and disinfectant (25, 50, 100, 200, 400, 800, 1600, 3200 ppm). Growht at 20°C and counting already after 24 hours. | 58        | The actual concentration of disinfectant probably only half of stated in article table. Generally speaking the neccessary concentration for disinfection in this paper is much higher than published in other papers. |
| 10 <sup>7</sup> CFU/ml | NaOCl            | 60 min          | 400 ppm                    |    | 20    | 5 log reduction | Dilution 1:1 of bacteria and disinfectant (25, 50, 100, 200, 400, 800, 1600, 3200 ppm). Growht at 20°C and counting already after 24 hours. | 58        | The actual concentration of disinfectant probably only half of stated in article table. Generally speaking the neccessary concentration for disinfection in this paper is much higher than published in other papers. |
| 10 <sup>7</sup> CFU/ml | CIO <sub>2</sub> | 20 min          | 3200 ppm                   |    | 20    | 4 log reduction | Dilution 1:1 of bacteria and disinfectant (25, 50, 100, 200, 400, 800, 1600, 3200 ppm). Growht at 20°C and counting already after 24        | 58        | The actual concentration of disinfectant probably only half of stated in article table. Generally speaking the neccessary   |

|                        |                        |        |          |    |                   | hours.  |    | concentration for<br>disinfection in this paper is<br>much higher than  |
|------------------------|------------------------|--------|----------|----|-------------------|---|----|---|
| 10 <sup>7</sup> CFU/ml | CIO <sub>2</sub>       | 60 min | 3200 ppm | 20 | 4 log reduction   | Dilution 1:1 of bacteria and disinfectant (25, 50, 100, 200, 400, 800, 1600, 3200 ppm). Growht at 20°C and counting already after 24 hours. | 58 | published in other papers.  The actual concentration of disinfectant probably only half of stated in article table. Generally speaking the neccessary concentration for disinfection in this paper is much higher than published in other papers. |
| 10 <sup>7</sup> CFU/ml | Quartenary<br>ammonium | 20 min | 400 ppm  | 20 | > 5 log reduction | Dilution 1:1 of bacteria and disinfectant (25, 50, 100, 200, 400, 800, 1600, 3200 ppm). Growht at 20°C and counting already after 24 hours. | 58 | The actual concentration of disinfectant probably only half of stated in article table. Generally speaking the neccessary concentration for disinfection in this paper is much higher than published in other papers.                             |
| 10 <sup>7</sup> CFU/ml | Quartenary<br>ammonium | 60 min | 200 ppm  | 20 | 4 log reduction   | Dilution 1:1 of bacteria and disinfectant (25, 50, 100, 200, 400, 800, 1600, 3200 ppm). Growht at 20°C and counting already after 24 hours. | 58 | The actual concentration of disinfectant probably only half of stated in article table. Generally speaking the neccessary concentration for disinfection in this paper is much higher than published in other papers.                             |

Conclusion: According to this paper *E. tarda* is quite resistant to chlorine!

## Indigenious flora (heterothophic bacteria)

| Concentration pathogen       | Disinfectant     | Contact<br>time | Concentration disinfectant | рН | Temp. | Result          | Method  | Reference | Comments                                 |
|------------------------------|------------------|-----------------|----------------------------|----|-------|-----------------|---|-----------|--|
|                              | OCI <sup>-</sup> | 1 min           | 0,54 mg/l                  |    |       | >99% reduction  | Hypoclorite produced by use of batch electrolytic system. | 57        | hatchery inlet water                     |
|                              | OCI <sup>-</sup> | 1 min           | 0,64 mg/l                  |    |       | >99% reduction  | Hypoclorite produced by use of batch electrolytic system. | 57        | waste-seawater                           |
| 4,7 x 10 <sup>4</sup> cfu/ml | OCI <sup>-</sup> | 1 min           | 1,28 mg/l                  |    |       | 3 log reduction | flowrate 2,0 m³/t, el. 2,5 A<br>(Electrolyzed salt water) | 56        | Natural flora in wastewater from hathing |

|                          |                  |               |     |                  |                              |    | facility.                |
|--------------------------|------------------|---------------|-----|------------------|------------------------------|----|--------------------------|
| 10 <sup>3,5</sup> cfu/ml | OCI <sup>-</sup> |               | 8,2 | Stable           | flowrate 4 l/min, el. 0,1 A  | 53 | Sea water, filtrated (10 |
| 10 Clu/IIII              | OCI              |               | 0,2 | Stable           | (Electrolyzed salt water)    | 55 | μm).                     |
| 10 <sup>3,5</sup> cfu/ml | OCI <sup>-</sup> |               | 8,2 | 99,4% reduction  | flowrate 4 l/min, el. 1,2 A. | 53 | Sea water, filtrated (10 |
| 10 Clu/IIII              | OCI              |               | 0,2 | 99,470 reduction | (Electrolyzed salt water)    | 55 | μm).                     |
| 10 <sup>3,5</sup> cfu/ml | OCI <sup>-</sup> | 2,13 mg Cl/l  | 8,2 | "inactivated"    | flowrate 4 l/min, el. 1,3 A. | 53 | Sea water, filtrated (10 |
| 10 Clu/IIII              | OCI              | 2,13 Hig Ci/I | 0,2 | mactivateu       | (Electrolyzed salt water)    | 55 | μm).                     |

Conclustion: When electrolysing saltwater it seems possible to obtain a 3 log reduction of the natural flora. Wether this will also be the case in wastewater from fish cutting plants is unknown.

### Lactococcus garviae

| Concentration pathogen | Disinfectant | Contact<br>time | Concentration disinfectant | рН | Temp. | Result            | Method   | Reference | Comments |
|------------------------|--------------|-----------------|----------------------------|----|-------|-------------------|--|-----------|----------|
|                        | chloramine-T | 30 min          | 0,08 - 0,5 % (v/v)         |    | 4     | > 5 log reduction | Tested at 0,001, 0,01, 0,05, 0,08, 0,1, 0,5, 0,7 and 1%. Hard water, organic loaded. | 49        |          |

#### Renibacterium salmoninarum

| Concentration pathogen                                   | Disinfectant | Contact<br>time | Concentration disinfectant        | рН   | Temp. | Result          | Method  | Reference | Comments  |
|--|--------------|-----------------|-----------------------------------|------|-------|-----------------|---|-----------|---|
| 3 x 10 <sup>4</sup> - 2 x 10 <sup>6</sup><br>bacteria/ml | NaOCl        | 26 sec          | 0,05 mg/l free<br>chlorine        | 7    | 15    | 3 log reduction | in vitro in PBS   | 82        | Read off a graph.                                       |
| 3 x 10 <sup>4</sup> - 2 x 10 <sup>6</sup><br>bacteria/ml | NaOCI        | 42 sec          | 0,6 mg/l free<br>chlorine         | 7    | 15    | 5 log reduction | in vitro in PBS   | 82        | Read off a graph.                                       |
| 5 x 10 <sup>4</sup> bacteria/ml                          | NaOCI        | 20 sec          | 0,06 mg/l free<br>chlorine        | 7    | 15    | 3 log reduction | in vitro in PBS   | 82        | Read off a graph.                                       |
| 5 x 10 <sup>4</sup> bacteria/ml                          | NaOCI        | 120 sec         | 0,07 mg/l free<br>chlorine        | 7    | 7,5   | 3 log reduction | in vitro in PBS   | 82        | Read off a graph.                                       |
| 5 x 10 <sup>4</sup> bacteria/ml                          | NaOCI        | 54 sec          | 0,41 - 0,53 mg/l<br>free chlorine | 6    | 15    | 3 log reduction | in vitro in PBS   | 82        | Read off a graph.                                       |
| 5 x 10 <sup>4</sup> bacteria/ml                          | NaOCl        | ≥ 60 sec        | 0,41 - 0,53 mg/l<br>free chlorine | 7    | 15    | 3 log reduction | in vitro in PBS   | 82        | Read off a graph., at<br>t=120 sec 2,7 log<br>reduction |
| 5 x 10 <sup>4</sup> bacteria/ml                          | NaOCl        | 92 sec          | 0,41 - 0,53 mg/l<br>free chlorine | 8    | 15    | 1 log reduction | in vitro in PBS   | 82        | Read off a graph.                                       |
| 5 x 10 <sup>6</sup> cfu/ml                               | NaOCl        | 15 min          | 10 mg/l free<br>chlorine          | 10,3 | 15    | "inactivated"   | Autoclaved tank water, pH<br>measured after addition of<br>NaOCl. | 39        | Growth tested on KDM2 agar plate.                       |
| 5 x 10 <sup>6</sup> cfu/ml                               | NaOCl        | 15 min          | 10 mg/l free                      | 6,3  | 15    | "inactivated"   | Distilled water, pH   | 39        | Growth tested on KDM2                                   |

|                            |       |          | chlorine                  |      |    |                | measurement after adding NaOCl.                                   |    | agar plate.  |
|----------------------------|-------|----------|---------------------------|------|----|----------------|---|----|--|
| 5 x 10 <sup>6</sup> cfu/ml | NaOCl | 5 min    | 200 mg/l free<br>chlorine | 11,8 | 15 | "inactivated"  | Autoclaved tank water, pH<br>measured after addition of<br>NaOCl. | 39 | Growth tested on KDM2 agar plate.  |
| 5 x 10 <sup>6</sup> cfu/ml | NaOCl | 5 min    | 200 mg/l free<br>chlorine | 12,0 | 15 | "inactivated"  | Distilled water, pH<br>measurement after adding<br>NaOCl.         | 39 | Growth tested on KDM2 agar plate.  |
| 5 x 10 <sup>6</sup> cfu/ml | NaOCl | 24 hours | 200 mg/l free<br>chlorine | 11,8 | 15 | Few survivors. | Autoclaved tank water, pH<br>measured after addition of<br>NaOCl. | 39 | Growth tested on KDM2<br>and SKDM agar plates<br>after culture in KDM2<br>buillon. |
| 5 x 10 <sup>6</sup> cfu/ml | NaOCl | 15 min   | 200 mg/l free<br>chlorine | 12,0 | 15 | Few survivors. | Distilled water, pH<br>measurement after adding<br>NaOCl.         | 39 | Growth tested on KDM2<br>and SKDM agar plates<br>after culture in KDM2<br>buillon. |

Conclusion: Under clean conditions in PBS *R. salmoninarum* seem very sensitive to chlorine. In autoclaved tank water 10 mg/l for 15 min or 200 mg/l for 5 min was able to reduce the titer > 3 log.

## Streptococcus iniae

| Concentration pathogen | Disinfectant  | Contact time | Concentration disinfectant | рН | Temp. | Result                       | Method | Reference | Comments |
|------------------------|---------------|--------------|----------------------------|----|-------|------------------------------|--------|-----------|----------|
|                        | NaOCl         | ≥ 15 min     | 3-5 ppm                    |    |       | "powerfull<br>disinfectants" |        | 93        |          |
|                        | Chlorhexidine | ≥ 15 min     | 3-5 ppm                    |    |       | "powerfull disinfectants"    |        | 93        |          |

#### Streptococcus sp.

| Concentration pathogen | Disinfectant | Contact<br>time | Concentration disinfectant | рН | Temp. | Result            | Method  | Reference | Comments  |
|------------------------|--------------|-----------------|----------------------------|----|-------|-------------------|---|-----------|---|
| 10 <sup>7</sup> CFU/ml | NaOCl        | 20 min          | 1600 ppm                   |    | 20    | > 5 log reduction | Dilution 1:1 of bacteria and disinfectant (25, 50, 100, 200, 400, 800, 1600, 3200 ppm). Growht at 20°C and counting already after 24 hours. | 58        | The actual concentration of disinfectant probably only half of stated in article table. Generally speaking the neccessary concentration for disinfection in this paper is much higher than published in other |

|                        |                        |        |          |    |                   |   |    | papers.   |
|------------------------|------------------------|--------|----------|----|-------------------|---|----|---|
| 10 <sup>7</sup> CFU/ml | NaOCI                  | 60 min | 1600 ppm | 20 | > 5 log reduction | Dilution 1:1 of bacteria and disinfectant (25, 50, 100, 200, 400, 800, 1600, 3200 ppm). Growht at 20°C and counting already after 24 hours. | 58 | The actual concentration of disinfectant probably only half of stated in article table. Generally speaking the neccessary concentration for disinfection in this paper is much higher than published in other papers. |
| 10 <sup>7</sup> CFU/ml | CIO <sub>2</sub>       | 20 min | 3200 ppm | 20 | Stable            | Dilution 1:1 of bacteria and disinfectant (25, 50, 100, 200, 400, 800, 1600, 3200 ppm). Growht at 20°C and counting already after 24 hours. | 58 | The actual concentration of disinfectant probably only half of stated in article table. Generally speaking the neccessary concentration for disinfection in this paper is much higher than published in other papers. |
| 10 <sup>7</sup> CFU/ml | CIO <sub>2</sub>       | 60 min | 3200 ppm | 20 | Stable            | Dilution 1:1 of bacteria and disinfectant (25, 50, 100, 200, 400, 800, 1600, 3200 ppm). Growht at 20°C and counting already after 24 hours. | 58 | The actual concentration of disinfectant probably only half of stated in article table. Generally speaking the neccessary concentration for disinfection in this paper is much higher than published in other papers. |
| 10 <sup>7</sup> CFU/ml | Quartenary<br>ammonium | 20 min | 200 ppm  | 20 | 4 log reduction   | Dilution 1:1 of bacteria and disinfectant (25, 50, 100, 200, 400, 800, 1600, 3200 ppm). Growht at 20°C and counting already after 24 hours. | 58 | The actual concentration of disinfectant probably only half of stated in article table. Generally speaking the neccessary concentration for disinfection in this paper is much higher than published in other papers. |
| 10 <sup>7</sup> CFU/ml | Quartenary ammonium    | 60 min | 200 ppm  | 20 | 5 log reduction   | Dilution 1:1 of bacteria and disinfectant (25, 50, 100, 200,  | 58 | The actual concentration of disinfectant probably   |

|  |  |  | 400, 800, 1600, 3200 ppm).<br>Growht at 20°C and counting<br>already after 24 hours. | only half of stated in<br>article table. Generally<br>speaking the neccessary<br>concentration for |
|--|--|--|--|--|
|  |  |  |  | disinfection in this paper<br>is much higher than<br>published in other<br>papers.                 |

Conclusion: According to this paper *Streptococcus* is quite resistant to chlorine!

### Vibrio anguillarum

| Concentration pathogen                  | Disinfectant            | Contact<br>time | Concentration disinfectant | рН  | Temp. | Result            | Method   | Reference | Comments  |
|---|-------------------------|-----------------|----------------------------|-----|-------|-------------------|--|-----------|---|
| 10 <sup>5</sup> cfu/ml                  | Benzalkonium<br>chlorid | 4 min           | 0,01%                      |     | 20    | > 4 log reduction |  | 85        | Concentration of commercial product.                      |
| 10 <sup>5</sup> cfu/ml                  | Benzalkonium chloride   | 1 min           | 0,03%                      |     | 20    | > 4 log reduction |  | 85        | Concentration of commercial product.                      |
| 10 <sup>8</sup> -10 <sup>9</sup> CFU/ml | chloramine-T<br>(SETAX) | 1 min           | 2 g/l                      |     | 4     | ≥ 6 log reduction | Sea water added 10% salmon blood.                                  | 22        |   |
| 1,1 x 10 <sup>6</sup> CFU/ml            | NaOCl                   | 60 min          | 150 mg/l added             |     |       | "inactivated"     | Test medium: sterilized<br>wastewater from fish<br>slaughterhouse. | 48        | Tested only at this dose/time combination.                |
| 10⁵ cfu/ml                              | NaOCl                   | 2 min           | 3 ppm                      |     | 20    | > 4 log reduction |  | 85        | Concentration of commercial product. (Purelox)            |
| 10⁵ cfu/ml                              | NaOCl                   | 1 min           | 10 ppm                     |     | 20    | > 4 log reduction |  | 85        | Concentration of commercial product. (Purelox)            |
|   | OCI <sup>-</sup>        | 1 min           | 0,21 mg/l                  |     |       | >99% reduction    |  | 57        | Hypoclorite produced by use of batch electrolytic system. |
| 4,5 x 10 <sup>6</sup> cfu/ml            | OCI <sup>-</sup>        | 1 min           | 0,07 mg/l                  |     |       | > 4 log reduction | flowrate 3,5 m <sup>3</sup> /t, el. 0,5 A                          | 54        | Electrolyzed salt water.                                  |
| 5 x 10 <sup>4</sup> cfu/ml              | OCI <sup>-</sup>        |                 |                            | 8,2 |       | "inactivated"     | Sea water, filtrated (10 μm).<br>Flowrate 4 l/min, el. 1,3 A       | 53        | Electrolyzed salt water.                                  |

Conclusion: Under loboratory conditions *V. anguillarum* is sensitive to chlorine. In steriliset wastewater from a fish slaugtherhouse 150 mg/l for 60 min was able to reduce the titer to undetectable. No other dose/time combinations were testet.

#### Vibrio ordalii

| Concentration pathogen | Disinfectant            | Contact<br>time | Concentration disinfectant | рН | Temp. | Result            | Method | Reference | Comments                                       |
|------------------------|-------------------------|-----------------|----------------------------|----|-------|-------------------|--------|-----------|--|
| 10 <sup>5</sup> cfu/ml | NaOCl                   | 2 min           | 3 ppm                      |    | 20    | > 4 log reduction |        | 85        | Concentration of commercial product. (Purelox) |
| 10 <sup>5</sup> cfu/ml | NaOCl                   | 1 min           | 10 ppm                     |    | 20    | > 4 log reduction |        | 85        | Concentration of commercial product. (Purelox) |
| 10 <sup>5</sup> cfu/ml | Benzalkonium<br>chlorid | 2 min           | 0,03%                      |    | 20    | > 4 log reduction |        | 85        | Concentration af kommercielt product           |
| 10 <sup>5</sup> cfu/ml | Benzalkonium chlorid    | 1 min           | 0,1%                       |    | 20    | > 4 log reduction |        | 85        | Concentration af kommercielt product           |

Conclusion: Under clean conditions *V. ordalii* is sensitive to chlorine in a low dose.

### Vibrio salmonicida

| Concentration pathogen                  | Disinfectant            | Contact time | Concentration disinfectant | рН | Temp. | Result          | Method                            | Reference | Comments |
|---|-------------------------|--------------|----------------------------|----|-------|-----------------|-----------------------------------|-----------|----------|
| 10 <sup>8</sup> -10 <sup>9</sup> CFU/ml | chloramine-T<br>(SETAX) | 1 min        | 3 g/l                      |    | 4     | 6 log reduction | Sea water added 10% salmon blood. | 22        |          |

## Vibrio sp.

| Concentration pathogen | Disinfectant | Contact<br>time | Concentration disinfectant | рН | Temp. | Result          | Method  | Reference | Comments  |
|------------------------|--------------|-----------------|----------------------------|----|-------|-----------------|---|-----------|---|
| 10 <sup>7</sup> CFU/ml | NaOCl        | 20 min          | 800 ppm                    |    | 20    | 5 log reduction | Dilution 1:1 of bacteria and disinfectant (25, 50, 100, 200, 400, 800, 1600, 3200 ppm). Growht at 20°C and counting already after 24 hours. | 58        | The actual concentration of disinfectant probably only half of stated in article table. Generally speaking the neccessary concentration for disinfection in this paper is much higher than published in other papers. |
| 10 <sup>7</sup> CFU/ml | NaOCl        | 60 min          | 400 ppm                    |    | 20    | 4 log reduction | Dilution 1:1 of bacteria and disinfectant (25, 50, 100, 200, 400, 800, 1600, 3200 ppm).   | 58        | The actual concentration of disinfectant probably only half of stated in  |

|                        |                        |        |          |    |    |                   | Growht at 20°C and counting already after 24 hours.   |    | article table. Generally speaking the neccessary concentration for disinfection in this paper is much higher than published in other papers.  |
|------------------------|------------------------|--------|----------|----|----|-------------------|---|----|---|
| 10 <sup>7</sup> CFU/ml | CIO <sub>2</sub>       | 20 min | 3200 ppm | 20 | 20 | 3 log reduction   | Dilution 1:1 of bacteria and disinfectant (25, 50, 100, 200, 400, 800, 1600, 3200 ppm). Growht at 20°C and counting already after 24 hours. | 58 | The actual concentration of disinfectant probably only half of stated in article table. Generally speaking the neccessary concentration for disinfection in this paper is much higher than published in other papers. |
| 10 <sup>7</sup> CFU/ml | CIO <sub>2</sub>       | 60 min | 3200 ppm | 20 | 20 | 3 log reduction   | Dilution 1:1 of bacteria and disinfectant (25, 50, 100, 200, 400, 800, 1600, 3200 ppm). Growht at 20°C and counting already after 24 hours. | 58 | The actual concentration of disinfectant probably only half of stated in article table. Generally speaking the neccessary concentration for disinfection in this paper is much higher than published in other papers. |
| 10 <sup>7</sup> CFU/ml | Quartenary<br>ammonium | 20 min | 400 ppm  | 20 | 20 | 4 log reduction   | Dilution 1:1 of bacteria and disinfectant (25, 50, 100, 200, 400, 800, 1600, 3200 ppm). Growht at 20°C and counting already after 24 hours. | 58 | The actual concentration of disinfectant probably only half of stated in article table. Generally speaking the neccessary concentration for disinfection in this paper is much higher than published in other papers. |
| 10 <sup>7</sup> CFU/ml | Quartenary<br>ammonium | 60 min | 400 ppm  | 20 | 20 | > 5 log reduction | Dilution 1:1 of bacteria and disinfectant (25, 50, 100, 200, 400, 800, 1600, 3200 ppm). Growht at 20°C and counting already after 24 hours. | 58 | The actual concentration of disinfectant probably only half of stated in article table. Generally speaking the neccessary concentration for   |

|  |  |  |  | disinfection in this paper |
|--|--|--|--|----------------------------|
|  |  |  |  | is much higher than        |
|  |  |  |  | published in other         |
|  |  |  |  | papers.                    |

Conclusion: According to this paper *Vibrio* is quite resistant to chlorine!

### Yersinia ruckeri

| Concentration pathogen                  | Disinfectant            | Contact<br>time | Concentration disinfectant | рН   | Temp. | Result                                | Method   | Reference | Comments   |
|---|-------------------------|-----------------|----------------------------|------|-------|---------------------------------------|--|-----------|--|
|   | chloramine-T            | 30 min          | 0,08 - 0,5 % (v/v)         |      | 4     | > 5 log reduction                     | Tested at 0,001, 0,01, 0,05, 0,08, 0,1, 0,5, 0,7 and 1%. Hard water, organic loaded. | 49        |  |
| 10 <sup>6,77</sup> cfu/ml               | chloramine-T<br>(SETAX) | 24 hours        | 50 mg/l                    | 7,5  |       | Stable                                | Full-scale trial (Vikan<br>Akvavet), wastewater from<br>fish slaugtherhouse.         | 28        | Salinity 20 %, "bløggevand" (blood, fish slime and epithelial cells in salt water) diluted with fresh water. |
| 10 <sup>4,69</sup> cfu/ml               | chloramine-T<br>(SETAX) | 24 hours        | 50 mg/l                    | 8,96 | 7     | 1½ log reduction                      | Full-scale trial (Norskagfisk),<br>blood water from fish<br>slaugtherhouse.          | 28        | Salinity 14-15 ‰   |
| 10 <sup>6,77</sup> cfu/ml               | chloramine-T<br>(SETAX) | 24 hours        | 100 mg/l                   | 7,5  |       | Stable                                | Full-scale trial (Vikan<br>Akvavet), wastewater from<br>fish slaugtherhouse.         | 28        | Salinity 20 %, "bløggevand" (blood, fish slime and epithelial cells in salt water) diluted with fresh water. |
| 10 <sup>4,69</sup> cfu/ml               | chloramine-T<br>(SETAX) | 24 hours        | 200 mg/l                   | 8,96 | 7     | 3 log reduction                       | Full-scale trial (Norskagfisk),<br>blood water from fish<br>slaugtherhouse.          | 28        | Salinity 14-15 ‰   |
| 10 <sup>6,77</sup> cfu/ml               | chloramine-T<br>(SETAX) | 24 hours        | 250 mg/l                   | 7,5  |       | Not detectable<br>(> 6 log reduction) | Full-scale trial (Vikan<br>Akvavet), wastewater from<br>fish slaugtherhouse.         | 28        | Salinity 20 %, "bløggevand" (blood, fish slime and epithelial cells in salt water) diluted with fresh water. |
| 10 <sup>4,69</sup> cfu/ml               | chloramine-T<br>(SETAX) | 24 hours        | 1000 mg/l                  | 8,96 | 7     | 4 log reduction                       | Full-scale trial (Norskagfisk),<br>blood water from fish<br>slaugtherhouse.          | 28        | Salinity 14-15 ‰   |
| 10 <sup>8</sup> -10 <sup>9</sup> CFU/ml | chloramine-T<br>(SETAX) | 1 min           | 2 g/l                      |      | 4     | 6 log reduction                       | Sea water added 10% salmon blood.  | 22        |  |
| 10 <sup>3</sup> cells/ml                | NaOCI                   | 10 min          | 0,01 mg/l<br>residual      | 6,9  | 20    | Stable                                | Distilled water.   | 97, 98    | Samples tested after ½,<br>1, 2, 5, 10, 20 and 30<br>min.  |
| 10 <sup>3</sup> cells/ml                | NaOCl                   | 30 sec          | 0,05 mg/l                  | 6,9  | 20    | "inactivated"                         | Distilled water.   | 97, 98    | Samples tested after ½,  |

|                            |       |           | residual              |      |    |                                       |   |        | 1, 2, 5, 10, 20 and 30 min.  |
|----------------------------|-------|-----------|-----------------------|------|----|---------------------------------------|---|--------|--|
| 10 <sup>3</sup> cells/ml   | NaOCl | 10 min    | 0,05 mg/l<br>residual | 6,9  | 20 | Stable                                | Soft lake water., 30 mg/l<br>CaCO₃  | 97, 98 |  |
| 10 <sup>3</sup> cells/ml   | NaOCl | 10 min    | 0,05 mg/l<br>residual | 8,2  | 20 | Stable                                | Hard lake water., 120 mg/l<br>CaCO₃   | 97, 98 |  |
| 10 <sup>3</sup> cells/ml   | NaOCl | 2 min     | 0,1 mg/l residual     | 6,9  | 20 | "inactivated"                         | Soft lake water., 30 mg/l<br>CaCO₃  | 97, 98 |  |
| 10 <sup>3</sup> cells/ml   | NaOCl | 2 min     | 0,1 mg/l residual     | 8,2  | 20 | "inactivated"                         | Hard lake water., 120 mg/l<br>CaCO₃   | 97, 98 |  |
| 10 <sup>8,28</sup> cfu/ml  | NaOCl | 10 hours  | 43 mg/l               |      | 7  | > 3 log reduction                     | Laboratory trial, 1 part<br>process water + 2 parts<br>"bløggevand" from fish<br>slaugtherhouse.                      | 28     |  |
|                            | NaOCl | 0,5 hours | 48 mg/l               |      |    | "inactivated"                         | Fuldskalaforsøg. Kemisk<br>fældet blodvand (Hævning til<br>pH 12, derefter fældning med<br>jernklorid til pH 6,5-7,5) | 28     |  |
| 10 <sup>6,77</sup> cfu/ml  | NaOCI | 24 hours  | 50 mg/l               | 7,5  |    | Stable                                | Full-scale trial (Vikan<br>Akvavet), wastewater from<br>fish slaugtherhouse.  | 28     | Salinity 20 %, "bløggevand" (blood, fish slime and epithelial cells in salt water) diluted with fresh water.             |
| 10 <sup>6,77</sup> cfu/ml  | NaOCl | 24 hours  | 100 mg/l              | 7,5  |    | Stable                                | Full-scale trial (Vikan<br>Akvavet), wastewater from<br>fish slaugtherhouse.  | 28     | Salinity 20 ‰,<br>"bløggevand" (blood, fish<br>slime and epithelial cells<br>in salt water) diluted with<br>fresh water. |
| 10 <sup>4,69</sup> cfu/ml  | NaOCl | 30 min    | 100 mg/l              | 8,96 | 7  | Not detectable<br>(≥ 4 log reduction) | Full-scale trial (Norskagfisk),<br>blood water from fish<br>slaugtherhouse.   | 28     | Salinity 14-15 ‰   |
| 10 <sup>8,28</sup> cfu/ml  | NaOCl | 10 hours  | 130 mg/l              |      | 7  | > 3 log reduction                     | Laboratory trial, 1 part<br>process water + 2 parts<br>"bløggevand" from fish<br>slaugtherhouse.                      | 28     |  |
| 6 x 10 <sup>6</sup> CFU/ml | NaOCl | 60 min    | 200 mg/l added        |      |    | Vækst                                 |   | 48     | Test medium: sterilized wastewater from fish slaughterhouse.   |
| 2 x 10 <sup>7</sup> CFU/ml | NaOCI | 24 hours  | 200 mg/l added        |      |    | Vækst                                 |   | 48     | Test medium:<br>unsterilized wastewater<br>from fish slaughterhouse,<br>frozen before use.                               |

| 6 x 10 <sup>6</sup> CFU/ml   | NaOCl | 60 min   | 250 mg/l added |      |   | "inactivated"                         |  | 48 | Test medium: sterilized wastewater from fish slaughterhouse.   |
|------------------------------|-------|----------|----------------|------|---|---------------------------------------|--|----|--|
| 10 <sup>6,77</sup> cfu/ml    | NaOCl | 2 t      | 250 mg/l       | 7,5  |   | > 3 log reduction                     | Full-scale trial (Vikan<br>Akvavet), wastewater from<br>fish slaugtherhouse.                     | 28 | Salinity 20 %,<br>"bløggevand" (blood, fish<br>slime and epithelial cells<br>in salt water) diluted with<br>fresh water. |
| 10 <sup>6,77</sup> cfu/ml    | NaOCl | 24 hours | 250 mg/l       | 7,5  |   | > 4 log reduction                     | Full-scale trial (Vikan<br>Akvavet), wastewater from<br>fish slaugtherhouse.                     | 28 | Salinity 20 ‰, "bløggevand" (blood, fish slime and epithelial cells in salt water) diluted with fresh water.             |
| 10 <sup>4,69</sup> cfu/ml    | NaOCl | 2 t      | 250 mg/l       | 8,96 | 7 | 3 log reduction                       | Full-scale trial (Norskagfisk),<br>blood water from fish<br>slaugtherhouse.                      | 28 | Salinity 14-15 ‰   |
| 2 x 10 <sup>7</sup> CFU/ml   | NaOCl | 24 hours | 250 mg/l added |      |   | "inactivated"                         |  | 48 | Test medium:<br>unsterilized wastewater<br>from fish slaughterhouse,<br>frozen before use.                               |
| 10 <sup>4,69</sup> cfu/ml    | NaOCl | 24 hours | 250 mg/l       | 8,96 | 7 | Not detectable<br>(≥ 4 log reduction) | Full-scale trial (Norskagfisk),<br>blood water from fish<br>slaugtherhouse.                      | 28 | Salinity 14-15 ‰   |
| 10 <sup>8,28</sup> cfu/ml    | NaOCl | 10 hours | 260 mg/l       |      | 7 | 5 log reduction                       | Laboratory trial, 1 part<br>process water + 2 parts<br>"bløggevand" from fish<br>slaugtherhouse. | 28 |  |
| 6,5 x 10 <sup>7</sup> CFU/ml | NaOCl | 30 min   | 280 mg/l added |      |   | Vækst                                 |  | 48 | Testmedium: usteriliseret spildevand fra fiskeslagteri   |
| 6,5 x 10 <sup>7</sup> CFU/ml | NaOCl | 60 min   | 280 mg/l added |      |   | Vækst                                 |  | 48 | Testmedium: usteriliseret<br>spildevand fra<br>fiskeslagteri   |
| 6,5 x 10 <sup>7</sup> CFU/ml | NaOCl | 30 min   | 350 mg/l added |      |   | "inactivated"                         |  | 48 | Testmedium: usteriliseret spildevand fra fiskeslagteri   |
| 6,5 x 10 <sup>7</sup> CFU/ml | NaOCl | 60 min   | 350 mg/l added |      |   | "inactivated"                         |  | 48 | Testmedium: usteriliseret spildevand fra fiskeslagteri   |
| 10 <sup>4,69</sup> cfu/ml    | NaOCl | 30 min   | 350 mg/l       | 8,96 | 7 | 4 log reduction                       | Full-scale trial (Norskagfisk),<br>blood water from fish<br>slaugtherhouse.                      | 28 | Salinity 14-15 ‰   |
| 10 <sup>4,69</sup> cfu/ml    | NaOCl | 2 t      | 350 mg/l       | 8,96 | 7 | Not detectable                        | Full-scale trial (Norskagfisk),  | 28 | Salinity 14-15 ‰   |

|  |  |  | (≥ 4 log reduction) | blood water from fish |  |
|--|--|--|---------------------|-----------------------|--|
|  |  |  | , ,                 | slaugtherhouse.       |  |

Conclusion: In full-scale trials under highly contaminated conditions as is found in process wastewater from fish slaugtherhouses 250 mg/l chlorine (administered) as NaOCl for 2 hours were able to decimate *Y. ruckeri* 2 log and after 24 hours to inactivate the bacterium. If the water was pretreated with first pH 12 followed by precipitation with ferrichlorid to pH 6,5 only 50 mg/l chlorine was needed to decimate > 3 log.

When using chloramines-T it was necessary to use a dose of 250-1000 mg/l chlorine for 24 hours to obtain the same degree of inactivation (no pretreatment of water).

#### **Parasites**

#### Gyrodactylus salaris

| Concentration pathogen | Disinfectant | Contact time | Concentration disinfectant | рН | Temp. | Result | Method | Reference | Comments   |
|------------------------|--------------|--------------|----------------------------|----|-------|--------|--------|-----------|--|
|                        | chlor        |              |                            |    |       | følsom |        | 1         | From OIE diagnostic<br>manual. No reference<br>stated! |

#### Myxosoma cerebralis

| Concentration pathogen | Disinfectant         | Contact time | Concentration disinfectant | рН  | Temp.         | Result               | Method   | Reference | Comments                       |
|------------------------|----------------------|--------------|----------------------------|-----|---------------|----------------------|--|-----------|--------------------------------|
|                        | Ca(CIO) <sub>2</sub> | 14 days      | 400 ppm                    |     | 22            | Survival             | In vitro – spores  | 42        |                                |
|                        | CaOCl                | 18 hours     | 1200 ppm                   |     |               | 28% of fish infected | Infected mud, chlorine added to water.                                 | 44        | Control fish 100% infected.    |
|                        | CaOCl                | 30 min       | 10 ppm                     |     | 12            | No spores in fish    | Myxosoma cerebralis free in<br>water, fish added after<br>disinfection | 44        | Control fish 100%<br>infected. |
|                        | Cl                   | 14 days      | 200 ppm                    |     | 22            | Survival             | In vitro – spores  | 42        |                                |
|                        | NaOCl                | 15 min       | 200 mg/l                   |     | 15            | 1 log reduction      | Infectivity of myxospores in tubifex.                                  | 37        | Myxospore suspension           |
|                        | NaOCl                | 15 min       | 500 mg/l                   |     | 15            | 5 log reduction      | Infectivity of myxospores in tubifex.                                  | 37        | Myxospore suspension           |
|                        | NaOCl                | 15 min       | 2500 mg/l                  | 8,1 | 15            | 100% reduction       | Infectivity of myxospores in tubifex.                                  | 37        | Myxospore suspension           |
|                        | NaOCl                | 1 min        | 131 ppm                    |     | room<br>temp. | All dead             | in vitro. Triactinomyxon<br>spores                                     | 96        |                                |

| Quartenary ammonium | 14 days | 0,1%      | 22 | Survival                     | In vitro – spores                     | 42 |   |
|---------------------|---------|-----------|----|------------------------------|---------------------------------------|----|---|
| Quartenary ammonium | 10 min  | 1000 mg/l | 22 | 1 log reduction              | Infectivity of myxospores in tubifex. | 37 | alkyl dimethyl benzyl<br>ammonium chlorid |
| Quartenary ammonium | 10 min  | 1500 mg/l | 22 | ingen infektion i<br>tubifex | Infectivity of myxospores in tubifex. | 37 | alkyl dimethyl benzyl<br>ammonium chlorid |

Conclusion: Using a concentration of 500-2500 mg/l it will likely be possible to use chlorine for desinfection of *M. cerebralis*.

### Trichodina jadranica

| Concentration pathogen | Disinfectant | Contact<br>time | Concentration disinfectant | рН | Temp. | Result         | Method      | Reference | Comments   |
|------------------------|--------------|-----------------|----------------------------|----|-------|----------------|-------------|-----------|--|
| 2,2                    | chloramine-T | 24 hours        | 50 ppm                     |    | 25    | Survival (0,5) | In vivo, ål | 75        | Catergorization<br>(category/number of<br>parasites on ell): 0/0,<br>1/1-10, 2/11-100, 3/100-<br>1000, 4/>1000 |

Conclusion: It seems as if it will be possible to use chlorine for desinfection of Trichodina but the dose has to be bigger than the one used here.

## **Temperature**

#### Virus

#### **VHSV**

| Concentration pathogen                 | Disinfectant | Contact<br>time | Concentration disinfectant | рН | Temp. | Result               | Method            | Reference                 | Comments          |
|--|--------------|-----------------|----------------------------|----|-------|----------------------|-------------------|---------------------------|-------------------|
| 10 <sup>6</sup> TCID <sub>50</sub> /ml | Heating      | 3 days          |                            |    | 20    | > 4 log reduction    | MEM without serum | 51                        | Read off a graph. |
| 10 <sup>6</sup> TCID <sub>50</sub> /ml | Heating      | 2½ uge          |                            |    | 20    | app. 4 log reduction | MEM with serum    | 51                        | Read off a graph. |
| 10 <sup>6</sup> TCID <sub>50</sub> /ml | Heating      | 3 hours         |                            |    | 30    | Survival             | MEM with serum    | 51                        |                   |
| 10 <sup>6</sup> TCID <sub>50</sub> /ml | Heating      | 24 hours        |                            |    | 30    | Not detectable       | MEM with serum    | 51                        |                   |
| 10 <sup>6</sup> TCID <sub>50</sub> /ml | Heating      | 5 min           |                            |    | 50    | Survival             | MEM with serum    | 51                        |                   |
| 10 <sup>6</sup> TCID <sub>50</sub> /ml | Heating      | 10 min          |                            |    | 50    | Not detectable       | MEM with serum    | 51                        |                   |
|  | Heating      | 1 hour          |                            |    | 60    | "inactivated"        |                   | 20, Dixon<br>(pers. com.) |                   |
| 10 <sup>6</sup> TCID <sub>50</sub> /ml | Heating      | 1 min           |                            |    | 70    | Not detectable       | MEM with serum    | 51                        |                   |

Conclusion: VHSV is heat sensitive and is inactivated at 60°C for 10 min to 1 hour.

#### *IHNV*

| Concentration pathogen                       | Disinfectant | Contact<br>time | Concentration disinfectant | рН  | Temp. | Result          | Method       | Reference                 | Comments           |
|--|--------------|-----------------|----------------------------|-----|-------|-----------------|--------------|---------------------------|--------------------|
| 10 <sup>7</sup> TCID <sub>50</sub> /ml       | Heating      | 5 hours         |                            | 7,2 | 8     | Stable          | MEM-1 medium | 32                        |                    |
| 10 <sup>7</sup> TCID <sub>50</sub> /ml       | Heating      | 5 hours         |                            | 7,2 | 22    | Stable          | MEM-1 medium | 32                        |                    |
|  | Heating      | 8 hours         |                            |     | 32    | "inactivated"   | MEM medium   | 83                        |                    |
| 2,5 x 10 <sup>7</sup> TCID <sub>50</sub> /ml | Heating      | 1 døgn          |                            | 7,2 | 32    | "inactivated"   | MEM-1 medium | 32                        | Karluk Lake isolat |
| 1,5 x 10 <sup>7</sup> TCID <sub>50</sub> /ml | Heating      | 1 døgn          |                            | 7,2 | 32    | "inactivated"   | MEM-1 medium | 32                        | Cedar River isolat |
| 10 <sup>7</sup> TCID <sub>50</sub> /ml       | Heating      | 7,3 hours       |                            | 7,2 | 32    | 4 log reduction | MEM-1 medium | 32                        |                    |
|  | Heating      | 5 hours         |                            | 7   | 35    | "inactivated"   |              | 100                       |                    |
| 2,5 x 10 <sup>7</sup> TCID <sub>50</sub> /ml | Heating      | 140 min         |                            | 7,2 | 38    | "inactivated"   | MEM-1 medium | 32                        | Karluk Lake isolat |
| 1,5 x 10 <sup>7</sup> TCID <sub>50</sub> /ml | Heating      | 140 min         |                            | 7,2 | 38    | "inactivated"   | MEM-1 medium | 32                        | Cedar River isolat |
|  | Heating      | 20 min          |                            | 7   | 40    | "inactivated"   |              | 100                       |                    |
|  | Heating      | 10 min          |                            | 7   | 45    | "inactivated"   |              | 100                       |                    |
|  | Heating      | 90 sec          |                            | 7   | 50    | "inactivated"   |              | 100                       |                    |
|  | Heating      | 30 sec          |                            | 7   | 55    | "inactivated"   |              | 100                       |                    |
|  | Heating      | 1 hour          |                            |     | 60    | "inactivated"   |              | 20, Dixon<br>(pers. com.) |                    |

Conclusion: IHNV is heat sensitive and is reported inactivated at 55°C for 30 sec and 60°C for 1 hour.

#### *IPNV*

| Concentration pathogen                   | Disinfectant | Contact<br>time | Concentration disinfectant | рН    | Temp. | Result                             | Method   | Reference                 | Comments                                    |
|--|--------------|-----------------|----------------------------|-------|-------|------------------------------------|--|---------------------------|---|
|  | Heating      | 20 t            |                            | 7,2   | 37,5  | 1 log reduction                    | MEM-1 medium                                     | 32'32                     | Read off a graph.                           |
|  | Heating      | 20 t            |                            | 7,2   | 50    | 2 log reduction                    | MEM-1 medium                                     | 32′32                     | Read off a graph.                           |
|  | Heating      | < 20 min        |                            | 7,2   | 60    | 95 % reduction                     |  | 32′32                     |   |
| 10 <sup>7,2</sup> TCID <sub>50</sub> /ml | Heating      | 30 min          |                            | 6,8   | 60    | 99,9% reduction                    |  | 74′74                     | Isolate VR-299                              |
| 10 <sup>7,2</sup> TCID <sub>50</sub> /ml | Heating      | 1 hour          |                            | 3     | 60    | 6 log reduction                    | virus in EMEM with serum                         | 74′74                     | Isolate VR-299.<br>Result Read off a graph. |
| 10 <sup>7,2</sup> TCID <sub>50</sub> /ml | Heating      | 4 hours         |                            | 9     | 60    | 6 log reduction                    | virus in EMEM with serum                         | 74′74                     | Isolate VR-299.<br>Result Read off a graph. |
| 10 <sup>7,2</sup> TCID <sub>50</sub> /ml | Heating      | 5 hours         |                            | 6,8-7 | 60    | 6 log reduction                    | virus in EMEM with and without serum             | 74′74                     | Isolate VR-299                              |
|  | Heating      | 8 hours         |                            | 7     | 60    | "inactivated"                      |  | 100′100                   | Isolate VR-299                              |
|  | Heating      | 16 hours        |                            | 7,2   | 60    | "inactivated"                      |  | 32′32                     |   |
|  | Heating      | 24 hours        |                            |       | 60    | Survival                           |  | 20, Dixon<br>(pers. com.) |   |
|  | Heating      | 48 hours        |                            |       | 60    | "inactivated"                      |  | 20, Dixon<br>(pers. com.) |   |
| 10 <sup>6,6</sup> TCID <sub>50</sub> /ml |              | 1 min           |                            |       | 65    | Not detectable (> 5 log reduction) | Laboratory trial, 1 part process water + 2 parts | 28                        |   |

|  |         |           |   |    |                   | "bløggevand" from fish slaugtherhouse.                                      |         |             |
|--|---------|-----------|---|----|-------------------|---|---------|-------------|
| 10 <sup>5,9</sup> TCID <sub>50</sub> /ml | Heating | 5 min     |   | 65 | 1½ log reduction  | Full-scale trial (Norskagfisk),<br>blood water from fish<br>slaugtherhouse. | 28      |             |
|  | Heating | 3,5 hours | 7 | 65 | "inactivated"     |   | 100′100 | Type VR-299 |
| 10 <sup>5,9</sup> TCID <sub>50</sub> /ml | Heating | 5 min     |   | 70 | 1,9 log reduction | Full-scale trial (Norskagfisk),<br>blood water from fish<br>slaugtherhouse. | 28      |             |
|  | Heating | 2 t       | 7 | 70 | "inactivated"     |   | 100′100 | Type VR-299 |
| 10 <sup>5,9</sup> TCID <sub>50</sub> /ml | Heating | 3 min     |   | 75 | 2,3 log reduction | Full-scale trial (Norskagfisk),<br>blood water from fish<br>slaugtherhouse. | 28      |             |
|  | Heating | 10 min    | 7 | 80 | "inactivated"     |   | 100′100 | Type VR-299 |

Conclusion: IPNV is more heat resistant the VHSV and IHNV. The virus is reported to be reduced by 3 log when heated to 60°C for 30 min. Another author reports survival after 24 h at 60°C. In laboratory trials using process water including fish slime, skin scrapings and blood > 5 log reduction were achieved after heating to 65°C for 1 min. In full-scale trials using blood water, 2,3 log reduction were achieved after treatment of IPNV for 3 min.

#### **ISAV**

| Concentration pathogen                       | Disinfectant | Contact<br>time | Concentration disinfectant | рН | Temp. | Result                         | Method  | Reference                 | Comments                                     |
|--|--------------|-----------------|----------------------------|----|-------|--------------------------------|---|---------------------------|--|
| 1 x 10 <sup>6</sup> TCID <sub>50</sub> /ml   | Temperature  | 14 days         |                            |    | 4     | Stable                         | Virus in L15-medium   | 27                        |  |
| 1 x 10 <sup>6</sup> TCID <sub>50</sub> /ml   | Temperature  | 10 days         |                            |    | 15    | Stable                         | Virus in L15-medium   | 27                        |  |
| 1 x 10 <sup>6</sup> TCID <sub>50</sub> /ml   | Temperature  | 2 days          |                            |    | 37    | Survival.<br>4-5 log reduction | Virus in L15-medium   | 27                        | 3,2 x 10 <sup>1</sup> TCID50/ml på<br>dag 2. |
|  | Heating      | 2 min           |                            |    | 50    | "inactivated"                  | Tissue homogenate of liver,<br>kidney and spleen from<br>moribund ISA-fish, treated<br>and IP-injected in fish. | 94                        | Tested at 45-60°C in 1, 2<br>and 5 min.      |
|  | Heating      | 1 min           |                            |    | 55    | "inactivated"                  | Tissue homogenate of liver,<br>kidney and spleen from<br>moribund ISA-fish, treated<br>and IP-injected in fish. | 94                        | Tested at 45-60°C in 1, 2<br>and 5 min.      |
| 2,5 x 10 <sup>6</sup> TCID <sub>50</sub> /ml | Heating      | 5 min           |                            |    | 56    | Not detectable                 | Virus in L15-medium   | 27                        |  |
|  | Heating      | 1 hour          |                            |    | 60    | "inactivated"                  |   | 20, Dixon<br>(pers. com.) |  |

Conclusion: ISAV is heat sensitive with reported inactivation times of 56°C for 5 min and 60°C for 1 hour.

#### KHV

| Concentration | Disinfectant | Contact | Concentration | рН | Temp. | Result | Method | Reference | Comments |
|---------------|--------------|---------|---------------|----|-------|--------|--------|-----------|----------|

| pathogen                     |         | time  | disinfectant |      |                |   |    |              |
|------------------------------|---------|-------|--------------|------|----------------|---|----|--------------|
| 1,6 x 10 <sup>4</sup> PFU/ml | Heating | 1 min |              | > 50 | Not detectable | Tested at 40, 50, 60 og 70°C i ½, 1, 3 and 5 min. | 55 | Strain KHV-I |

### Nodavirus

| Concentration pathogen                 | Disinfectant | Contact<br>time | Concentration disinfectant | рН | Temp. | Result   | Method   | Reference | Comments                       |
|--|--------------|-----------------|----------------------------|----|-------|--|--|-----------|--------------------------------|
|  | Temperature  | 1 year          |                            |    | -20   | Stable   | Grown virus. Tested after 4 weeks, 3 and 6 months and 1 year.                        | 30        | Isolate: sea bass<br>nodavirus |
|  | Temperature  | 6 months        |                            |    | 4     | Stable   | Grown virus. Tested after 1, 4<br>and 7 days, 4 weeks, 3 and 6<br>months and 1 year. | 30        | lsolate: sea bass<br>nodavirus |
|  | Temperature  | 1 year          |                            |    | 4     | Survival (4-5 log<br>reduction)  | Grown virus. Tested after 1, 4<br>and 7 days, 4 weeks, 3 and 6<br>months and 1 year. | 30        | Isolate: sea bass<br>nodavirus |
|  | Temperature  | 4 uger          |                            |    | 25    | Survival (2-3 log<br>reduction)  | Grown virus. Tested after 1, 4<br>and 7 days, 4 weeks, 3 and 6<br>months and 1 year. | 30        | Isolate: sea bass<br>nodavirus |
|  | Temperature  | 3 months        |                            |    | 25    | Not detectable   | Grown virus. Tested after 1, 4 and 7 days, 4 weeks, 3 and 6 months and 1 year.       | 30        | lsolate: sea bass<br>nodavirus |
|  | Temperature  | 1 day           |                            |    | 37    | Survival (2-3 log<br>reduction)  | Grown virus. Tested after 1, 4 and 7 days, 4 weeks, 3 and 6 months and 1 year.       | 30        | lsolate: sea bass<br>nodavirus |
|  | Temperature  | 4 days          |                            |    | 37    | Not detectable   | Grown virus. Tested after 1, 4 and 7 days, 4 weeks, 3 and 6 months and 1 year.       | 30        | Isolate: sea bass<br>nodavirus |
| 10 μg purified virus                   | Heating      | 30 min          |                            | 7  | 50    | Not inactivated<br>(0/800 larvae<br>survived, control<br>230/800)              | Diluted in 1 ml PBS. Used for infection trial in day old striped jack larvae.        | 9         | Isolate: SJNNV                 |
| 10 μg purified virus                   | Heating      | 30 min          |                            | 7  | 60    | "Effective" (390/800 larvae survived, antigen ELISA negativ - control 230/800) | Diluted in 1 ml PBS. Used for infection trial in day old striped jack larvae.        | 9         | Isolate: SJNNV                 |
| 10 <sup>7</sup> TCID <sub>50</sub> /ml | Heating      | 30 min          |                            |    | 60    | Not detectable   | Hanks balanced salt solution   | 30        | Isolate: sea bass<br>nodavirus |
| 10 <sup>8 TCID</sup> <sub>50</sub> /ml | Heating      | 30 min          |                            |    | 60    | 6½ log reduction   | Hanks balanced salt solution   | 30        | Isolate: sea bass              |

|  |         |          |  |    |                | med serum                              |                           | nodavirus                      |
|--|---------|----------|--|----|----------------|--|---------------------------|--------------------------------|
| 10 <sup>8 TCID</sup> <sub>50</sub> /ml | Heating | 1 hour   |  | 60 | Not detectable | Hanks balanced salt solution med serum | 30                        | Isolate: sea bass<br>nodavirus |
|  | Heating | 24 hours |  | 60 | "inactivated"  |  | 20, Dixon<br>(pers. com.) |                                |

Conclusion: nodavirus is heat sensitive although more resistant than VHSV and IPNV, with reported inactivation times of 1-24 hours at 60°C. Treatment of virus for 30 min at 50°C was not sufficient inhibit disease in an infection trial using day-old striped jack larvae. Increasing the Temperature to 60°C for 30 min was effective in inhibiting disease.

#### Ranavirus

| Concentration pathogen | Disinfectant | Contact<br>time | Concentration disinfectant | рН | Temp. | Result        | Method                        | Reference                    | Comments                                 |
|------------------------|--------------|-----------------|----------------------------|----|-------|---------------|-------------------------------|------------------------------|--|
|                        | Temperature  | 2 years         |                            |    | -70   | Survival      | Virus in cell culture medium. | 67                           | EHNV. CPE development slow,<br>8-10 days |
|                        | Temperature  | 2 years         |                            |    | -70   | Survival      | Virus in fish tissue.         | 67                           | EHNV. CPE development slow,<br>8-10 days |
|                        | Temperature  | 2 years         |                            |    | -20   | Survival      | Virus in cell culture medium. | 67                           | EHNV. CPE development slow,<br>8-10 days |
|                        | Temperature  | 2 years         |                            |    | -20   | Survival      | Virus in fish tissue.         | 67                           | EHNV. CPE development slow,<br>8-10 days |
|                        | Temperature  | 300 days        |                            |    | 4     | Survival      | Virus in RTG-2 celler         | 67                           | EHNV. CPE development slow,<br>8-10 days |
|                        | Heating      | 24 hours        |                            |    | 40    | "inactivated" | Virus in cell culture medium. | 67                           | EHNV                                     |
|                        | Heating      | 15 min          |                            |    | 60    | "inactivated" | Virus in cell culture medium. | 67                           | EHNV                                     |
|                        | Heating      | 24 hours        |                            |    | 60    | "inactivated" |                               | 20, Dixon<br>(pers.<br>com.) |  |

Conclusion: Ranavirus is reported inactivated by heat treatment for 15 min to 24 hours at 60°C.

#### Salmonid alphavirus

| Concentration pathogen | Disinfectant | Contact<br>time | Concentration disinfectant | рН | Temp. | Result         | Method   | Reference | Comments                                |
|------------------------|--------------|-----------------|----------------------------|----|-------|----------------|--|-----------|---|
|                        | Temperature  | 30 min          |                            |    | 4-25  | Stable         | Heating to 15, 25, 37, 45, 50, 55 and 60°C in 15 min followed by cooling in ice. | 79        | Salmon pancreas disease<br>virus (SPDV) |
|                        | Heating      | 30 min          |                            |    | 37-45 | reduced        | Heating to 15, 25, 37, 45, 50, 55 and 60°C in 15 min followed by cooling in ice. | 79        | Salmon pancreas disease<br>virus (SPDV) |
|                        | Heating      | 30 min          |                            |    | 50    | Not detectable | Heating to 15, 25, 37, 45, 50, 55 and 60°C in 15 min followed by cooling in ice. | 79        | Salmon pancreas disease<br>virus (SPDV) |
|                        | Heating      | 1 hour          |                            |    | 60    | "inactivated"  |  | 33        | SAV1                                    |

Conclusion: SAV is reported inactivated at 50°C for 30 min and 60°C for 1 hour.

### **SVCV**

| Concentration pathogen                   | Disinfectant | Contact<br>time | Concentration disinfectant | рН | Temp. | Result             | Method                        | Reference | Comments |
|--|--------------|-----------------|----------------------------|----|-------|--------------------|-------------------------------|-----------|----------|
|  |              |                 |                            |    |       |                    | Cell culture medium without   |           |          |
| 10 <sup>6,5</sup> TCID <sub>50</sub> /ml | Temperature  | 180 days        |                            |    | -74   | 2 log reduction    | serum. Titrated day 3, 7, 14, | 3         |          |
|  |              |                 |                            |    |       |                    | 21, 28, 75, 110 and 180.      |           |          |
|  |              |                 |                            |    |       |                    | Cellekulturmedium med 5%      |           |          |
| 10 <sup>7,8</sup> TCID <sub>50</sub> /ml | Temperature  | 180 days        |                            |    | -74   | Stable             | serum. Titreret day 3, 7, 14, | 3         |          |
|  |              |                 |                            |    |       |                    | 21, 28, 75, 110 og 180 days   |           |          |
|  |              |                 |                            |    |       |                    | Cell culture medium without   |           |          |
| 10 <sup>6,5</sup> TCID <sub>50</sub> /ml | Temperature  | 180 days        |                            |    | -20   | > 3½ log reduction | serum. Titrated day 3, 7, 14, | 3         |          |
|  |              |                 |                            |    |       |                    | 21, 28, 75, 110 and 180.      |           |          |
|  |              |                 |                            |    |       |                    | Cellekulturmedium med 5%      |           |          |
| 10 <sup>7,8</sup> TCID <sub>50</sub> /ml | Temperature  | 180 days        |                            |    | -20   | 2 log reduction    | serum. Titreret day 3, 7, 14, | 3         |          |
|  |              |                 |                            |    |       | -                  | 21, 28, 75, 110 og 180 days   |           |          |
|  |              |                 |                            |    |       |                    | Cell culture medium without   |           |          |
| 10 <sup>6,5</sup> TCID <sub>50</sub> /ml | Temperature  | 110 days        |                            |    | 4     | > 4 log reduction  | serum. Titrated day 3, 7, 14, | 3         |          |
|  | ·            | -               |                            |    |       |                    | 21, 28, 75, 110 and 180.      |           |          |
|  |              |                 |                            |    |       |                    | Cell culture medium without   |           |          |
| 10 <sup>6,5</sup> TCID <sub>50</sub> /ml | Temperature  | 180 days        |                            |    | 4     | "inactivated"      | serum. Titrated day 3, 7, 14, | 3         |          |
| 307                                      | '            | ,               |                            |    |       |                    | 21, 28, 75, 110 and 180.      |           |          |
|  |              |                 |                            |    |       |                    | Cellekulturmedium med 5%      |           |          |
| 10 <sup>7,8</sup> TCID <sub>50</sub> /ml | Temperature  | 180 days        |                            |    | 4     | > 3 log reduction  | serum. Titreret day 3, 7, 14, | 3         |          |
| 507                                      |              | , ,             |                            |    |       |                    | 21, 28, 75, 110 og 180 days   |           |          |
|  |              |                 |                            |    |       |                    | Cell culture medium without   |           |          |
| 10 <sup>6,5</sup> TCID <sub>50</sub> /ml | Temperature  | 7 days          |                            |    | 22-24 | 2 log reduction    | serum. Titrated day 3, 7, 14, | 3         |          |
| 507                                      |              | , , ,           |                            |    |       |                    | 21, 28, 75, 110 and 180.      |           |          |
|  |              |                 |                            |    |       |                    | Cell culture medium without   |           |          |
| 10 <sup>6,5</sup> TCID <sub>50</sub> /ml | Temperature  | 14 days         |                            |    | 22-24 | "inactivated"      | serum. Titrated day 3, 7, 14, | 3         |          |
| 307                                      | '            | ,               |                            |    |       |                    | 21, 28, 75, 110 and 180.      |           |          |
|  |              |                 |                            |    |       |                    | Cellekulturmedium med 5%      |           |          |
| 10 <sup>7,8</sup> TCID <sub>50</sub> /ml | Temperature  | 21 days         |                            |    | 22-24 | > 3 log reduction  | serum. Titreret day 3, 7, 14, | 3         |          |
|  |              |                 |                            |    |       | 2 128 12222        | 21, 28, 75, 110 og 180 days   |           |          |
|  |              |                 |                            |    |       |                    | Cellekulturmedium med 5%      |           |          |
| 10 <sup>7,8</sup> TCID <sub>50</sub> /ml | Temperature  | 75 days         |                            |    | 22-24 | "inactivated"      | serum. Titreret day 3, 7, 14, | 3         |          |
|  | 3            |                 |                            |    | '     |                    | 21, 28, 75, 110 og 180 days   |           |          |
|  |              |                 |                            |    |       |                    | Cell cultur medium with 5%    |           |          |
| 7.5                                      |              |                 |                            |    |       |                    | serum. Titrated after 5, 10,  |           |          |
| 10 <sup>7,5</sup> TCID <sub>50</sub> /ml | Heating      | 480 min         |                            |    | 30    | ½ log reduction    | 20, 30, 60, 120, 240 and 480  | 3         |          |
|  |              |                 |                            |    |       |                    | min.                          |           |          |

| 10 <sup>7,5</sup> TCID <sub>50</sub> /ml | Heating | 480 min | 35 | 1 log reduction   | Cell cultur medium with 5% serum. Titrated after 5, 10, 20, 30, 60, 120, 240 and 480 min. | 3                         |  |
|--|---------|---------|----|-------------------|---|---------------------------|--|
| 10 <sup>7,5</sup> TCID <sub>50</sub> /ml | Heating | 240 min | 40 | 3 log reduction   | Cell cultur medium with 5% serum. Titrated after 5, 10, 20, 30, 60, 120, 240 and 480 min. | 3                         |  |
| 10 <sup>7,5</sup> TCID <sub>50</sub> /ml | Heating | 480 min | 40 | > 4 log reduction | Cell cultur medium with 5% serum. Titrated after 5, 10, 20, 30, 60, 120, 240 and 480 min. | 3                         |  |
| 10 <sup>7,5</sup> TCID <sub>50</sub> /ml | Heating | 60 min  | 45 | 3 log reduction   | Cell cultur medium with 5% serum. Titrated after 5, 10, 20, 30, 60, 120, 240 and 480 min. | 3                         |  |
| 10 <sup>7,5</sup> TCID <sub>50</sub> /ml | Heating | 180 min | 45 | > 5 log reduction | Cell cultur medium with 5% serum. Titrated after 5, 10, 20, 30, 60, 120, 240 and 480 min. | 3                         |  |
| 10 <sup>7,5</sup> TCID <sub>50</sub> /ml | Heating | 60 min  | 50 | "inactivated"     | Cell cultur medium with 5% serum. Titrated after 5, 10, 20, 30, 60, 120, 240 and 480 min. | 3                         |  |
|  | Heating | 1 hour  | 60 | "inactivated"     |   | 20, Dixon<br>(pers. com.) |  |

Conclusion: SVCV is inactivated at  $\leq 60$ °C after 1 h.

## Channel catfish virus

| Concentration pathogen | Disinfectant | Contact<br>time | Concentration disinfectant | рН | Temp. | Result        | Method | Reference                 | Comments |
|------------------------|--------------|-----------------|----------------------------|----|-------|---------------|--------|---------------------------|----------|
|                        | Heating      | 24 hours        |                            |    | 60    | "inactivated" |        | 20, Dixon<br>(pers. com.) |          |

#### **Bacteria**

### Aeromonas salmonicida

| Concentration pathogen       | Disinfectant | Contact time | Concentration disinfectant | рН | Temp. | Result        | Method | Reference | Comments |
|------------------------------|--------------|--------------|----------------------------|----|-------|---------------|--------|-----------|----------|
| 1,4 x 10 <sup>6</sup> cfu/ml | Heating      | 48 hours     |                            | 7  | 35    | "inactivated" |        | 100       |          |

| 1,4 x 10 <sup>6</sup> cfu/ml | Heating | 3 hours | 7 | 40 | "inactivated" | 100          |
|------------------------------|---------|---------|---|----|---------------|--------------|
| 1,4 x 10 <sup>6</sup> cfu/ml | Heating | 10 min  | 7 | 45 | "inactivated" | 100          |
| 1,4 x 10 <sup>6</sup> cfu/ml | Heating | 2 min   | 7 | 50 | "inactivated" | 100          |
|                              | Heating | 1 hour  | • | 60 | Survival      | 20, Dixon    |
|                              | Heating | 1 11001 |   | 00 | Jui vivai     | (pers. com.) |

Conclusion: *A. salmonicida* is reported inactivated after heat treatment for 2 min at 50°C. This is disputed by another report stating survival after 1 hour at 60°C.

#### Lactococcus garviae

|   | Concentration pathogen | Disinfectant | Contact<br>time | Concentration disinfectant | рН | Temp. | Result        | Method | Reference              | Comments |
|---|------------------------|--------------|-----------------|----------------------------|----|-------|---------------|--------|------------------------|----------|
| ĺ |                        | Heating      | 24 hours        |                            |    | 60    | Survival      |        | 20, Dixon (pers. com.) |          |
| İ |                        | Heating      | 48 hours        |                            |    | 60    | "inactivated" |        | 20, Dixon (pers. com.) |          |

### Vibrio anguillarum

| Concentration pathogen | Disinfectant | Contact<br>time | Concentration disinfectant | рН | Temp. | Result        | Method | Reference                 | Comments  |
|------------------------|--------------|-----------------|----------------------------|----|-------|---------------|--------|---------------------------|---|
| 10 <sup>6</sup> CFU/ml | Heating      | 1 min           |                            |    | 60    | "inactivated" |        | 48                        | Test medium: sterilized wastewater from fish slaughterhouse.            |
| 10 <sup>7</sup> CFU/ml | Heating      | 2 min           |                            |    | 60    | Growth        |        | 48                        | Test medium:<br>unsterilized wastewater<br>from fish<br>slaughterhouse. |
|                        | Heating      | 1 hour          |                            |    | 60    | Survival      |        | 20, Dixon<br>(pers. com.) |   |
| 10 <sup>6</sup> CFU/ml | Heating      | 15 sec          |                            |    | 72    | "inactivated" |        | 48                        | Test medium: sterilized wastewater from fish slaughterhouse.            |
| 10 <sup>7</sup> CFU/ml | Heating      | 15 sec          |                            |    | 72    | "inactivated" |        | 48                        | Test medium:<br>unsterilized wastewater<br>from fish<br>slaughterhouse. |

## Mycobacterium chelonei

| Concentration | Disinfectant | Contact | Concentration | рН | Temp. | Result | Method | Reference | Comments |
|---------------|--------------|---------|---------------|----|-------|--------|--------|-----------|----------|

| pathogen                     |         | time     | disinfectant |   |    |               |                           |
|------------------------------|---------|----------|--------------|---|----|---------------|---------------------------|
| 7,5 x 10 <sup>6</sup> cfu/ml | Heating | 24 hours |              | 7 | 40 | "inactivated" | 100                       |
| 7,5 x 10 <sup>6</sup> cfu/ml | Heating | 4 hours  |              | 7 | 45 | "inactivated" | 100                       |
| 7,5 x 10 <sup>6</sup> cfu/ml | Heating | 60 min   |              | 7 | 50 | "inactivated" | 100                       |
| 7,5 x 10 <sup>6</sup> cfu/ml | Heating | 15 min   |              | 7 | 55 | "inactivated" | 100                       |
| 7,5 x 10 <sup>6</sup> cfu/ml | Heating | 2,5 min  |              | 7 | 60 | "inactivated" | 100                       |
|                              | Heating | 6 hours  |              |   | 60 | "inactivated" | 20, Dixon<br>(pers. com.) |
| 7,5 x 10 <sup>6</sup> cfu/ml | Heating | < 30 sec |              | 7 | 65 | "inactivated" | 100                       |

#### Photobacterium damselae

| Concentration pathogen | Disinfectant | Contact time | Concentration disinfectant | рН | Temp. | Result   | Method | Reference                 | Comments |
|------------------------|--------------|--------------|----------------------------|----|-------|----------|--------|---------------------------|----------|
|                        | Heating      | 1 hour       |                            |    | 60    | Survival |        | 20, Dixon<br>(pers. com.) |          |

### Renibacterium salmoninarum

| Concentration pathogen   | Disinfectant | Contact<br>time | Concentration disinfectant | рН | Temp. | Result        | Method | Reference                 | Comments |
|--------------------------|--------------|-----------------|----------------------------|----|-------|---------------|--------|---------------------------|----------|
| ≥ 10 <sup>5</sup> cfu/ml | Heating      | > 2 t           |                            | 7  | 40    | "inactivated" |        | 100                       |          |
| ≥ 10 <sup>5</sup> cfu/ml | Heating      | >6 t            |                            | 7  | 45    | "inactivated" |        | 100                       |          |
| $\geq 10^5$ cfu/ml       | Heating      | > 4 hours       |                            | 7  | 50    | "inactivated" |        | 100                       |          |
| $\geq 10^5$ cfu/ml       | Heating      | > 3 hours       |                            | 7  | 55    | "inactivated" |        | 100                       |          |
|                          | Heating      | 1 hour          |                            |    | 60    | "inactivated" |        | 20, Dixon<br>(pers. com.) |          |
| ≥ 10 <sup>5</sup> cfu/ml | Heating      | > 15 min        |                            | 7  | 65    | "inactivated" |        | 100                       |          |

## Streptococcus iniae

| Concentration pathogen | Disinfectant | Contact<br>time | Concentration disinfectant | рН | Temp. | Result        | Method | Reference                 | Comments |
|------------------------|--------------|-----------------|----------------------------|----|-------|---------------|--------|---------------------------|----------|
|                        | Heating      | 5 min           |                            |    | 60    | "inactivated" |        | 20, Dixon<br>(pers. com.) |          |

### Yersinia ruckeri

| Concentrat | on Disinfectant | Contact | Concentration | рН | Temp. | Result | Method | Reference | Comments |
|------------|-----------------|---------|---------------|----|-------|--------|--------|-----------|----------|

| pathogen                     |         | time       | disinfectant |      |    |                                    |  |                           |  |
|------------------------------|---------|------------|--------------|------|----|------------------------------------|--|---------------------------|--|
| 6,6 x 10 <sup>7</sup> CFU/ml | Heating | 1 min      |              |      | 60 | "inactivated"                      |  | 48                        | Test medium: sterilized wastewater from fish slaughterhouse.         |
| 6,5 x 10 <sup>7</sup> CFU/ml | Heating | 2 min      |              |      | 60 | "inactivated"                      |  | 48                        | Test medium:<br>unsterilized wastewater<br>from fish slaughterhouse. |
|                              | Heating | 1 hour     |              |      | 60 | Survival                           |  | 20, Dixon<br>(pers. com.) |  |
| 10 <sup>8,28</sup> cfu/ml    | Heating | 1 min      |              |      | 65 | 5 log reduction                    | Laboratory trial, 1 part<br>process water + 2 parts<br>"bløggevand" from fish<br>slaugtherhouse. | 28                        |  |
| 10 <sup>4,69</sup> cfu/ml    | Heating | 3 min      |              | 8,96 | 65 | Not detectable (≥ 4 log reduction) | Full-scale trial (Norskagfisk),<br>blood water from fish<br>slaugtherhouse.                      | 28                        | Salinity 14-15 ‰   |
| 10 <sup>8,28</sup> cfu/ml    | Heating | 5 min      |              |      | 65 | 7½ log reduction                   | Laboratory trial, 1 part<br>process water + 2 parts<br>"bløggevand" from fish<br>slaugtherhouse. | 28                        |  |
| 10 <sup>5,04</sup> cfu/ml    | Heating | 24 hours   |              |      | 65 | Stable                             | Full-scale trial (Norskagfisk),<br>blood water from fish<br>slaugtherhouse.                      | 28                        |  |
| 10 <sup>4,69</sup> cfu/ml    | Heating | 1 min      |              | 8,96 | 70 | 4 log reduction                    | Full-scale trial (Norskagfisk),<br>blood water from fish<br>slaugtherhouse.                      | 28                        | Salinity 14-15 ‰   |
| 10 <sup>5,04</sup> cfu/ml    | Heating | 24 hours   |              |      | 70 | Stable                             | Full-scale trial (Norskagfisk),<br>blood water from fish<br>slaugtherhouse.                      | 28                        |  |
| 6,6 x 10 <sup>7</sup> CFU/ml | Heating | 15 seconds |              |      | 72 | "inactivated"                      |  | 48                        | Test medium: sterilized wastewater from fish slaughterhouse.         |
| 6,5 x 10 <sup>7</sup> CFU/ml | Heating | 15 sec     |              |      | 72 | "inactivated"                      |  | 48                        | Test medium:<br>unsterilized wastewater<br>from fish slaughterhouse. |
| 10 <sup>4,69</sup> cfu/ml    | Heating | 1 min      |              | 8,96 | 75 | 3½ log reduction                   | Full-scale trial (Norskagfisk),<br>blood water from fish<br>slaugtherhouse.                      | 28                        | Salinity 14-15 ‰   |
| 10 <sup>4,69</sup> cfu/ml    | Heating | 2 min      |              | 8,96 | 75 | Not detectable (≥ 4 log reduction) | Full-scale trial (Norskagfisk),<br>blood water from fish<br>slaugtherhouse.                      | 28                        | Salinity 14-15 ‰   |
| 10 <sup>5,04</sup> cfu/ml    | Heating | 24 hours   |              |      | 75 | Not detectable (> 6 log reduction) | Full-scale trial (Norskagfisk),<br>blood water from fish   | 28                        |  |

|  | slaugthernouse.    |  |
|--|--------------------|--|
|  | Jidagtiici iioasc. |  |

#### **Parasites**

# Gyrodactylus salaris

| Concentration pathogen | Disinfectant | Contact<br>time | Concentration disinfectant | рН | Temp. | Result   | Method              | Reference | Comments |
|------------------------|--------------|-----------------|----------------------------|----|-------|----------|---------------------|-----------|----------|
| 75                     | Temperature  | 60 hours        |                            |    | 3     | All dead | Free, without host. | 80        |          |
| 422                    | Temperature  | 365 hours       |                            |    | 3     | All dead | On dead host.       | 80        |          |
| 88                     | Temperature  | 45 hours        |                            |    | 12    | All dead | Free, without host. | 80        |          |
| 315                    | Temperature  | 142 hours       |                            |    | 12    | All dead | On dead host.       | 80        |          |
| 65                     | Temperature  | 27 hours        |                            |    | 18    | All dead | Free, without host. | 80        |          |
| 204                    | Temperature  | 72 t            |                            |    | 18    | All dead | On dead host.       | 80        |          |

# Myxosoma cerebralis

| Concentration pathogen | Disinfectant | Contact<br>time | Concentration disinfectant | рН | Temp. | Result                       | Method                                | Reference | Comments  |
|------------------------|--------------|-----------------|----------------------------|----|-------|------------------------------|---------------------------------------|-----------|---|
|                        | Temperature  | 7 days          |                            |    | -80   | No infection in tubifex.     | Infectivity of myxospores in tubifex. | 37        | Myxospore suspension                            |
|                        | Temperature  | 2 months        |                            |    | -80   | No infection in tubifex.     | Infectivity of myxospores in tubifex. | 37        | Myxospore in head tissue from fish.             |
|                        | Temperature  | 7 days          |                            |    | -20   | No infection in tubifex.     | Infectivity of myxospores in tubifex. | 37        | Myxospore suspension                            |
|                        | Temperature  | 2 months        |                            |    | -20   | No infection in tubifex.     | Infectivity of myxospores in tubifex. | 37        | Myxospore in head tissue from fish.             |
| 96-192                 | Temperature  | 105 min         |                            |    | -20   | Survival?                    | in vitro. Triactinomyxon spores       | 96        | $1.0 \pm 0.8\%$ probably alive, remaining dead. |
|                        | Temperature  | 2 months        |                            |    | 4     | Active infection in tubifex. | Infectivity of myxospores in tubifex. | 37        | Myxospore in head tissue from fish.             |
|                        | Temperature  | 7 days          |                            |    | 5     | Active infection in tubifex. | Infectivity of myxospores in tubifex. | 37        | Myxospore suspension                            |
| 45-131                 | Temperature  | 105 min         |                            |    | 7     | Alive 31%, dead<br>46%       | in vitro. Triactinomyxon spores       | 96        |   |
| 51-124                 | Temperature  | 60 min          |                            |    | 19-21 | Alive 72%, dead 5%           | in vitro. Triactinomyxon spores       | 96        |   |
|                        | Temperature  | 2 months        |                            |    | 20    | No infection in tubifex.     | Infectivity of myxospores in tubifex. | 37        | Myxospore in head tissue from fish.             |
|                        | Temperature  | 7 days          |                            |    | 22    | Active infection in          | Infectivity of myxospores in          | 37        | Myxospore suspension                            |

|                 |         |        |    | tubifex. | tubifex.   |    |  |
|-----------------|---------|--------|----|----------|--|----|--|
|                 | Heating | 5 min  | 58 | Survival | in vitro. Triactinomyxon spores  | 96 |  |
| 100 spores talt | Heating | 10 min | 70 | 60% dead | In vitro - spores farvet med<br>methylenblå som tegn på<br>død (skal eftervises) | 43 |  |
|                 | Heating | 5 min  | 75 | All dead | in vitro. Triactinomyxon spores  | 96 |  |
| 100 spores talt | Heating | 10 min | 80 | 98% dead | In vitro - spores farvet med<br>methylenblå som tegn på<br>død (skal eftervises) | 43 | 4 trials where all dead, 1 trial where 88% dead. |
| 100 spores talt | Heating | 10 min | 90 | All dead | In vitro - spores farvet med<br>methylenblå som tegn på<br>død (skal eftervises) | 43 |  |

# **Percolation**

It has not been possible to find any references describing the decimating effect of percolating of fish pathogenic viruses. Furthermore it has not been possible to find publications describing the effect of percolating other birnaviruses. It is therefore not possible to validate if this procedure is safe to use.

# Other procedures:

### **Iodine based disinfectants**

#### **Virus**

#### **IHNV**

| Concentration pathogen                         | Disinfectant   | Contact time   | Concentration disinfectant    | рН | Temp. | Result                      | Method           | Reference | Comments                         |
|--|----------------|----------------|-------------------------------|----|-------|-----------------------------|------------------|-----------|----------------------------------|
| 10 <sup>4,53</sup> - 10 <sup>5,18</sup> pfu/ml | Povidon iodine | 7,5<br>seconds | 0,10 mg/l free<br>iodide      |    |       | 99,49 → 99,99%<br>reduction | Distilled water. | 11        | 6 isolates representing 5 types. |
| 10 <sup>4,62</sup> pfu/ml                      | Povidon iodine | 5 min          | 0,4 mg/l residual free iodide | 6  |       | > 99,99% reduction          |                  | 11        | Testing of effect of pH.         |
| 10 <sup>4,66</sup> pfu/ml                      | Povidon iodine | 5 min          | 0,4 mg/l residual free iodide | 7  |       | > 99,99% reduction          |                  | 11        | Testing of effect of pH.         |
| 10 <sup>4,74</sup> pfu/ml                      | Povidon iodine | 5 min          | 0,4 mg/l residual             | 8  |       | 99,89% reduction            |                  | 11        | Testing of effect of pH.         |

|  |                |         | free iodide                      |     |   |   |    |                                   |
|--|----------------|---------|----------------------------------|-----|---|---|----|-----------------------------------|
| 10 <sup>4,71</sup> pfu/ml                      | Povidon iodine | 5 min   | 0,4 mg/l residual free iodide    | 9   | 90,78% reduction                          |   | 11 | Testing of effect of pH.          |
|  | Povidon iodine | 5 min   | 0,4 mg/l residual free iodide    |     | > 4 log reduction for alle saliniteter    | Natural seawater containg 0, 4, 7½, 15½ and 32 ‰ salte.                       | 11 | Effect of BSA (dirty conditions). |
| 10 <sup>5,04</sup> pfu/ml                      | Povidon iodine | 5 min   | 0,4 mg/l residual<br>free iodide |     | > 4 log reduction for alle saliniteter    | Dirty conditions: iodide + calf<br>serum(0,002%) mixed before<br>virus added  | 11 | Effect of BSA (dirty conditions). |
| 10 <sup>5,04</sup> pfu/ml                      | Povidon iodine | 5 min   | 0,4 mg/l residual<br>free iodide |     | Stable                                    | Dirty conditions: iodide + calf<br>serum(0,016%) mixed before<br>virus added  | 11 | Effect of BSA (dirty conditions). |
| 10 <sup>4,81</sup> pfu/ml                      | Povidon iodine | 5 min   | 0,4 mg/l residual<br>free iodide |     | > 4 log reduction<br>for alle saliniteter | Dirty conditions: virus + calf<br>serum (0,002%) mixed<br>before iodide added | 11 | Effect of BSA (dirty conditions). |
| 10 <sup>4,81</sup> pfu/ml                      | Povidon iodine | 5 min   | 0,4 mg/l residual<br>free iodide |     | 99,96% reduction                          | Dirty conditions: virus + calf<br>serum (0,016%) mixed<br>before iodide added | 11 | Effect of BSA (dirty conditions). |
| 10 <sup>4,81</sup> pfu/ml                      | Povidon iodine | 5 min   | 0,4 mg/l residual<br>free iodide |     | 66,67% reduction                          | Dirty conditions: virus + calf<br>serum (0,064%) mixed<br>before iodide added | 11 | Effect of BSA (dirty conditions). |
| 10 <sup>4,41</sup> - 10 <sup>4,91</sup> pfu/ml | Povidon iodine | 7,5 sec | 0,4 mg/l residual<br>free iodide |     | Not detectable til<br>Stable              | Natural water from 8<br>different sources (fresh +<br>salt)                   | 11 |                                   |
| 10 <sup>4,41</sup> - 10 <sup>4,91</sup> pfu/ml | Povidon iodine | 7,5 sec | 0,8 mg/l residual<br>free iodide |     | ≥ 4 log reduction                         | Natural water from 8<br>different sources (fresh +<br>salt)                   | 11 |                                   |
|  | Iodophor       | 5 min   | 8 ppm                            | 6,0 | "inactivated"                             |   | 8  | 1 ppm = 1 mg/l                    |
|  | Iodophor       | 30 sec  | 12 ppm                           | 7,0 | "inactivated"                             |   | 8  |                                   |
|  | Iodophor       | 15 sec  | 25 ppm                           | 7,0 | "inactivated"                             |   | 8  |                                   |
|  | Iodophor       | 5 min   | 32 ppm                           | 8,6 | "inactivated"                             |   | 8  |                                   |
| 10 <sup>6</sup> PFU/ml                         | Iodophor       | 10 min  | 100 mg/l                         |     | > 3 log reduction                         | Green eggs and eyed eggs<br>treated in 10 or 60 min                           | 31 |                                   |

Conclusion: Under laboratory conditions IHNV is sensitive to disinfection with iodine. The higher the pH and the more organic waste the more iodine is needen to disinfect the same amount of virus. Recommended dose: 100 ppm, 10 min contact time.

#### **IPNV**

| Concentration pathogen                    | Disinfectant   | Contact<br>time | Concentration disinfectant | рН | Temp. | Result            | Method  | Reference | Comments |
|---|----------------|-----------------|----------------------------|----|-------|-------------------|---|-----------|----------|
| 10 <sup>7,41</sup> TCID <sub>50</sub> /ml | lodophor, acid | 30 min          | 0,033% (v/v)               |    | 4     | > 4 log reduction | Tested at 0,0055, 0,011,<br>0,022,0,033, 0,044 and<br>0,055%. Hard water, Dirty | 49        |          |

| 10 <sup>3,9</sup> TCID <sub>50</sub> /ml   lodophor   5 min   4 mg/l residual   21   "inactivated"   Testet using ½, 1,2, 4, 8, 16, 32 and 64 mg/l residual. Distilled water.   23   IPNV: Serot lodophor:   10 <sup>3,8</sup> TCID <sub>50</sub> /ml   lodophor   15 seconds   12 mg/l residual   21   "inactivated"   Tested at time 0, 15, 30, 60, 120 s. Distilled water.   23   IPNV: Serot lodophor:   10 <sup>3,9</sup> TCID <sub>50</sub> /ml   lodophor   5 min   16 mg/l residual   6-8,6   21   "inactivated"   Testet using ½, 1,2, 4, 8, 16, 32 and 64 mg/l residual. Distilled water.   23   IPNV: Serot lodophor:   10 <sup>3,9</sup> TCID <sub>50</sub> /ml   lodophor   5 min   30 ppm iodine   room temp.   "inactivated"   PBS   17   Wescot lodophor:   10 <sup>6,6</sup> TCID <sub>50</sub> /ml   lodophor   5 min   32 ppm   6,9   "inactivated"   PBS   17   Wescot lodophor:   10 <sup>6,6</sup> TCID <sub>50</sub> /ml   lodophor   3 min   45 ppm iodine   room temp.   Survival   PBS   17   Wescot lodophor:   10 <sup>5,2</sup> TCID <sub>50</sub> /ml   Actomar   5 min   0,01%   > 4 log reduction   5   Active iodid 150 and 200 Time 2, 4, 5, 5   and 30 min and 30 | Betadine<br>type Buhl.<br>Betadine<br>type Buhl.                  |
|--|---|
| 10 so/ml lodophor seconds 12 mg/l residual 21 mactivated 120 s. Distilled water. 23 lodophor:  10 so/ml lodophor 5 min 16 mg/l residual 6-8,6 21 "inactivated" Testet using ½, 1, 2, 4, 8, 16, 32 and 64 mg/l residual. Distilled water. 23 lodophor:  10 so/ml lodophor 5 min 30 ppm iodine room temp. "inactivated" PBS 17 Wesco 10 lodophor:  10 so/ml lodophor 5 min 32 ppm 6,9 "inactivated" PBS 17 Wesco 10 lodophor 10 lodophor 5 min 35 ppm iodine room temp. "inactivated" PBS 17 Wesco 10 lodophor 10 lo   | Betadine<br>type Buhl.  |
| 10 <sup>3,9</sup> TCID <sub>50</sub> /ml         lodophor         5 min         16 mg/l residual         6-8,6         21         "inactivated"         32 and 64 mg/l residual. Distilled water.         23         IPNV: Serot lodophor: Indophor: Indophor  |   |
| 10°50 ms         lodophor         5 min         30 ppm iodine         temp.         "inactivated"         PBS         17         Wesco           10°50 TCID <sub>50</sub> /ml         lodophor         5 min         35 ppm iodine         room temp.         "inactivated"         PBS         17         Wesco           10°50 TCID <sub>50</sub> /ml         lodophor         3 min         45 ppm iodine         room temp.         Survival         PBS         17         Wesco           Actomar         5 min         0,01%         > 4 log reduction         5           10°5.2 TCID <sub>50</sub> /ml         Actomar         20 min         50 ppm         "inactivated"         Without serum         6         Active iodid<br>150 and 200<br>Time 2, 4, 5,   |   |
| 10 <sup>6,6</sup> TCID <sub>50</sub> /ml         lodophor         5 min         35 ppm iodine         room temp.         "inactivated"         PBS         17         Wesco           10 <sup>6,6</sup> TCID <sub>50</sub> /ml         lodophor         3 min         45 ppm iodine         room temp.         Survival         PBS         17         Wesco           Actomar         5 min         0,01%         > 4 log reduction         5           10 <sup>5,2</sup> TCID <sub>50</sub> /ml         Actomar         20 min         50 ppm         "inactivated"         Without serum         6         Active iodid 150 and 200 Time 2, 4, 5,   | odyne   |
| 10% TCID <sub>50</sub> /ml lodophor 5 min 35 ppm iodine temp. "inactivated" PBS 17 Wesco 106,6 TCID <sub>50</sub> /ml lodophor 3 min 45 ppm iodine room temp. Survival PBS 17 Wesco 5 17 Survival PBS 17 Wesco 5 18 PBS 17 Wesco 19 PBS 17 Wesco 19 PBS  |   |
| 10 <sup>5/2</sup> TCID <sub>50</sub> /ml   Actomar   20 min   50 ppm   50 ppm   50 ppm   70 min   50 ppm   70 min   50 ppm   70 min   50 ppm   70 min   70 mi  | odyne   |
| 10 <sup>5,2</sup> TCID <sub>50</sub> /ml Actomar 20 min 50 ppm "inactivated" Without serum 6 Active iodid 150 and 200 Time 2, 4, 5,  | odyne   |
| 10 <sup>5,2</sup> TCID <sub>50</sub> /ml Actomar 20 min 50 ppm "inactivated" Without serum 6 150 and 200 Time 2, 4, 5,   |   |
|  | ppm testet.<br>6, 8, 10, 20                                       |
| 10 <sup>5,2</sup> TCID <sub>50</sub> /ml Actomar 2 min → 50 ppm 3 log reduction With 5 % serum 6 Active iodid 150 and 200 Time 2, 4, 5, and 30 mi  | ppm testet.<br>6, 8, 10, 20                                       |
| 10 <sup>5,8 TCID</sup> <sub>50</sub> /ml Actomar 6 min 150 ppm 3 log reduction With 5 % serum 37 Active iodid 150 and 200 Time 2, 4, 5, and 30 mi  | ppm testet.<br>6, 8, 10, 20                                       |
| 10 <sup>5,8 TCID</sup> <sub>50</sub> /ml Actomar 20 min 150 ppm "inactivated" With 5 % serum 6 Active iodid 150 and 200 Time 2, 4, 5, and 30 mi  | I- FO 400   |
| iodophor 5 min 80-100 ppm 5 > 99,9% Iodophor added virus 24 FAM (acid i  | ppm testet.<br>6, 8, 10, 20                                       |
| iodophor 5 min 80-100 ppm 5 > 99,9% Iodophor added virus 24 Buffodine iodop  | ppm testet.<br>6, 8, 10, 20<br>n tested.                          |
| iodophor 5 min 80-100 ppm 5 90% Eggs before hardening, surface infected with virus 24 iodop  | ppm testet.<br>6, 8, 10, 20<br>n tested.<br>iodophor)<br>(neutral |

Conclusion: Under laboratory conditions IPNV is sensitive for iodine. When conditions are dirty more iodine is needed. Recommended dose for 3 log reduction: 150 ppm, 10 min contact time.

#### **ISAV**

| Concentration pathogen | Disinfectant | Contact<br>time | Concentration disinfectant | рН | Temp. | Result              | Method  | Reference | Comments  |
|------------------------|--------------|-----------------|----------------------------|----|-------|---------------------|---|-----------|-----------|
| 5,1 log10 ffu/ml       | Iodophor     | 5 min           | 50 ppm                     |    | 4     | > 4,8 log reduction | Hard water, 342 ppm total<br>hardness Testet with 50, 100<br>and 200 ppm.                                 | 89        | Buffodine |
| 5,1 log10 ffu/ml       | lodine       | 5 min           | 100 ppm                    |    | 4     | > 4,8 log reduction | Hard water, 342 ppm total hardness, with and without addition of serum.                                   | 89        | Tegodyne  |
| 5,5 log10 ffu/ml       | lodine       | 5 min           | 100 ppm                    |    | 4     | > 5,2 log reduction | Hard water, 342 ppm total hardness, with and without addition of serum. Testet with 100, 200 and 400 ppm. | 89        | FAM 30    |

Conclusion: Under laboratory conditions ISAV is sensitive for iodine. Recommended dose: 100 ppm, 5 min contact time.

# **VHSV**

| Concentration pathogen                   | Disinfectant | Contact<br>time | Concentration disinfectant | рН  | Temp. | Result          | Method  | Reference | Comments  |
|--|--------------|-----------------|----------------------------|-----|-------|-----------------|---|-----------|---|
|  | Iodophor     | 5 min           | 8 ppm                      | 6,9 |       | "inactivated"   |   | 8         |   |
| 10 <sup>7</sup> TCID <sub>50</sub> /ml   | Iodophor     | 1 min           | 50 ppm                     |     | 15    | Not detectable  | Diluted in PBS. 1% fetal calf serum.                  | 65        | Isolat JF001Ehi1.<br>Dilution scale 1:50.<br>Isodine.                               |
| 10 <sup>7</sup> TCID <sub>50</sub> /ml   | Iodophor     | 1 min           | 50 ppm                     |     | 15    | Not detectable  | Fortyndet i kunstig havvand.<br>1% føtalt calf serum. | 65        | Isolat JF001Ehi1.<br>Dilution scale 1:50.<br>Isodine.                               |
| 10 <sup>5,8 TCID</sup> <sub>50</sub> /ml | Actomar      | 8 min →         | 50 ppm                     |     |       | 3 log reduction | With 5 % serum  | 6         | Active iodide, 50 and 100 ppm tested. Time 2, 4, 5, 6, 8, 10, 20 and 30 min tested. |
| 10 <sup>5,5 TCID</sup> <sub>50</sub> /ml | Actomar      | 4 min           | 50 ppm                     |     |       | "inactivated"   | Without serum   | 6         | Active iodide, 50 and 100 ppm tested. Time 2, 4, 5, 6, 8, 10, 20 and 30 min tested. |
|  | Actomar      | 5 min           | 100 ppm                    |     |       | "inactivated"   |   | 5         | Author claim 100% reduction.  |
| 10 <sup>6,5 TCID</sup> <sub>50</sub> /ml | Actomar      | 2 min           | 100 ppm                    |     |       | "inactivated"   | With 5 % serum  | 6         | Active iodide, 50 and 100 ppm tested. Time 2, 4, 5, 6, 8, 10, 20 and 30 min tested. |
| 10 <sup>8</sup> pfu/ml                   | Iodophor     | 10 min          | 100 ppm                    |     |       | Not detectable  | fiskeæg added virus                                   | 95        | genotype IVb  |
|  | iodophor     | 5 min           | 80-100 ppm                 |     | 5     | > 99,9%         | Iodophor added virus                                  | 24        | Buffodine (neutral  |

|          |       |            |   |          |  |    | iodophor)                    |
|----------|-------|------------|---|----------|--|----|------------------------------|
| iodophor | 5 min | 80-100 ppm | 5 | > 99,99% | Nystrøgne, Not hærdede æg<br>overflade inficeret med virus | 24 | Buffodine (neutral iodophor) |
| iodophor | 5 min | 80-100 ppm | 5 | > 99,9%  | Iodophor added virus                                       | 24 | FAM (acid iodophor)          |

Conclusion: lodophores can inactivate VHSV on fish eggs using a dose of 100 ppm and a contact time of 10 min. The results indicate that iodine will be used under dirty conditions minimizing the disinfecting effect. Recommended dose: 100 ppm, 10 min.

# SVCV

| Concentration pathogen                   | Disinfectant | Contact<br>time       | Concentration disinfectant | рН | Temp. | Result          | Method         | Reference | Comments  |
|--|--------------|-----------------------|----------------------------|----|-------|-----------------|----------------|-----------|---|
|  | Actomar      | 5 min                 | 100 ppm                    |    |       | 99 % reduction  |                | 5         |   |
| 10 <sup>7,2</sup> TCID <sub>50</sub> /ml | Actomar      | 10 min                | 100 ppm                    |    |       | "inactivated"   | Without serum  | 6         | Active iodide 100, 150<br>and 200 ppm tested.<br>Time 2, 4, 5, 6, 8, 10, 20<br>and 30 min tested. |
| 10 <sup>7,5 TCID</sup> <sub>50</sub> /ml | Actomar      | 2 min og<br>fremefter | 100 ppm                    |    |       | 2 log reduction | With 5 % serum | 6         | Active iodide 100, 150<br>and 200 ppm tested.<br>Time 2, 4, 5, 6, 8, 10, 20<br>and 30 min tested. |
| 10 <sup>7,8 TCID</sup> <sub>50</sub> /ml | Actomar      | 4 min                 | 150 ppm                    |    |       | 3 log reduction | With 5 % serum | 6         | Active iodide 100, 150<br>and 200 ppm tested.<br>Time 2, 4, 5, 6, 8, 10, 20<br>and 30 min tested. |
| 10 <sup>7,5 TCID</sup> <sub>50</sub> /ml | Actomar      | 10 min                | 200 ppm                    |    |       | "inactivated"   | With 5 % serum | 6         | Active iodide 100, 150<br>and 200 ppm tested.<br>Time 2, 4, 5, 6, 8, 10, 20<br>and 30 min tested. |

Conclusion: The results indicates that a dose of 100 ppm wil be used under dirty conditions rendering an acceptable disinfection impossible. Recommended dose: 200 ppm, 10 min.

#### **PFRV**

| Concentration pathogen                   | Disinfectant | Contact<br>time       | Concentration disinfectant | рН | Temp. | Result            | Method         | Reference | Comments  |
|--|--------------|-----------------------|----------------------------|----|-------|-------------------|----------------|-----------|---|
|  | Actomar      | 5 min                 | 100 ppm                    |    |       | 99,99 % reduction |                | 5         |   |
| 10 <sup>7,5 TCID</sup> <sub>50</sub> /ml | Actomar      | 4 min                 | 100 ppm                    |    |       | "inactivated"     | Without serum  | 6         | Active iodide 100, 150<br>and 200 ppm tested.<br>Time 2, 4, 5, 6, 8, 10, 20<br>and 30 min tested. |
| 10 <sup>7,5 TCID</sup> <sub>50</sub> /ml | Actomar      | 4 min og<br>fremefter | 100 ppm                    |    |       | 6 log reduction   | With 5 % serum | 6         | Active iodide 100, 150 and 200 ppm tested.  |

|  |         |        |         |  |               |                |   | Time 2, 4, 5, 6, 8, 10, 20<br>and 30 min tested.  |
|--|---------|--------|---------|--|---------------|----------------|---|---|
| 10 <sup>7,2</sup> TCID <sub>50</sub> /ml | Actomar | 10 min | 150 ppm |  | "inactivated" | With 5 % serum | 6 | Active iodide 100, 150<br>and 200 ppm tested.<br>Time 2, 4, 5, 6, 8, 10, 20<br>and 30 min tested. |

Conclusion: The results indicate that a dose of 100 ppm will be used under dirty conditions, but the obtained reduction was still satisfactory.

#### KHV

| Concentration pathogen           | Disinfectant | Contact<br>time | Concentration disinfectant | рН | Temp. | Result         | Method  | Reference | Comments  |
|----------------------------------|--------------|-----------------|----------------------------|----|-------|----------------|---|-----------|---|
| 1 - 1,5 x 10 <sup>4</sup> PFU/ml | lodophor     | 30<br>seconds   | 130 mg/l                   |    | 0     | Not detectable | Virus and disinfectant mixed 1:1, tested after 30 sec and 20 min. Diluted 1:10 using L15 medium and 200 µl inoculated . | 55        | Strain KHV-I. The method cannot detect a 3 log reduction. |
| 1 - 1,5 x 10 <sup>4</sup> PFU/ml | lodophor     | 30<br>seconds   | 200 mg/l                   |    | 25    | Not detectable | testet ved 30 sec og 20 min.  | 55        | Strain KHV-I. The method cannot detect a 3 log reduction. |

Conclusion: Although the method used is not capable of detecting a 3 log reduction the results indicate the KHV is sensitive to disinfection using iodophores.

#### **Nodavirus**

| Concentration pathogen                     | Disinfectant          | Contact<br>time | Concentration disinfectant | рН | Temp. | Result  | Method   | Reference | Comments                       |
|--|-----------------------|-----------------|----------------------------|----|-------|---|--|-----------|--------------------------------|
|  | Jod                   | 15 min          | 40 mg/l                    |    |       | Not inactivated (all<br>larvae dead after<br>15 days) | Washing of eggs from noda infected ovaries in seawater, followed after hatching.                                     | 9         | Isolate: SJNNV                 |
| 10 μg purified virus                       | Jod                   | 10 min          | 50 mg/ml                   |    | 20    | "Effective"   | Diluted in 1 ml PBS. Used for infection trial in day old striped jack larvae. Concentration testet: 2,5 - 100 mg/ml. | 9         | Isolate: SJNNV                 |
| 10 <sup>6,125</sup> TCID <sub>50</sub> /ml | iodophor,<br>buffered | 5 min           | 25 ppm I <sub>2</sub>      |    | 15    | Not detectable  | Distilled water. Tested after 5,<br>15 and 30 min.   | 30        | Isolate: sea bass<br>nodavirus |
| 10 <sup>6,125</sup> TCID <sub>50</sub> /ml | iodophor,<br>buffered | 30 min          | 100 ppm I <sub>2</sub>     |    | 15    | 4½ log reduction                                      | HBSS + calf serum. Tested after 5, 15 and 30 min.  | 30        | Isolate: sea bass<br>nodavirus |

Conclusion: Under laboratory conditions nodavirus is sensitive to disinfection with iodine products but it seems that nodavirus is a bit more resistant than VHSV and IHNV. Recommended concentration: 100 ppm, 30 min.

# Oncorhynchus masou virus

| Concentration pathogen | Disinfectant | Contact<br>time | Concentration disinfectant | рН | Temp. | Result        | Method | Reference | Comments |
|------------------------|--------------|-----------------|----------------------------|----|-------|---------------|--------|-----------|----------|
|                        | Iodophor     | 30 sec          | 40 ppm                     |    | 0     | "inactivated" |        | 35        |          |
|                        | Iodophor     | 30 sec          | 40 ppm                     |    | 25    | "inactivated" |        | 35        |          |

#### Bacteria

# Aeromonas liquefaciens

| Concentration pathogen  | Disinfectant   | Contact<br>time | Concentration disinfectant | рН | Temp. | Result                             | Method  | Reference | Comments  |
|-------------------------|----------------|-----------------|----------------------------|----|-------|------------------------------------|---|-----------|-----------|
| 1 x 10 <sup>7</sup> /ml | Iodophor       | 30 sec          | 25 ppm                     | 7  | 10-13 | > 5 log reduction                  | Distilled water. 4 strains tested. Time: 15, 30, 60, 120 and 300 seconds.       | 84        | Wescodyne |
| 1 x 10 <sup>7</sup> /ml | Iodophor       | 120 sec         | 25 ppm                     | 7  | 10-13 | Not detectable (> 7 log reduction) | Distilled water. 4 strains<br>tested. Time: 15, 30, 60, 120<br>and 300 seconds. | 84        | Wescodyne |
| 1 x 10 <sup>6</sup> /ml | Iodophor       | 300 sec         | 25 ppm                     | 8  | 10-13 | > 5 log reduction                  | Distilled water. 1 strain<br>tested. Time: 15, 30, 60, 120<br>and 300 seconds.  | 84        | Wescodyne |
| 1 x 10 <sup>7</sup> /ml | Povidon iodine | 15 sec          | 25 ppm                     | 7  | 10-13 | ≥ 6 log reduction                  | Distilled water. 4 strains<br>tested. Time: 15, 30, 60, 120<br>and 300 seconds. | 84        | Betadine  |
| 1 x 10 <sup>7</sup> /ml | Povidon iodine | 120 sec         | 25 ppm                     | 7  | 10-13 | Not detectable (> 7 log reduction) | Distilled water. 4 strains<br>tested. Time: 15, 30, 60, 120<br>and 300 seconds. | 84        | Betadine  |
| 1 x 10 <sup>7</sup> /ml | Povidon iodine | 300 sec         | 25 ppm                     | 8  | 10-13 | > 5 log reduction                  | Distilled water. 1 strain<br>tested. Time: 15, 30, 60, 120<br>and 300 seconds.  | 84        | Betadine  |

Conclusion: Under clean conditions 25 ppm for a few minutes will provide an aceptable reduction.

#### Aeromonas salmonicida

| Concentration pathogen | Disinfectant   | Contact<br>time | Concentration disinfectant | рН  | Temp. | Result           | Method   | Reference | Comments                 |
|------------------------|----------------|-----------------|----------------------------|-----|-------|------------------|--|-----------|--------------------------|
| 10 <sup>7</sup> CFU/ml | l <sub>2</sub> |                 | 1,0 mg/l added             | 7,2 | 7     | 99,9% reduction  | PBS  | 71        | Reduction rate: 0,21/sec |
| 10 <sup>7</sup> CFU/ml | l <sub>2</sub> | 36 sec          | 1 mg/l added               | 7,2 | 7     | 99,9 % reduction | PBS  | 71        |                          |
| 10 <sup>7</sup> CFU/ml | I <sub>2</sub> |                 | 1,3 mg/l added             | 7,8 | 7     | 99,9% reduction  | Wastewater from aquaculture (15,7 % salinity). | 71        | Reduction rate: 0,14/sec |
| 10 <sup>7</sup> CFU/ml | l <sub>2</sub> | 1 min           | 1,3 mg/l added             | 7,8 | 7     | 3½ log reduction | Wastewater from aquaculture                    | 71        | Read off a graph.        |

|                           |                |         |                |     |       |   | (15,7 ‰ salinity).  |    |  |
|---------------------------|----------------|---------|----------------|-----|-------|---|---|----|--|
| 10 <sup>7</sup> CFU/ml    | l <sub>2</sub> |         | 2,6 mg/l added | 7,8 | 7     | 99,9% reduction                         | Wastewater from aquaculture (15,7 % salinity).  | 71 | Reduction rate: 0,26/sec                       |
| 10 <sup>7</sup> CFU/ml    | I <sub>2</sub> | 40 sec  | 2,6 mg/l added | 7,8 | 7     | 4½ log reduction                        | Wastewater from aquaculture (15,7 % salinity).  | 71 | Read off a graph.                              |
|                           | lodophor, acid | 30 min  | 0,28 % (v/v)   |     | 4     | > 5 log reduction                       | Tested at 0,16, 0,2, 0,28, 0,4, 0,8, 1, 1,6, 2 and 3,2%. Hard water, high organic load. | 49 |  |
| 2,6 x 10 <sup>7</sup> /ml | lodophor       | 15 sec  | 25 ppm         | 7   | 10-13 | 6 log reduction                         | Distilled water. 4 strains tested. Time: 15, 30, 60, 120 and 300 seconds.               | 84 | Wescodyne                                      |
| 4 x 10 <sup>7</sup> /ml   | lodophor       | 60 sec  | 25 ppm         | 8   | 10-13 | Not detectable<br>(> 7 log reduction)   | Distilled water. 1 strain tested. Time: 15, 30, 60, 120 and 300 seconds.                | 84 | Wescodyne                                      |
| 2,6 x 10 <sup>7</sup> /ml | lodophor       | 120 sec | 25 ppm         | 7   | 10-13 | Not detectable<br>(> 7 log reduction)   | Distilled water. 4 strains tested. Time: 15, 30, 60, 120 and 300 seconds.               | 84 | Wescodyne                                      |
| 10 <sup>5</sup> cfu/ml    | Povidon iodine | 3 min   | 10 ppm         |     | 20    | > 4 log reduction                       |   | 85 | Concentration of commercial product. (Isodine) |
| 10 <sup>8</sup> cfu/ml    | Povidon iodine | 5 min   | 5 ppm          |     | 20    | Stable                                  | Test of effect of bacteria titer.   | 85 | Concentration of commercial product. (Isodine) |
| 10 <sup>6</sup> cfu/ml    | Povidon iodine | 5 min   | 5 ppm          |     | 20    | > 3 log reduction                       | Test of effect of bacteria titer.   | 85 | Concentration of commercial product. (Isodine) |
| 10 <sup>4</sup> cfu/ml    | Povidon iodine | 1 min   | 5 ppm          |     | 20    | > 3 log reduction                       | Test of effect of bacteria titer.   | 85 | Concentration of commercial product. (Isodine) |
| 10 <sup>5,5</sup> cfu/ml  | Povidon iodine | 4 min   | 5 ppm          |     | 20    | Stable                                  | 10 ppm calf serum added.  | 85 |  |
| 10 <sup>5,5</sup> cfu/ml  | Povidon iodine | 4 min   | 5 ppm          |     | 20    | 4½ log reduction                        | 0 ppm calf serum added.   | 85 |  |
| 10 <sup>5,5</sup> cfu/ml  | Povidon iodine | 3 min   | 5 ppm          |     | 5     | 3½ log reduction                        |   | 85 | Test of effect of<br>Temperature.              |
| 10 <sup>5,5</sup> cfu/ml  | Povidon iodine | 3 min   | 5 ppm          |     | 25    | 4½ log reduction                        |   | 85 | Test of effect of<br>Temperature.              |
| 10 <sup>5,5</sup> cfu/ml  | Povidon iodine | 5 min   | 5 ppm          |     | 15    | Stable                                  | Artificial sea water.   | 85 |  |
| 10 <sup>5,5</sup> cfu/ml  | Povidon iodine | 1-3 min | 5 ppm          |     | 15    | 3½ - 4 log<br>reduction                 | Hard water (300 ppm CaCO <sub>3</sub> )   | 85 |  |
| 10 <sup>5,5</sup> cfu/ml  | Povidon iodine | 1-3 min | 5 ppm          |     | 15    | 3½ - 4 log<br>reduction                 | Distilled water.  | 85 |  |
| 1 x 10 <sup>7</sup> /ml   | Povidon iodine | 15 sec  | 25 ppm         | 7   | 10-13 | > 5 log reduction<br>for 3 of 4 strains | Distilled water. 4 strains tested. Time: 15, 30, 60, 120                                | 84 | Betadine                                       |

|                         |                |         |        |   |       |                   | and 300 seconds.  |    |  |
|-------------------------|----------------|---------|--------|---|-------|-------------------|---|----|--|
| 1 x 10 <sup>7</sup> /ml | Povidon iodine | 300 sec | 25 ppm | 7 | 10-13 | Not detectable    | Distilled water. 4 strains<br>tested. Time: 15, 30, 60, 120<br>and 300 seconds. | 84 | Betadine                                       |
| 4 x 10 <sup>7</sup> /ml | Povidon iodine | 300 sec | 25 ppm | 7 | 10-13 | Not detectable    | Distilled water. 1 strain<br>tested. Time: 15, 30, 60, 120<br>and 300 seconds.  | 84 | Betadine                                       |
| 10 <sup>5</sup> cfu/ml  | Povidon iodine | 1 min   | 30 ppm |   | 20    | > 4 log reduction |   | 85 | Concentration of commercial product. (Isodine) |

Conclusion: The lower the Temperature, the highter the titer of the pathogen, the more organic dirt, the worse the obtaine disinfection. The results indicate that a dose of 25 ppm for 5 min will provide  $\geq$  3 log reduction.

# Carnobacterium piscicola

| Concentration pathogen | Disinfectant   | Contact<br>time | Concentration disinfectant | рН | Temp. | Result            | Method  | Reference | Comments |
|------------------------|----------------|-----------------|----------------------------|----|-------|-------------------|---|-----------|----------|
|                        | lodophor, acid | 30 min          | 0,4 - 1% (v/v)             |    | 4     | > 5 log reduction | Tested at 0,16, 0,2, 0,28, 0,4, 0,8, 1, 1,6, 2 and 3,2%. Hard water, high organic load. | 49        |          |

#### Flexibacter columnaris

| Concentration pathogen    | Disinfectant   | Contact<br>time | Concentration disinfectant | рН | Temp. | Result                             | Method  | Reference | Comments  |
|---------------------------|----------------|-----------------|----------------------------|----|-------|------------------------------------|---|-----------|-----------|
| 1,7 x 10 <sup>6</sup> /ml | Iodophor       | 15 sec          | 25 ppm                     | 7  | 10-13 | > 5 log reduction                  | Distilled water. 4 strains<br>tested. Time: 15, 30, 60, 120<br>and 300 seconds. | 84        | Wescodyne |
| 1,7 x 10 <sup>6</sup> /ml | Iodophor       | 300 sec         | 25 ppm                     | 7  | 10-13 | Not detectable (> 6 log reduction) | Distilled water. 4 strains<br>tested. Time: 15, 30, 60, 120<br>and 300 seconds. | 84        | Wescodyne |
| 4 x 10 <sup>4</sup> /ml   | Iodophor       | 30 sec          | 25 ppm                     | 8  | 10-13 | Not detectable (> 3 log reduction) | Distilled water. 1 strain<br>tested. Time: 15, 30, 60, 120<br>and 300 seconds.  | 84        | Wescodyne |
| 2 x 10 <sup>6</sup> /ml   | Povidon iodine | 15 sec          | 25 ppm                     | 7  | 10-13 | Not detectable (> 6 log reduction) | Distilled water. 4 strains<br>tested. Time: 15, 30, 60, 120<br>and 300 seconds. | 84        | Betadine  |
| 2 x 10 <sup>6</sup> /ml   | Povidon iodine | 120 sec         | 25 ppm                     | 7  | 10-13 | Not detectable (≥ 6 log reduction) | Distilled water. 4 strains<br>tested. Time: 15, 30, 60, 120<br>and 300 seconds. | 84        | Betadine  |
| 4 x 10 <sup>4</sup> /ml   | Povidon iodine | 120 sec         | 25 ppm                     | 8  | 10-13 | Not detectable (> 3                | Distilled water. 1 strain   | 84        | Betadine  |

|  |  |  | log reduction) | tested. Time: 15, 30, 60, 120 |   |
|--|--|--|----------------|-------------------------------|---|
|  |  |  |                | and 300 seconds.              | 1 |

Conclusion: Under clean conditions 25 ppm for 5 min will inactivate the bacteria.

# Cytophaga psychrophila (Flavabacterium psychrophilum)

| Concentration pathogen    | Disinfectant   | Contact<br>time | Concentration disinfectant | рН | Temp. | Result                             | Method  | Reference | Comments  |
|---------------------------|----------------|-----------------|----------------------------|----|-------|------------------------------------|---|-----------|-----------|
| 1,4 x 10 <sup>6</sup> /ml | Iodophor       | 15 sec          | 25 ppm                     | 7  | 10-13 | > 5 log reduction                  | Distilled water. 4 strains<br>tested. Time: 15, 30, 60, 120<br>and 300 seconds. | 84        | Wescodyne |
| 1,4 x 10 <sup>6</sup> /ml | Iodophor       | 60 sec          | 25 ppm                     | 7  | 10-13 | Not detectable (> 6 log reduction) | Distilled water. 4 strains<br>tested. Time: 15, 30, 60, 120<br>and 300 seconds. | 84        | Wescodyne |
| 5 x 10 <sup>3</sup> /ml   | Iodophor       | 15 sec          | 25 ppm                     | 8  | 10-13 | Not detectable (> 3 log reduction) | Distilled water. 1 strain<br>tested. Time: 15, 30, 60, 120<br>and 300 seconds.  | 84        | Wescodyne |
| 1,4 x 10 <sup>6</sup> /ml | Povidon iodine | 15 sec          | 25 ppm                     | 7  | 10-13 | 5 log reduction                    | Distilled water. 4 strains<br>tested. Time: 15, 30, 60, 120<br>and 300 seconds. | 84        | Betadine  |
| 1,4 x 10 <sup>6</sup> /ml | Povidon iodine | 30 sec          | 25 ppm                     | 7  | 10-13 | ≥ 6 log reduction                  | Distilled water. 4 strains<br>tested. Time: 15, 30, 60, 120<br>and 300 seconds. | 84        | Betadine  |
| 5 x 10 <sup>3</sup> /ml   | Povidon iodine | 30 sec          | 25 ppm                     | 8  | 10-13 | Not detectable (> 3 log reduction) | Distilled water. 1 strain<br>tested. Time: 15, 30, 60, 120<br>and 300 seconds.  | 84        | Betadine  |

Conclusion: Under clean conditions 25 ppm for 5 min will inactivate the bacteria.

# Edwardsiella tarda

| Concentration pathogen | Disinfectant   | Contact<br>time | Concentration disinfectant | рН | Temp. | Result          | Method  | Reference | Comments  |
|------------------------|----------------|-----------------|----------------------------|----|-------|-----------------|---|-----------|---|
| 10 <sup>7</sup> CFU/ml | Povidon iodine | 20 min          | 800 ppm                    |    | 20    | 5 log reduction | Dilution 1:1 of bacteria and disinfectant (25, 50, 100, 200, 400, 800, 1600, 3200 ppm). Growht at 20°C and counting already after 24 hours. | 58        | The actual concentration of disinfectant probably only half of stated in article table. Generally speaking the neccessary concentration for disinfection in this paper is much higher than published in other papers. |
| 10 <sup>7</sup> CFU/ml | Povidon iodine | 60 min          | 800 ppm                    |    | 20    | 5 log reduction | Dilution 1:1 of bacteria and  | 58        | The actual concentration  |

|  |  |  | disinfectant (25, 50, 100, 200, 400, 800, 1600, 3200 ppm). | of disinfectant probably only half of stated in |
|--|--|--|--|---|
|  |  |  | Growht at 20°C and counting                                | article table. Generally                        |
|  |  |  | already after 24 hours.                                    | speaking the neccessary                         |
|  |  |  |  | concentration for                               |
|  |  |  |  | disinfection in this paper                      |
|  |  |  |  | is much higher than                             |
|  |  |  |  | published in other                              |
|  |  |  |  | papers.   |

Conclusion: this paper states that *E. tarda* is much more resistant to iodine that other bacteria!

#### Kidney disease (Corynebacterium sp. - Renibacterium salmoninarum?)

| Concentration pathogen | Disinfectant   | Contact<br>time | Concentration disinfectant | рН | Temp. | Result         | Method  | Reference | Comments  |
|------------------------|----------------|-----------------|----------------------------|----|-------|----------------|---|-----------|-----------|
| Growth                 | Iodophor       | 15 sec          | 25 ppm                     | 7  | 10-13 | Not detectable | Distilled water. 2 strains tested. Time: 15, 30, 60, 120 and 300 seconds. | 84        | Wescodyne |
| Growth                 | Iodophor       | 300 sec         | 25 ppm                     | 8  | 10-13 | Not detectable | Distilled water. 1 strain tested. Time: 300 seconds.                      | 84        | Wescodyne |
| Growth                 | Povidon iodine | 15 sec          | 25 ppm                     | 7  | 10-13 | Not detectable | Distilled water. 2 strains tested. Time: 15, 30, 60, 120 and 300 seconds. | 84        | Betadine  |
| Growth                 | Povidon iodine | 300 sec         | 25 ppm                     | 8  | 10-13 | Not detectable | Distilled water. 1 strain tested. Time: 300 seconds.                      | 84        | Betadine  |

#### Renibacterium salmoninarum

| Concentration pathogen | Disinfectant   | Contact<br>time | Concentration disinfectant | рН | Temp. | Result   | Method                          | Reference | Comments |
|------------------------|----------------|-----------------|----------------------------|----|-------|--|---------------------------------|-----------|----------|
| > 1667 Bacteria/æg     | Povidon iodine | 15 min          | 500 mg/l                   |    | 15    | 166/170 (97,6%) of<br>the eggs sterile on<br>surface | Eggs from infected coho salmon. | 25        |          |

Conclusion: Disinfection of salmon egg seems to require a higher amount of iodine to be disinfected that do the bacteria under clean conditions (destilled water). Under clean conditions *R. salmoninarum* is comparable to other fish pathogenic bacteria requiring 25 ppm for 5 min. A dose of 500 mg/l did not completely inactivate *R. salmoniarum* on the surface of the eggs though most of the eggs were rendered sterile.

#### Lactococcus garviae

| Concentration | Disinfectant   | Contact | Concentration | На | Temp. | Result            | Method                          | Reference | Comments |
|---------------|----------------|---------|---------------|----|-------|-------------------|---------------------------------|-----------|----------|
| pathogen      | Distillectant  | time    | disinfectant  | рп | Temp. | Nesuit            | Wethou                          | Reference | Comments |
|               | lodophor, acid | 30 min  | 0,4 % (v/v)   |    | 4     | > 5 log reduction | Tested at 0,16, 0,2, 0,28, 0,4, | 49        |          |

|  |  |  | 0,8, 1, 1,6, 2 and 3,2%. Hard |  |
|--|--|--|-------------------------------|--|
|  |  |  | water, high organic load.     |  |

#### Streptococcus sp

| Concentration pathogen | Disinfectant   | Contact<br>time | Concentration disinfectant | рН | Temp. | Result            | Method  | Reference | Comments  |
|------------------------|----------------|-----------------|----------------------------|----|-------|-------------------|---|-----------|---|
| 10 <sup>7</sup> CFU/ml | Povidon iodine | 20 min          | 3200 ppm                   |    | 20    | > 5 log reduction | Dilution 1:1 of bacteria and disinfectant (25, 50, 100, 200, 400, 800, 1600, 3200 ppm). Growht at 20°C and counting already after 24 hours. | 58        | The actual concentration of disinfectant probably only half of stated in article table. Generally speaking the neccessary concentration for disinfection in this paper is much higher than published in other papers. |
| 10 <sup>7</sup> CFU/ml | Povidon iodine | 60 min          | 1600 ppm                   |    | 20    | 4 log reduction   | Dilution 1:1 of bacteria and disinfectant (25, 50, 100, 200, 400, 800, 1600, 3200 ppm). Growht at 20°C and counting already after 24 hours. | 58        | The actual concentration of disinfectant probably only half of stated in article table. Generally speaking the neccessary concentration for disinfection in this paper is much higher than published in other papers. |

Conclusion: this paper states that *Streprococcus* is much more resistant to iodine that other bacteria!

# Vibrio anguillarum

| Concentration pathogen  | Disinfectant   | Contact time | Concentration disinfectant | рН | Temp. | Result                                | Method  | Reference | Comments                                       |
|-------------------------|----------------|--------------|----------------------------|----|-------|---------------------------------------|---|-----------|--|
| 10 <sup>5</sup> cfu/ml  | Povidon iodine | 1 min        | 10 ppm                     |    | 20    | > 4 log reduction                     |   | 85        | Concentration of commercial product. (Isodine) |
| 3 x 10 <sup>7</sup> /ml | lodophor       | 15 sec       | 25 ppm                     | 7  | 10-13 | > 6 log reduction                     | Distilled water. 4 strains tested. Time: 15, 30, 60, 120 and 300 seconds. | 84        | Wescodyne                                      |
| 3 x 10 <sup>7</sup> /ml | lodophor       | 120 sec      | 25 ppm                     | 7  | 10-13 | Not detectable<br>(> 7 log reduction) | Distilled water. 4 strains tested. Time: 15, 30, 60, 120 and 300 seconds. | 84        | Wescodyne                                      |
| 5 x 10 <sup>6</sup> /ml | lodophor       | 60 sec       | 25 ppm                     | 8  | 10-13 | Not detectable (> 6 log reduction)    | Distilled water. 1 strain tested. Time: 15, 30, 60, 120 and 300 seconds.  | 84        | Wescodyne                                      |
| 1 x 10 <sup>5</sup> /ml | Povidon iodine | 15 sec       | 25 ppm                     | 7  | 10-13 | Not detectable<br>(> 5 log reduction) | Distilled water. 4 strains tested. Time: 15, 30, 60, 120 and 300 seconds. | 84        | Betadine                                       |

| 5 x 10 <sup>6</sup> /ml | Povidon iodine | 300 sec | 25 ppm | 8 | 10-13 | Not detectable (> 6 log reduction) | Distilled water. 1 strain<br>tested. Time: 15, 30, 60, 120<br>and 300 seconds. | 84 | Betadine |
|-------------------------|----------------|---------|--------|---|-------|------------------------------------|--|----|----------|
|-------------------------|----------------|---------|--------|---|-------|------------------------------------|--|----|----------|

Conclusion: Under clean conditions 25 ppm for 5 min will inactivate the bacteria.

#### Vibrio ordalii

| Concentration pathogen | Disinfectant   | Contact<br>time | Concentration disinfectant | рН | Temp. | Result            | Method | Reference | Comments                                       |
|------------------------|----------------|-----------------|----------------------------|----|-------|-------------------|--------|-----------|--|
| 10 <sup>5</sup> cfu/ml | Povidon iodine | 1 min           | 30 ppm                     |    | 20    | > 4 log reduction |        | 85        | Concentration of commercial product. (Isodine) |

Conclusion: Under clean conditions 25 ppm for 5 min will inactivate the bacteria.

#### Vibrio sp.

| Concentration pathogen | Disinfectant   | Contact<br>time | Concentration disinfectant | рН | Temp. | Result            | Method  | Reference | Comments  |
|------------------------|----------------|-----------------|----------------------------|----|-------|-------------------|---|-----------|---|
| 10 <sup>7</sup> CFU/ml | Povidon iodine | 20 min          | 1600 ppm                   |    | 20    | > 5 log reduction | Dilution 1:1 of bacteria and disinfectant (25, 50, 100, 200, 400, 800, 1600, 3200 ppm). Growht at 20°C and counting already after 24 hours. | 58        | The actual concentration of disinfectant probably only half of stated in article table. Generally speaking the neccessary concentration for disinfection in this paper is much higher than published in other papers. |
| 10 <sup>7</sup> CFU/ml | Povidon iodine | 60 min          | 800 ppm                    |    | 20    | 4 log reduction   | Dilution 1:1 of bacteria and disinfectant (25, 50, 100, 200, 400, 800, 1600, 3200 ppm). Growht at 20°C and counting already after 24 hours. | 58        | The actual concentration of disinfectant probably only half of stated in article table. Generally speaking the neccessary concentration for disinfection in this paper is much higher than published in other papers. |

Conclusion: this paper states that Vibrio is much more resistant to iodine than stated in other papers!

#### Yersinia ruckeri

| Concentration pathogen  | Disinfectant   | Contact<br>time | Concentration disinfectant | рН | Temp. | Result                                | Method  | Reference | Comments  |
|-------------------------|----------------|-----------------|----------------------------|----|-------|---------------------------------------|---|-----------|-----------|
| 3 x 10 <sup>7</sup> /ml | Iodophor       | 15 sec          | 25 ppm                     | 7  | 10-13 | Not detectable (> 7<br>log reduction) | Distilled water. 4 strains<br>tested. Time: 15, 30, 60, 120<br>and 300 seconds.         | 84        | Wescodyne |
| 5 x 10 <sup>6</sup> /ml | Iodophor       | 15 sec          | 25 ppm                     | 8  | 10-13 | Not detectable (> 6 log reduction)    | Distilled water. 1 strain<br>tested. Time: 15, 30, 60, 120<br>and 300 seconds.          | 84        | Wescodyne |
|                         | lodophor, acid | 30 min          | 0,28 % (v/v)               |    | 4     | > 5 log reduction                     | Tested at 0,16, 0,2, 0,28, 0,4, 0,8, 1, 1,6, 2 and 3,2%. Hard water, high organic load. | 126       |           |
| 2 x 10 <sup>7</sup> /ml | Povidon iodine | 15 sec          | 25 ppm                     | 7  | 10-13 | Not detectable<br>(> 7 log reduction) | Distilled water. 4 strains<br>tested. Time: 15, 30, 60, 120<br>and 300 seconds.         | 84        | Betadine  |
| 5 x 10 <sup>6</sup> /ml | Povidon iodine | 15 sec          | 25 ppm                     | 8  | 10-13 | Not detectable<br>(> 6 log reduction) | Distilled water. 1 strain<br>tested. Time: 15, 30, 60, 120<br>and 300 seconds.          | 84        | Betadine  |

Conclusion: Under clean conditions 25 ppm for 5 min will inactivate the bacteria.

#### **Parasites**

# Gyrodactylus salaris

| Concentration pathogen | Disinfectant | Contact<br>time | Concentration disinfectant | рН | Temp. | Result    | Method | Reference | Comments   |
|------------------------|--------------|-----------------|----------------------------|----|-------|-----------|--------|-----------|--|
|                        | lodine       |                 |                            |    |       | Sensitive |        |           | From OIE diagnostic manual. No reference stated! |

# Myxosoma cerebralis

| Concentration pathogen | Disinfectant   | Contact<br>time | Concentration disinfectant | рН | Temp.         | Result   | Method                             | Reference | Comments                  |
|------------------------|----------------|-----------------|----------------------------|----|---------------|----------|------------------------------------|-----------|---------------------------|
|                        | Povidon iodine | 10 min          | 500 ppm                    |    | room<br>temp. | Survival | in vitro. Triactinomyxon<br>spores | 96        | 5% of commercial product. |

| Povidon iodine  | 10 min    | 5000 ppm   | room  | Survival | in vitro. Triactinomyxon | 96 | 50% of commercial |
|-----------------|-----------|------------|-------|----------|--------------------------|----|-------------------|
| r ovidon lodine | 10 111111 | 3000 ppiii | temp. | Survivar | spores                   | 30 | product.          |

#### **Fungae**

#### Phoma herbarum

| Concentration pathogen | Disinfectant   | Contact time | Concentration disinfectant | рН | Temp. | Result    | Method  | Reference | Comments  |
|------------------------|----------------|--------------|----------------------------|----|-------|-----------|---|-----------|-----------|
| Growth                 | Povidon iodine | 120 sec      | 25 ppm                     | 7  | 10-13 | No growth | Distilled water. 1 strain tested (spores). Time: 15, 30, 60, 120 and 300 seconds. | 84        | Betadine  |
| Growth                 | Iodophor       | 120 sec      | 25 ppm                     | 7  | 10-13 | No growth | Distilled water. 1 strain tested (spores). Time: 15, 30, 60, 120 and 300 seconds. | 84        | Wescodyne |

# Saprolegnia parasitica

| Concentration pathogen | Disinfectant   | Contact<br>time | Concentration disinfectant | рН | Temp. | Result | Method   | Reference | Comments  |
|------------------------|----------------|-----------------|----------------------------|----|-------|--------|--|-----------|-----------|
| Growth                 | Povidon iodine | 300 sec         | 25 ppm                     | 7  | 10-13 | Growth | Distilled water 1 stamme testet (mycelium). Tid: 15, 30, 60, 120 og 300 seconds. | 84        | Betadine  |
| Growth                 | Iodophor       | 300 sec         | 25 ppm                     | 7  | 10-13 | Growth | Distilled water 1 stamme testet (mycelium). Tid: 15, 30, 60, 120 og 300 seconds. | 84        | Wescodyne |

# **Ozone**

#### **Virus**

#### **VHSV**

It has not been possible to find any references.

#### *IHNV*

| Concentration pathogen                                   | Disinfectant | Contact<br>time | Concentration disinfectant | рН  | Temp. | Result        | Method                    | Reference | Comments             |
|--|--------------|-----------------|----------------------------|-----|-------|---------------|---------------------------|-----------|----------------------|
| 10 <sup>4</sup> - 10 <sup>5</sup> TCID <sub>50</sub> /ml | ozone        | 30 sec          | total residual             | 6,9 | 10    | "inactivated" | Ozone tilført: 70 mg/h/L, | 99, 98    | CT value: 0,3 mg*s/l |

|  |       |        | oxidants 0,01<br>mg/l               |     |    |                           | Distilled water.  |        |                       |
|--|-------|--------|-------------------------------------|-----|----|---------------------------|---|--------|-----------------------|
|  | ozone | 15 sec | total residual<br>oxidants 0,5 mg/l |     |    | 99% infectivity reduction |   | 102    | C T value: 7,5 mg*s/l |
| 10 <sup>4</sup> - 10 <sup>5</sup> TCID <sub>50</sub> /mI | ozone | 10 min | 70 mg/h/L                           | 6,9 | 10 | "inactivated"             | Not possible to obtain a stable ozone residual. Soft lake water, 30 mg/l CaCO <sub>3</sub> .  | 99, 98 |                       |
| 10 <sup>4</sup> - 10 <sup>5</sup> TCID <sub>50</sub> /ml | ozone | 10 min | 70 mg/h/L                           | 8,2 | 10 | "inactivated"             | Not possible to obtain a stable ozone residual. Hard lake water, 120 mg/l CaCO <sub>3</sub> . | 99, 98 |                       |

Conclusion: IHNV is sensitive to treatment with ozone. Based on these figures the dose needed for a 3 log reduction is unknown.

#### *IPNV*

| Concentration pathogen                   | Disinfectant | Contact<br>time | Concentration disinfectant              | рН  | Temp. | Result                    | Method  | Reference | Comments              |
|--|--------------|-----------------|---|-----|-------|---------------------------|---|-----------|-----------------------|
| 10 <sup>5</sup> TCID <sub>50</sub> /ml   | ozone        | 60 sec          | total residual<br>oxidants 0,01<br>mg/l | 6,9 | 10    | "inactivated"             | Ozone added: 70 mg/h/L,<br>Distilled water.   | 99, 98    | C T value: 0,6 mg*s/l |
| 10 <sup>5,5</sup> TCID <sub>50</sub> /ml | ozone        | 60 sec          | 0,20 mg/l                               |     | 9-12  | > 4 log reduction         | Lake water, autoclaved.   | 70        | C T value: 12 mg*s/l  |
| 10 <sup>5,5</sup> TCID <sub>50</sub> /ml | ozone        | 120 sec         | 0,20 mg/l                               |     | 9-12  | > 5 log reduction         | Lake water, autoclaved.   | 70        | C T value: 24 mg*s/l  |
| 10 <sup>5,5</sup> TCID <sub>50</sub> /ml | ozone        | 60 sec          | 0,20 mg/l                               |     | 9-12  | > 5 log reduction         | Brackish water, salinity 15 ‰, autoclaved.  | 70        | C T value: 12 mg*s/l  |
| 10 <sup>5,5</sup> TCID <sub>50</sub> /ml | ozone        | 60 sec          | 0,20 mg/l                               |     | 9-12  | > 5 log reduction         | Sea water, salinity 32 ‰, autoclaved.   | 70        | C T value: 12 mg*s/l  |
|  | ozone        | 1 min           | total residual oxidants 0,5 mg/l        |     |       | 99% infectivity reduction |   | 102       | C T value: 30 mg*s/l  |
| 10 <sup>5</sup> TCID <sub>50</sub> /ml   | ozone        | 30 sec          | 90 mg/h/L                               | 6,9 | 10    | "inactivated"             | Not possible to obtain a stable ozone residual. Soft lake water, 30 mg/l CaCO <sub>3</sub> .  | 99, 98    |                       |
| 10 <sup>5</sup> TCID <sub>50</sub> /ml   | ozone        | 10 min          | 90 mg/h/L                               | 8,2 | 10    | "inactivated"             | Not possible to obtain a stable ozone residual. Hard lake water, 120 mg/l CaCO <sub>3</sub> . | 99, 98    |                       |

Conclusion: IPNV is sensitive to treatment with ozone. Based on these figures the dose needed for a 3 log reduction is unknown.

#### **ISAV**

| Concentration pathogen | Disinfectant | Contact<br>time | Concentration disinfectant | рН  | Temp. | Result           | Method                       | Reference | Comments                                       |
|------------------------|--------------|-----------------|----------------------------|-----|-------|------------------|------------------------------|-----------|--|
|                        | ozone        | 15 sec          | 0,33 mg/l TRO              | 7,9 | 5     | 99,0 % reduction | Sea water, sterile filtered. | 73        | C T value: 5,0 mg*s/l,<br>written in article.  |
|                        | ozone        | 31 min          | 2,5 mg/l TRO               | 7,9 | 5     | 98,4 % reduction | Sea water, sterile filtered. | 73        | C T value: 4650 mg*s/l,<br>written in article. |

| ozone | 14 min | 6,7 mg/l TRO | 7,9 | 5 | 98,0 % reduction | Sea water, sterile filtered. | 73 | C T value: 5628 mg*s/l,<br>written in article.  |
|-------|--------|--------------|-----|---|------------------|------------------------------|----|---|
| ozone | 17 min | 7,9 mg/l TRO | 7,9 | 5 | 98,7 % reduction | Sea water, sterile filtered. | 73 | C T value: 8058 mg*s/,<br>written in article. I |

Conclusion: Based on this paper ISAV is sensitive to ozone but it will not be possible to obtain more than a 2 log reduction.

#### **Nodavirus**

| Concentration pathogen | Disinfectant | Contact<br>time | Concentration disinfectant | рН  | Temp. | Result   | Method   | Reference | Comments                                       |
|------------------------|--------------|-----------------|----------------------------|-----|-------|--|--|-----------|--|
| 10 μg purified virus   | ozone        | 30 sec          | 0,1 mg/ml TRO              |     | 20    | Not inactivated<br>(0/800 larvae<br>survived, control<br>238/800)  | Virus added to 1 ml ozone treated sea water. Used for infection trial in day old striped jack larvae.          | 9         | Isolate: SJNNV.<br>C T value: 3 mg*s/l         |
|                        | ozone        | 1 min           | 0,2 mg/ml TRO              |     |       | "Effective" (136/800 larvae survived, antigen ELISA negative, untreated eggs 0/800 survived, antigen ELISA positive) | Washihng of eggs from noda infected ovaries i sea water, followed after hatching.                              | 9         | Isolate: SJNNV<br>C T value: 12 mg*s/l         |
| 10 µg purified virus   | ozone        | 2,5 min         | 0,1 mg/ml TRO              |     | 20    | "Effective" (334/800 larvae survived, antigen ELISA negativ, control 238/800)  | Virus added to 1 ml ozone<br>treated sea water. Used for<br>infection trial in day old<br>striped jack larvae. | 9         | Isolate: SJNNV.<br>C T value: 15 mg*s/l        |
| 10 μg purified virus   | ozone        | 30 sec          | 0,5 mg/ml TRO              |     | 20    | "Effective" (150/800 larvae survived, antigen ELISA negativ, control 238/800)  | Virus added to 1 ml ozone<br>treated sea water. Used for<br>infection trial in day old<br>striped jack larvae. | 9         | Isolate: SJNNV.<br>C T value: 15 mg*s/l        |
|                        | ozone        | 31,5 min        | 1,6 mg/l TRO               | 7,9 | 5     | 98,0 % reduction   | Sea water, sterile filtered.   | 73        | C T value: 3043 mg*s/l,<br>written in article. |

Conclusion: Nodavirus is sensitive towards ozone. One papers state that a dose of 12-15 mg\*s/l is effective in prohibiting disease in striped jack larvae. The other paper states that when using a dose of 3000 mg\*s/l only a 2 log reduction is obtainable.

#### Bacteria

#### Aeromons licquefaciens

| - |               |              |         |               |    |       |        |        |           |          |
|---|---------------|--------------|---------|---------------|----|-------|--------|--------|-----------|----------|
|   | Concentration | Disinfectant | Contact | Concentration | pН | Temp. | Result | Method | Reference | Comments |

| pathogen            |       | time    | disinfectant |   |                      |   |    |   |
|---------------------|-------|---------|--------------|---|----------------------|---|----|---|
| 10 <sup>7</sup> /ml | ozone | > 6 min | 0,1 mg/l     | 7 | app. 4 log reduction | Distilled water, continous ozonation.                   | 16 | Read off a graph.<br>C T value: > 36 mg*s/l                         |
| 10 <sup>7</sup> /ml | ozone | 3 min   | 0,15 mg/l    | 7 | app. 4 log reduction | Distilled water, continous ozonation.                   | 16 | Read off a graph.<br>C T value: 27 mg*s/l                           |
| 10 <sup>7</sup> /ml | ozone | 2 min   | 0,2 mg/l     | 7 | app. 4 log reduction | Distilled water, continous ozonation.                   | 16 | Read off a graph.<br>C T value: 24 mg*s/l                           |
| 10 <sup>7</sup> /ml | ozone | 3½ min  | 0,2 mg/l     | 7 | "inactivated"        | Distilled water, continous ozonation.                   | 16 | Read off a graph.<br>C T value: 42 mg*s/l                           |
| 10 <sup>8</sup> /ml | ozone | 1 min   | 1 mg/l       | 7 | app. 3 log reduction | Distilled water, ozonation stopped when bacteria added. | 16 | No further reduction<br>during the next 4 min.<br>Read off a graph. |

Conclusion: Based on this paper a dose of 30 mg\*s/l is capable of a 4 log reduction of A. licquefaciens.

# Aeromonas salmonicida

| Concentration pathogen   | Disinfectant | Contact<br>time | Concentration disinfectant | рН  | Temp. | Result               | Method  | Reference | Comments   |
|--------------------------|--------------|-----------------|----------------------------|-----|-------|----------------------|---|-----------|--|
| 10 <sup>3</sup> cells/ml | ozone        | 10 min          | 0,01 mg/l<br>residual      | 6,9 | 20    | "inactivated"        | Distilled water Samples tested after ½, 1, 2, 5, 10, 20 and 30 min. | 98, 97    | C T value: 6 mg*s/l  |
| 10 <sup>3</sup> cells/ml | ozone        | 30 sec          | 0,04 mg/l<br>residual      | 6,9 | 20    | "inactivated"        | Distilled water Samples tested after ½, 1, 2, 5, 10, 20 and 30 min. | 98, 97    | C T value: 1,2 mg*s/l  |
| 10 <sup>7</sup> CFU/ml   | ozone        | 1 min           | 0,065 mg/l/sec             | 7,8 | 7     | 3½ log reduction     | Wastewater from aquaculture (15,7 % salinity).                      | 71        | Read off a graph.  |
| 10 <sup>7</sup> CFU/ml   | ozone        |                 | 0,065 mg/l/sec             | 7,8 | 7     | 99,9 % reduction     | Wastewater from aquaculture (15,7 % salinity).                      | 71        | Reduction rate: 0,12/sec   |
| 10 <sup>7</sup> /ml      | ozone        | 6 min           | 0,05 mg/l                  | 7   |       | app. 4 log reduction | Distilled water, continous ozonation.                               | 16        | Read off a graph.<br>C T value: 18 mg*s/l  |
| 10 <sup>7</sup> CFU/ml   | ozone        |                 | 0,1 mg/l/sec               | 7,2 | 7     | 99,9 % reduction     | PBS   | 71        | Reduction rate: 0,32/sec   |
| Unknown                  | ozone        |                 | 0,1 mg/l residual          |     |       | Not detectable       | Test af laboratoriespildevand                                       | 10        | A. salm is known to be part of the wastewater, but it has not been tested whether it was possible to re-isolate the bacteria before ozonation. |
| 10 <sup>7</sup> /ml      | ozone        | 1½ min          | 0,1 mg/l                   | 7   |       | app. 4 log reduction | Distilled water, continous ozonation.                               | 16        | Read off a graph.<br>C T value: 9 mg*s/l   |
| 10 <sup>7</sup> /ml      | ozone        | 2½ min          | 0,1 mg/l                   | 7   |       | "inactivated"        | Distilled water, continous ozonation.                               | 16        | Read off a graph.<br>C T value: 15 mg*s/l  |

| 3 x 10 <sup>6</sup> CFU/ml | ozone | 180 sec | 0,15 mg/l |     | 9-12 | 4 log reduction | Sea water, salinity 32 ‰, autoclaved.  | 70     | C T value: 27 mg*s/l                      |
|----------------------------|-------|---------|-----------|-----|------|-----------------|--|--------|---|
| 3 x 10 <sup>6</sup> CFU/ml | ozone | 120 sec | 0,15 mg/l |     | 9-12 | 4 log reduction | Brackish water, salinity 15 ‰, autoclaved.   | 70     | Read off a graph.<br>C T value: 18 mg*s/l |
| 3 x 10 <sup>6</sup> CFU/ml | ozone | 60 sec  | 0,20 mg/l |     | 9-12 | 4 log reduction | Lake water, autoclaved.  | 70     | Read off a graph.<br>C T value: 12 mg*s/l |
| 10 <sup>8</sup> /ml        | ozone | 1 min   | 1 mg/l    | 7   |      | "inactivated"   | Distilled water, ozonation stopped when bacteria added.  | 16     | Read off a graph.                         |
| 10 <sup>3</sup> cells/ml   | ozone | 30 min  | 20 mg/h/l | 8,2 | 20   | "inactivated"   | Hard lake water., 120 mg/l CaCO <sub>3</sub> Samples tested after ½, 1, 2, 5, 10, 20 and 30 min. | 98, 97 |   |
| 10 <sup>3</sup> cells/ml   | ozone | 5 min   | 90 mg/h/l | 8,2 | 20   | "inactivated"   | Hard lake water., 120 mg/l CaCO <sub>3</sub> Samples tested after ½, 1, 2, 5, 10, 20 and 30 min. | 98, 97 |   |
| 10 <sup>3</sup> cells/ml   | ozone | 15 min  | 90 mg/h/l | 6,9 | 20   | "inactivated"   | Soft lake water., 30 mg/l CaCO <sub>3</sub> Samples tested after ½, 1, 2, 5, 10, 20 and 30 min.  | 98, 97 |   |

Conclusion: A. salmonicida is sensitive to treatment with ozone. Based on these figures the dose needed for a 4 log reduction is 10-30 mg\*s/l.

#### Enterococcus seriolicida

| Concentration pathogen   | Disinfectant | Contact time | Concentration disinfectant | рН | Temp. | Result          | Method   | Reference | Comments                                     |
|--------------------------|--------------|--------------|----------------------------|----|-------|-----------------|--|-----------|--|
| 10 <sup>6,5</sup> cfu/ml | ozone        | 6 min        | 0,018 mg/l TRO             |    |       | Stable          | Sea water, sterile filtered.                   | 92        | Read off a graph.<br>C T value: 6,48 mg*s/l  |
| 10 <sup>6,5</sup> cfu/ml | ozone        | 4 min        | 0,096 mg/l TRO             |    |       | "inactivated"   | Sea water, sterile filtered.                   | 92        | Read off a graph.<br>C T value: 23,04 mg*s/l |
| 10 <sup>6,5</sup> cfu/ml | ozone        | 1 min        | 0,536 mg/l TRO             |    |       | "inactivated"   | Sea water, sterile filtered.                   | 92        | Read off a graph.<br>C T value: 32,16 mg*s/l |
|                          | ozone        | 1 min        | 0,393 mg/l TRO             |    |       | 6 log reduction | Estimated based on Chick-<br>Watson parametre. | 92        | C T value: 23,58 mg*s/l                      |

# Natural flora (heterotrophic bacteria)

| Concentration pathogen       | Disinfectant | Contact<br>time | Concentration disinfectant | рН  | Temp. | Result             | Method                      | Reference | Comments   |
|------------------------------|--------------|-----------------|----------------------------|-----|-------|--------------------|-----------------------------|-----------|--|
| 116 ± 25 cfu/ml              | ozone        | 8,3 min         | 0,21 mg/l                  | 7,5 | 14,3  | 1,35 log reduction | fish farm, recirculation    | 87        | C T value: 105 mg*s/l                                    |
| 4,7 x 10 <sup>4</sup> cfu/ml | ozone        | 1 min           | 0,5 mg/l TRO               |     |       | 4 log reduction    | flowrate 2,0 m <sup>3</sup> | 56        | Natural flora in<br>wastewater from hathing<br>facility. |

|                          |       |         |                   |  |               |         |    | C T value: 30 mg*s/l                         |
|--------------------------|-------|---------|-------------------|--|---------------|---------|----|--|
| 10 <sup>5,5</sup> cfu/ml | ozone | 3 min   | 0,773 mg/l TRO    |  | "inactivated" | havvand | 92 | Read off a graph.                            |
|                          |       |         | _                 |  |               |         |    | C T value: 140 mg*s/l                        |
| Unknown                  | ozone |         | 1,0 mg/l residual |  | Survival      |         | 10 |  |
| 10 <sup>5,5</sup> cfu/ml | ozone | < 1 min | 1,933 mg/l TRO    |  | "inactivated" | havvand | 92 | Read off a graph.<br>C T value: < 120 mg*s/l |

# Pasteurella piscicida

| Concentration pathogen | Disinfectant | Contact<br>time | Concentration disinfectant | рН | Temp. | Result          | Method   | Reference | Comments                                   |
|------------------------|--------------|-----------------|----------------------------|----|-------|-----------------|--|-----------|--|
| 10 <sup>6</sup> cfu/ml | ozone        | 6 min           | 0,018 mg/l TRO             |    |       | Stable          | Sea water, sterile filtered.                   | 92        | Read off a graph.<br>C T value: 6,5 mg*s/l |
|                        | ozone        | 1 min           | 0,165 mg/l TRO             |    |       | 6 log reduction | Estimated based on Chick-<br>Watson parametre. | 92        | C T value: 10 mg*s/l                       |
| 10 <sup>6</sup> cfu/ml | ozone        | 1 min           | 0,370 mg/l TRO             |    |       | "inactivated"   | Sea water, sterile filtered.                   | 92        | Read off a graph.<br>C T value: 22 mg*s/l  |

# Pseudomonas flourescens

| Concentration pathogen | Disinfectant | Contact<br>time | Concentration disinfectant | рН | Temp. | Result               | Method  | Reference | Comments  |
|------------------------|--------------|-----------------|----------------------------|----|-------|----------------------|---|-----------|---|
| 10 <sup>7</sup> /ml    | ozone        | 4½ min          | 0,1 mg/l                   | 7  |       | app. 4 log reduction | Distilled water, continous ozonation.                         | 16        | Read off a graph.<br>C T value: 27 mg*s/l                           |
| 10 <sup>7</sup> /ml    | ozone        | 2½ min          | 0,15 mg/l                  | 7  |       | app. 4 log reduction | Distilled water, continous ozonation.                         | 16        | Read off a graph.<br>C T value: 22,5 mg*s/l                         |
| 10 <sup>7</sup> /ml    | ozone        | 2 min           | 0,2 mg/l                   | 7  |       | app. 4 log reduction | Distilled water, continous ozonation.                         | 16        | Read off a graph.<br>C T value: 24 mg*s/l                           |
| 10 <sup>7</sup> /ml    | ozone        | 2½ min          | 0,15 mg/l                  | 7  |       | "inactivated"        | Distilled water, continous ozonation.                         | 16        | Read off a graph.<br>C T value: 22,5 mg*s/l                         |
| 108/ml                 | ozone        | 1 min           | 1 mg/l                     | 7  |       | app. 3 log reduction | Distilled water, ozonation<br>stopped when bacteria<br>added. | 16        | No further reduction<br>during the next 4 min.<br>Read off a graph. |

Conclusion: *P. flourescens* is sensitive to treatment with ozone. Based on these figures the dose needed for a 4 log reduction is 20-30 mg\*s/l.

#### Renibacterium salmoninarum

| Concentration pathogen | Disinfectant | Contact<br>time | Concentration disinfectant | рН | Temp. | Result         | Method                        | Reference | Comments   |
|------------------------|--------------|-----------------|----------------------------|----|-------|----------------|-------------------------------|-----------|--|
| unknown                | ozone        |                 | 0,1 mg/l residual          |    |       | Not detectable | Test af laboratoriespildevand | 10        | R. salm is known to be part of the wastewater, but it has not been tested whether it was possible to re-isolate the bacteria before ozonation. |

# Vibrio anguillarum

| Concentration pathogen     | Disinfectant | Contact time | Concentration disinfectant | рН | Temp. | Result               | Method   | Reference | Comments  |
|----------------------------|--------------|--------------|----------------------------|----|-------|----------------------|--|-----------|---|
| 10 <sup>6</sup> cfu/ml     | ozone        | 6 min        | 0,018 mg/l TRO             |    |       | Stable               | Sea water, sterile filtered.                   | 92        | Read off a graph.<br>C T value: 6,5 mg*s/l  |
|                            | ozone        | 1 min        | 0,029 mg/I TRO             |    |       | 6 log reduction      | Estimated based on Chick-<br>Watson parametre. | 92        | C T value: 1,8 mg*s/l   |
| unknown                    | ozone        |              | 0,1 mg/l residual          |    |       | Not detectable       | Test af laboratoriespildevand                  | 10        | V. ang is known to be part of the wastewater, but it has not been tested whether it was possible to re-isolate the bacteria before ozonation. |
| 3 x 10 <sup>8</sup> CFU/ml | ozone        | 60 sec       | 0,15 mg/l                  |    | 9-12  | app. 5 log reduction | Sea water, salinity 32 ‰, autoclaved.          | 70        | Read off a graph.<br>C T value: 9 mg*s/l  |
| 3 x 10 <sup>8</sup> CFU/ml | ozone        | 60 sec       | 0,15 mg/l                  |    | 9-12  | app. 6 log reduction | Brackish water, salinity 15 ‰, autoclaved.     | 70        | Read off a graph.<br>C T value: 9 mg*s/l  |
| 10 <sup>6</sup> cfu/ml     | ozone        | 1½ min       | 0,196 mg/l TRO             |    |       | "inactivated"        | Sea water, sterile filtered.                   | 92        | Read off a graph.<br>C T value: 18 mg*s/l   |
| 3 x 10 <sup>8</sup> CFU/ml | ozone        | 60 sec       | 0,20 mg/l                  |    | 9-12  | app. 5 log reduction | Lake water, autoclaved.                        | 70        | Read off a graph.<br>C T value: 12 mg*s/l   |

Conclusion: *V. anguillarum* is sensitive to treatment with ozone. Based on these figures the dose needed for a 5-6 log reduction is 10-20 mg\*s/l. The results indicate that there is a minimum TRO needed in order for the ozone to inactivate the microorganism. When *V. anguillarum* was treated with

0,018 mg/l TRO (6m5 mg\*s/l) for 6 min the titer was stable, whereas treatment using 0,029 mg/l TRO (1,8 mg\*s/l) for 1 min the bacteria was inactivated.

#### Vibrio salmoicida

| Concentration pathogen     | Disinfectant | Contact time | Concentration disinfectant | рН | Temp. | Result          | Method                                     | Reference | Comments             |
|----------------------------|--------------|--------------|----------------------------|----|-------|-----------------|--|-----------|----------------------|
| 3 x 10 <sup>6</sup> CFU/ml | ozone        | 60 sec       | 0,20 mg/l                  |    | 9-12  | 4 log reduction | Lake water, autoclaved.                    | 70        | C T value: 12 mg*s/l |
| 3 x 10 <sup>6</sup> CFU/ml | ozone        | 120 sec      | 0,15 mg/l                  |    | 9-12  | 4 log reduction | Sea water, salinity 32 ‰, autoclaved.      | 70        | C T value: 18 mg*s/l |
| 3 x 10 <sup>6</sup> CFU/ml | ozone        | 180 sec      | 0,15 mg/l                  |    | 9-12  | 4 log reduction | Brackish water, salinity 15 ‰, autoclaved. | 70        | C T value: 27 mg*s/l |

Conclusion: V. salmonicida is sensitive to treatment with ozone. Based on these figures the dose needed for a 4 log reduction is 10-30 mg\*s/l.

#### Yersinia ruckeri

| Concentration pathogen     | Disinfectant | Contact<br>time | Concentration disinfectant | рН  | Temp. | Result                   | Method   | Reference | Comments  |
|----------------------------|--------------|-----------------|----------------------------|-----|-------|--------------------------|--|-----------|---|
| 10 <sup>3</sup> cells/ml   | ozone        | 30 sec          | 0,01 mg/l residual         | 6,9 | 20    | "inactivated"            | Distilled water Samples tested after ½, 1, 2, 5, 10, 20 and 30 min.  | 98, 97    | C T value: 0,3 mg*s/l   |
| 10 <sup>7</sup> /ml        | ozone        | 5½ min          | 0,05 mg/l                  | 7   |       | app. 4 log reduction     | Distilled water, continous ozonation.                                | 16        | Read off a graph.<br>C T value: 16,5 mg*s/l                         |
| 10 <sup>7</sup> /ml        | ozone        | 4 min           | 0,1 mg/l                   | 7   |       | app. 4 log reduction     | Distilled water, continous ozonation.                                | 16        | Read off a graph.<br>C T value: 24 mg*s/l                           |
| 10 <sup>7</sup> /ml        | ozone        | 2½ min          | 0,15 mg/l                  | 7   |       | app. 4 log reduction     | Distilled water, continous ozonation.                                | 16        | Read off a graph.<br>C T value: 22,5 mg*s/l                         |
| 3 x 10 <sup>9</sup> CFU/ml | ozone        | 30 sec          | 0,15 mg/l                  |     | 9-12  | app. 6 log reduction     | Sea water, salinity 32 ‰, autoclaved.                                | 70        | Read off a graph.<br>C T value: 4,5 mg*s/l                          |
| 3 x 10 <sup>9</sup> CFU/ml | ozone        | 60 sec          | 0,15 mg/l                  |     | 9-12  | app. 7 log reduction     | Brackish water, salinity 15 ‰, autoclaved.                           | 70        | Read off a graph.<br>C T value: 9 mg*s/l                            |
| 10 <sup>7</sup> /ml        | ozone        | 1½ min          | 0,2 mg/l                   | 7   |       | app. 4 log reduction     | Distilled water, continous ozonation.                                | 16        | Read off a graph.<br>C T value: 18 mg*s/l                           |
| 10 <sup>7</sup> /ml        | ozone        | 1½ min          | 0,2 mg/l                   | 7   |       | "inactivated"            | Distilled water, continous ozonation.                                | 16        | Read off a graph.<br>C T value: 18 mg*s/l                           |
| 3 x 10 <sup>9</sup> CFU/ml | ozone        | 60 sec          | 0,20 mg/l                  |     | 9-12  | app. 7 log reduction     | Lake water, autoclaved.  | 70        | Read off a graph.<br>C T value: 12 mg*s/l                           |
| 10 <sup>8</sup> /ml        | ozone        | 1 min           | 1 mg/l                     | 7   |       | app. 3½ log<br>reduction | Distilled water, ozonation stopped when bacteria added.              | 16        | No further reduction<br>during the next 4 min.<br>Read off a graph. |
| 10 <sup>3</sup> cells/ml   | ozone        | 25 min          | 20 mg/h/l                  | 8,2 | 20    | "inactivated"            | Hard lake water., 120 mg/l CaCO <sub>3</sub> Samples tested after ½, | 98, 97    |   |

|                          |       |        |           |     |    |               | 1, 2, 5, 10, 20 and 30 min.   |        |  |
|--------------------------|-------|--------|-----------|-----|----|---------------|---|--------|--|
| 10 <sup>3</sup> cells/ml | ozone | 25 min | 20 mg/h/l | 6,9 | 20 | "inactivated" | Soft lake water., 30 mg/l $CaCO_3$ Samples tested after $\frac{1}{2}$ , 1, 2, 5, 10, 20 and 30 min. | 98, 97 |  |
| 10 <sup>3</sup> cells/ml | ozone | 10 min | 90 mg/h/l | 8,2 | 20 | "inactivated" | Hard lake water., 120 mg/l CaCO <sub>3</sub> Samples tested after ½, 1, 2, 5, 10, 20 and 30 min.    | 98, 97 |  |
| 10 <sup>3</sup> cells/ml | ozone | 10 min | 90 mg/h/l | 6,9 | 20 | "inactivated" | Soft lake water., 30 mg/l $CaCO_3$ Samples tested after $\frac{1}{2}$ , 1, 2, 5, 10, 20 and 30 min. | 98, 97 |  |

Conclusion: *Y. ruckeri* is sensitive to treatment with ozone. Based on these figures the dose needed for a  $\geq$  4 log reduction is 10-30 mg\*s/l.

# Other oxidising disinfectants

#### Virus

#### *IPNV*

| Concentration pathogen                         | Disinfectant   | Contact<br>time | Concentration disinfectant | рН | Temp. | Result            | Method   | Reference | Comments   |
|--|--|-----------------|----------------------------|----|-------|-------------------|--|-----------|--|
| 10 <sup>6,08-7,41</sup> TCID <sub>50</sub> /ml | pentakalium<br>bis<br>(peroxymonsul<br>fate) bis<br>(sulfat) | 30 min          | 0,5 % (v/v)                |    | 4     | > 4 log reduction | Tested at 0,1, 0,2 and 0,5%.<br>Hard water, high organic load. | 49        | Contains > 10% available oxygen.   |
|  | peracetic acid<br>(divosan forte)                            | 39 days         | 0,5%                       |    | 4     | Survival          | Mixing of virus and fish silage.                               | 91        | Type Sp. Titer reduction<br>2,75. Titer day 39<br>without Divosan Forte:<br>5,45, with DF 2,70 |
|  | peracetic acid<br>(divosan forte)                            | 16 days         | 5%                         |    | 4     | > 2,45 reduction  | Mixing of virus and fish silage.                               | 91        | Type Sp. Titer reduction > 2,45. Titer day 16 without Divosan Forte: 5,45, with DF < 3.00      |
| 10 <sup>6,58-6,74</sup> TCID <sub>50</sub> /ml | peracetic<br>acid/hydrogen<br>peroxid                        | 30 min          | 0,276% (v/v)               |    | 4     | > 4 log reduction | Tested at 0,16, 0,276 and 1,6%. Hard water, high organic load. | 49        |  |
| 4 x 10 <sup>6</sup> pfu/ml                     | VirkonS in fish<br>silage treated<br>with formic<br>acid and | 30 min          | 1/100 w/v                  | ?  | ?     | "inactivated"     | Mixing of virus and fish silage.                               | 90, 91    | Startdosis: 4x10 <sup>6</sup> ,<br>slutdosis <400  |

|  | propionic acid                                       |        |      |   |                   |  |    |  |
|--|--|--------|------|---|-------------------|--|----|--|
| 10 <sup>5,00-6,23</sup> TCID <sub>50</sub> /ml | Hydrogenperox<br>id/acetic<br>acid/peracetic<br>acid | 30 min | 1,0% | 4 | > 4 log reduction | Sea water. Contact time 15 and 30 min. Koncentration: 0,5, 1,0, 1,5 and 2,0%. 1% BSA + 1% yeast extract. | 18 | IPNV isolat N1.<br>Kick-Start2: $H_2O_2$ 20%,<br>organic acids > 10%,<br>peracetic acid 5%,<br>surfactant, stabilizing and<br>complex inducing agents. |

#### **ISAV**

| Concentration pathogen | Disinfectant  | Contact time | Concentration disinfectant | рН | Temp. | Result              | Method  | Reference | Comments  |
|------------------------|---|--------------|----------------------------|----|-------|---------------------|---|-----------|-----------|
| 5,0 log10 ffu/ml       | peracetic<br>acid/H <sub>2</sub> O <sub>2</sub> /acetic<br>acid | 5 min        | 1:80                       |    | 4     | > 4,7 log reduction | Hard water, 342 ppm total hardness, no addition of serum. | 89        | Proxitane |
| 5,0 log10 ffu/ml       | peracetic<br>acid/H <sub>2</sub> O <sub>2</sub> /acetic<br>acid | 5 min        | 1:80                       |    | 4     | > 4,7 log reduction | Hard water, 342 ppm total hardness, addition of serum.    | 89        | Proxitane |

# Nodavirus

| Concentration pathogen                     | Disinfectant          | Contact<br>time | Concentration disinfectant | рН | Temp. | Result            | Method  | Reference | Comments                       |
|--|-----------------------|-----------------|----------------------------|----|-------|-------------------|---|-----------|--------------------------------|
| 10 <sup>6,375</sup> TCID <sub>50</sub> /ml | Virkon<br>(peroxygen) | 5 min           | 1:125 w/v                  |    | 15    | 3,8 log reduction | Distilled water Tested after 5, 15 and 30 min.  | 30        | Isolate: sea bass<br>nodavirus |
| 10 <sup>6,375</sup> TCID <sub>50</sub> /ml | Virkon<br>(peroxygen) | 30 min          | 1:125 w/v                  |    | 15    | 3,3 log reduction | Distilled water Tested after 5, 15 and 30 min.  | 30        | Isolate: sea bass<br>nodavirus |
| 10 <sup>6,375</sup> TCID <sub>50</sub> /ml | Virkon<br>(peroxygen) | 5 min           | 1:500 w/v                  |    | 15    | Stable            | Distilled water Tested after 5, 15 and 30 min.  | 30        | Isolate: sea bass<br>nodavirus |
| 10 <sup>6,375</sup> TCID <sub>50</sub> /ml | Virkon<br>(peroxygen) | 30 min          | 1:500 w/v                  |    | 15    | 3,3 log reduction | Distilled water Tested after 5, 15 and 30 min.  | 30        | Isolate: sea bass<br>nodavirus |
| 10 <sup>6,375</sup> TCID <sub>50</sub> /ml | Virkon<br>(peroxygen) | 5 min           | 1:125 w/v                  |    | 15    | 2,8 log reduktoin | HBSS+calf serum. Tested after 5, 15 and 30 min. | 30        | Isolate: sea bass<br>nodavirus |
| 10 <sup>6,375</sup> TCID <sub>50</sub> /ml | Virkon<br>(peroxygen) | 30 min          | 1:125 w/v                  |    | 15    | 2,8 log reduktoin | HBSS+calf serum. Tested after 5, 15 and 30 min. | 30        | Isolate: sea bass<br>nodavirus |
| 10 <sup>6,375</sup> TCID <sub>50</sub> /ml | Virkon<br>(peroxygen) | 30 min          | 1:500 w/v                  |    | 15    | Stable            | HBSS+calf serum. Tested after 5, 15 and 30 min. | 30        | Isolate: sea bass<br>nodavirus |

# Oncorhynchus masou virus

| Concentration pathogen | Disinfectant      | Contact time | Concentration disinfectant | рН | Temp. | Result        | Method | Reference | Comments |
|------------------------|-------------------|--------------|----------------------------|----|-------|---------------|--------|-----------|----------|
|                        | kaliumpermanganat | 30 sec       | 32                         |    | 0     | "inactivated" |        | 35        |          |
|                        | kaliumpermanganat | 30 sec       | 16                         |    | 15    | "inactivated" |        | 35        |          |
|                        | kaliumpermanganat | 30 sec       | 16                         |    | 25    | "inactivated" |        | 35        |          |

#### Ranavirus

| Concentration pathogen     | Disinfectant | Contact<br>time | Concentration disinfectant | рН | Temp. | Result        | Method | Reference | Comments                          |
|----------------------------|--------------|-----------------|----------------------------|----|-------|---------------|--------|-----------|-----------------------------------|
| 1 x 10 <sup>7</sup> PFU/ml | VirkonS      | 1 min           | 1%                         |    | 22    | "inactivated" |        | 14        | Isolate from American<br>bullfrog |

#### Aeromonas salmonicida

| Concentration pathogen | Disinfectant   | Contact<br>time | Concentration disinfectant | рН | Temp. | Result            | Method   | Reference | Comments                         |
|------------------------|--|-----------------|----------------------------|----|-------|-------------------|--|-----------|----------------------------------|
|                        | pentakalium<br>bis<br>(peroxymonsul<br>fate) bis<br>(sulfat) | 30 min          | 0,5 % (w/v)                |    | 4     | > 5 log reduction | Tested at 0,01, 0,05, 0,1, 0,2, 0,5 and 1%. Hard water, high organic load.   | 49        | Contains > 10% available oxygen. |
|                        | peracetic<br>acid/hydrogen<br>peroxid                        | 30 min          | 0,1% (w/v)                 |    | 4     | > 5 log reduction | Tested at 0,05, 0,1, 0,2, 0,33<br>and 0,5%. Hard water, high<br>organic load | 49        |                                  |

# Carnobacterium piscicola

| Concentration pathogen | Disinfectant   | Contact<br>time | Concentration disinfectant | рН | Temp. | Result            | Method   | Reference | Comments                         |
|------------------------|--|-----------------|----------------------------|----|-------|-------------------|--|-----------|----------------------------------|
|                        | pentakalium<br>bis<br>(peroxymonsul<br>fate) bis<br>(sulfat) | 30 min          | 0,5 - 1% (w/v)             |    | 4     | > 5 log reduction | Tested at 0,01, 0,05, 0,1, 0,2, 0,5 and 1%. Hard water, high organic load.   | 49        | Contains > 10% available oxygen. |
|                        | peracetic<br>acid/hydrogen<br>peroxid                        | 30 min          | 0,2 % (v/v)                |    | 4     | > 5 log reduction | Tested at 0,05, 0,1, 0,2, 0,33<br>and 0,5%. Hard water, high<br>organic load | 49        |                                  |

#### Edwardsiella tarda

| Concentration pathogen | Disinfectant                  | Contact<br>time | Concentration disinfectant | рН | Temp. | Result            | Method  | Reference | Comments  |
|------------------------|-------------------------------|-----------------|----------------------------|----|-------|-------------------|---|-----------|---|
| 10 <sup>7</sup> CFU/ml | H <sub>2</sub> O <sub>2</sub> | 20 min          | 1600 ppm                   |    | 20    | > 5 log reduction | Dilution 1:1 of bacteria and disinfectant (25, 50, 100, 200, 400, 800, 1600, 3200 ppm). Growht at 20°C and counting already after 24 hours. | 58        | The actual concentration of disinfectant probably only half of stated in article table. Generally speaking the neccessary concentration for disinfection in this paper is much higher than published in other papers. |
| 10 <sup>7</sup> CFU/ml | H <sub>2</sub> O <sub>2</sub> | 60 min          | 1600 ppm                   |    | 20    | > 5 log reduction | Dilution 1:1 of bacteria and disinfectant (25, 50, 100, 200, 400, 800, 1600, 3200 ppm). Growht at 20°C and counting already after 24 hours. | 58        | The actual concentration of disinfectant probably only half of stated in article table. Generally speaking the neccessary concentration for disinfection in this paper is much higher than published in other papers. |

# Lactococcus garviae

| Concentration pathogen | Disinfectant   | Contact time | Concentration disinfectant | рН | Temp. | Result            | Method   | Reference | Comments                         |
|------------------------|--|--------------|----------------------------|----|-------|-------------------|--|-----------|----------------------------------|
|                        | pentakalium<br>bis<br>(peroxymonsul<br>fate) bis<br>(sulfat) | 30 min       | 0,5 - >1% (w/v)            |    | 4     | > 5 log reduction | Tested at 0,01, 0,05, 0,1, 0,2, 0,5 and 1%. Hard water, high organic load.   | 49        | Contains > 10% available oxygen. |
|                        | peracetic<br>acid/hydrogen<br>peroxid                        | 30 min       | 0,2 - 0,3 % (v/v)          |    | 4     | > 5 log reduction | Tested at 0,05, 0,1, 0,2, 0,33<br>and 0,5%. Hard water, high<br>organic load | 49        |                                  |

# Streptococcus sp

| Concentration pathogen | Disinfectant                  | Contact<br>time | Concentration disinfectant | рН | Temp. | Result          | Method  | Reference | Comments  |
|------------------------|-------------------------------|-----------------|----------------------------|----|-------|-----------------|---|-----------|---|
| 10 <sup>7</sup> CFU/ml | H <sub>2</sub> O <sub>2</sub> | 20 min          | 3200 ppm                   |    | 20    | 5 log reduction | Dilution 1:1 of bacteria and disinfectant (25, 50, 100, 200, 400, 800, 1600, 3200 ppm). Growht at 20°C and counting already after 24 hours. | 58        | The actual concentration of disinfectant probably only half of stated in article table. Generally speaking the neccessary concentration for disinfection in this paper is much higher than published in other papers. |
| 10 <sup>7</sup> CFU/ml | H <sub>2</sub> O <sub>2</sub> | 60 min          | 1600 ppm                   |    | 20    | 4 log reduction | Dilution 1:1 of bacteria and disinfectant (25, 50, 100, 200, 400, 800, 1600, 3200 ppm). Growht at 20°C and counting already after 24 hours. | 58        | The actual concentration of disinfectant probably only half of stated in article table. Generally speaking the neccessary concentration for disinfection in this paper is much higher than published in other papers. |

# Vibrio sp.

| Concentration pathogen | Disinfectant                  | Contact time | Concentration disinfectant | рН | Temp. | Result          | Method  | Reference | Comments  |
|------------------------|-------------------------------|--------------|----------------------------|----|-------|-----------------|---|-----------|---|
| 10 <sup>7</sup> CFU/ml | H <sub>2</sub> O <sub>2</sub> | 20 min       | 1600 ppm                   |    | 20    | 4 log reduction | Dilution 1:1 of bacteria and disinfectant (25, 50, 100, 200, 400, 800, 1600, 3200 ppm). Growht at 20°C and counting already after 24 hours. | 58        | The actual concentration of disinfectant probably only half of stated in article table. Generally speaking the neccessary concentration for disinfection in this paper is much higher than published in other papers. |
| 10 <sup>7</sup> CFU/ml | H <sub>2</sub> O <sub>2</sub> | 60 min       | 800 ppm                    |    | 20    | 4 log reduction | Dilution 1:1 of bacteria and disinfectant (25, 50, 100, 200, 400, 800, 1600, 3200 ppm).   | 58        | The actual concentration<br>of disinfectant probably<br>only half of stated in  |

|  |  |  | Growht at 20°C and counting | article table. Generally   |
|--|--|--|-----------------------------|----------------------------|
|  |  |  | already after 24 hours.     | speaking the neccessary    |
|  |  |  |                             | concentration for          |
|  |  |  |                             | disinfection in this paper |
|  |  |  |                             | is much higher than        |
|  |  |  |                             | published in other         |
|  |  |  |                             | papers.                    |

# Yersinia ruckeri

| Concentration pathogen | Disinfectant   | Contact<br>time | Concentration disinfectant | рН | Temp. | Result            | Method   | Reference | Comments                         |
|------------------------|--|-----------------|----------------------------|----|-------|-------------------|--|-----------|----------------------------------|
|                        | pentakalium<br>bis<br>(peroxymonsu<br>Ifate) bis<br>(sulfat) | 30 min          | 0,5 % (w/v)                |    | 4     | > 5 log reduction | Tested at 0,01, 0,05, 0,1, 0,2, 0,5 and 1%. Hard water, high organic load.   | 49        | Contains > 10% available oxygen. |
|                        | peracetic<br>acid/hydrogen<br>peroxid                        | 30 min          | 0,2 % (v/v)                |    | 4     | > 5 log reduction | Tested at 0,05, 0,1, 0,2, 0,33<br>and 0,5%. Hard water, high<br>organic load | 49        |                                  |

#### **Parasites**

# Ichthybodo necator

| Concentration pathogen | Disinfectant                         | Contact time | Concentration disinfectant | рН | Temp. | Result   | Method                                    | Reference | Comments |
|------------------------|--------------------------------------|--------------|----------------------------|----|-------|----------|---|-----------|----------|
|                        | Detarox (20%                         |              |                            |    |       |          | 2 treatments of naturally                 |           |          |
|                        | H <sub>2</sub> O <sub>2</sub> , 4-5% |              |                            |    |       | All dead | 2 treatments of naturally infected trout. | 45        |          |
|                        | peracetic acid)                      |              |                            |    |       |          | imected trout.                            |           |          |

# Ichthyophthirius multifiliis

| Concentration pathogen | Disinfectant | Contact<br>time | Concentration disinfectant | рН | Temp. | Result | Method               | Reference | Comments  |
|------------------------|--------------|-----------------|----------------------------|----|-------|--------|----------------------|-----------|---|
|                        | H2O2         | 10 hours        | 50 μl/l                    |    | 20    | Stable | In vitro - trophonts | 66        |   |
|                        | perotan      | 10 hours        | 50 μl/l                    |    | 20    | Stable | In vitro - trophonts | 66        | perotan: $H_2O_2$ + acetic acid                 |
|                        | perotan      | 10 hours        | 100 μΙ/Ι                   |    | 20    | Døde   | In vitro - trophonts | 66        | perotan: H <sub>2</sub> O <sub>2</sub> + acetic |

|  |         |          |         |    |        |                      |    | acid |
|--|---------|----------|---------|----|--------|----------------------|----|------|
|  | VirkonS | 10 hours | 50 μl/l | 20 | Stable | In vitro - trophonts | 66 |      |

# Myxosoma cerebralis

| Concentration pathogen | Disinfectant                  | Contact<br>time | Concentration disinfectant | рН | Temp.         | Result   | Method                             | Reference | Comments |
|------------------------|-------------------------------|-----------------|----------------------------|----|---------------|----------|------------------------------------|-----------|----------|
|                        | H <sub>2</sub> O <sub>2</sub> | 10 min          | 8,5 %                      |    | room<br>temp. | Survival | in vitro. Triactinomyxon<br>spores | 96        |          |
|                        | H <sub>2</sub> O <sub>2</sub> | 10 min          | 10,2%                      |    | room<br>temp. | All dead | in vitro. Triactinomyxon<br>spores | 96        |          |
|                        | KMnO <sub>4</sub>             | 14 days         | 1%                         |    | 22            | Survival | In vitro - spores                  | 42        |          |

# Trichodina jadranica

| Concentration pathogen | Disinfectant                                  | Contact<br>time | Concentration disinfectant | рН | Temp. | Result         | Method      | Reference | Comments   |
|------------------------|---|-----------------|----------------------------|----|-------|----------------|-------------|-----------|--|
| 2,4                    | Detarox (20%<br>H2O2, 4-5%<br>peracetic acid) | 24 hours        | 45 ppm                     |    | 25    | Survival (0,4) | In vivo, ål | 75        | Catergorization<br>(category/number of<br>parasites on ell): 0/0,<br>1/1-10, 2/11-100, 3/100-<br>1000, 4/>1000 |
| 2,7                    | H2O2  | 4 hours         | 1000 ppm                   |    | 25    | Survival (2,3) | In vivo, ål | 75        | Catergorization<br>(category/number of<br>parasites on ell): 0/0,<br>1/1-10, 2/11-100, 3/100-<br>1000, 4/>1000 |
| 2,0                    | kaliumperman<br>ganat                         | 24 hours        | 20 ppm                     |    | 25    | All dead       | In vivo, ål | 75        | Catergorization<br>(category/number of<br>parasites on ell): 0/0,<br>1/1-10, 2/11-100, 3/100-<br>1000, 4/>1000 |
| 2,6                    | VirkonPF                                      | 24 hours        | 20 ppm                     |    | 25    | All dead       | ln vivo, ål | 75        | Catergorization<br>(category/number of<br>parasites on ell): 0/0,<br>1/1-10, 2/11-100, 3/100-<br>1000, 4/>1000 |

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