# Field trial evaluation of povidone iodine as an effective disinfectant for different stages of returning spawners salmon

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Received July 25, 2007

**Abstract**

Reaching with the fertilized gametes to the production stage is a critical process for the success of aquaculture and restoration of wild fish programs worldwide. Iodophors are routinely used to disinfect both broodstocks and fertilized eggs to control vertical as well as horizontal transmission of fish pathogens. During three successive spawning cycles, the specific fish pathogens *Aeromonas salmonicida* and *Renibacterium salmoninarum* associated with typical clinical picture have been isolated from examined salmon before gamete collection. The prevalence approximately reached 51% and 79% for *A. salmonicida* and *R. salmoninarum* respectively. On the other hand, the *R. salmoninarum* was isolated from the collected eggs of the broadstocks used for spawning in the above mentioned cycles. Average prevalence of infection in tested eggs was 15%. At the pre-rinsing cycles, the bacterial load was determined as $1.2 \times 10^3$ and $0.6 \times 10^2$ CFU/plate for *A. salmonicida* and *R. salmoninarum* in spawners respectively. On the level of fertilized eggs, the load was determined as $0.2 \times 10^3$ CFU/plate and $0.1 \times 10^3$ for *A. salmonicida* and *R. salmoninarum* respectively. In the following spawning cycle, the efficacy of povidone iodine as surface disinfectant for returning spawners and fertilized eggs of Chinook salmon were tested. Concentrations of 60 mg/L povidone iodine for 30 minutes as initial dose followed by a maintenance dose of 70 mg/L for 10 minutes were applied as rinsing solutions for ♂ and ♀ Chinook salmon spawners. Eggs were shell-hardened in 80 mg /L povidone iodine for 30 minutes. Isolation trials from equal number of post rinsing spawner Chinook salmon and fertilized eggs indicated a sharp decline in bacterial colonies number per plate for spawners and eggs. The achieved post-rinsing results are highly indicative for the efficacy of povidone iodine as efficient disinfectant for both fish and eggs. Ultimately, the current study will ensure the production of fish populations with less bacterial load and consequent potent health status. [Life Science Journal. 2007; 4(3): 87 – 93 ] (ISSN: 1097 – 8135).

**Keywords:** iodine; spawners salmon; BKD

## 1 Introduction

Fish health poses major challenges to development and progress of the salmon restoration in the Great Lakes basin. Among these health challenges, Bacterial Kidney Disease(BKD), caused by *Renibacterium salmoninarum* (*R. salmoninarum*) and Furunculosis caused by *Aeromonas salmonicida* (*A. salmonicida*) . Both diseases represent an eminent threat due to the enzootic nature of their pathogens within Great Lakes basin (Eissa, 2005). Moreover, the obligate intracellular nature and the selective affinity of *R. salmoninarum* for the kidney makes this pathogen and its soluble antigens a major threat to the host by compromising the fish immune response (Ellis, 1999; Fredriksen, 1997; Grayson, 2002).

Both BKD and Furunculosis, usually result in massive mortalities when hit a large salmonid population especially during the spawning season. Spawning is a stressful physiological process for both female and male broodstocks. Female Chinook salmon are usually stressed with their relatively large sized egg-laden ovaries that occupy more than 60% of the fish body during the spawning season. Mature ripened male Chinook traveling along the

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river during their migration trip might show up a severe form of cannibalism and spatial disorientation (Reno, 1998). The cohabitating Chinook salmon are coexisting together in very tiny space while crossing the different natural strata and electric dams along the river till reaching their terminal fate in the weir (Reno, 1998). These cohabitating gravid salmon are under continuous physical stress. This physical kind of stress as well as growing sex-hormonal changes associated with spawning are potent stimulus for long-lasting corticosterol release which initiates a cascade of immunosuppressive events involving both humoral and cellular mediated immunity (Eissa, 2005; Pickering, 1989).

The eminent threats posed to salmonid population due to enzootic nature and multiple routes of infection (horizontal and vertical) exhibited by the two diseases were a rich media for pathogen-host-management interaction studies during the preceding few decades. The precise hygienic regime formed an infrastructure for maintaining a successful management plan for any fish rearing system worldwide. Disinfectants are among the cornerstones that maintain such successful hygienic strategy. Throughout the past half century, a remarkable number of disinfectants were applied in fish hatcheries to combat fish pathogens with different degrees of success. Chlorine, aldehydes, phenolic compounds, ozone and others were among the used disinfectants. A large number of these compounds and their derivatives proved their toxicity to the fish and their gametes when applied in close contact to their live tissues. Thus, the look for a new safe disinfectant that can be used efficiently for fish, gametes as well as hatchery utensils was an evolving demand.

Long term studies performed in this field during the preceding years indicated that iodophores are the most reliable surface disinfectants that can efficiently used to control the spread of fish pathogens with wide safety margin. However, some authors indicated minimal adverse effects on fish eggs when exposed to unjust concentrations of iodophores. Others indicated that rinsing of broodstocks in iodophore solution before collecting gametes could minimize the *R. salmoninarum* as well as other egg-shell contaminating pathogens (Ross and Smith, 1972). Moreover; few authors such as Evelyn (1984) indicated the inability of iodophores to completely reduce the intraovum infection of *R. salmoninarum*. Further, Jodun and Millard (2001) concluded that, if a greater disinfection efficacy is desired, an increase in iodophor concentration may be preferable to an increase in contact time.

The importance of the current study originates from the fact that most of the previous studies involved the use of iodophores on only one stage of the fish production cycle for a shorter duration while ours presents a long term study that involves different stages of fish production cycle including broodstocks, fertilized eggs and embryos.

## 2 Materials and Methods

### 2.1 Fish and eggs

Throughout three years period a total of 680 feral spawning salmonid were collected from Michigan weirs. Fish included 300, 200, 150 and 200 returning Chinook salmon (*Oncorhynchus tshawytscha*) collected from the Little Manistee River Weir (LMRW), Manistee County, Michigan (Lake Michigan watershed). The sacrifice of the feral spawners entailed exposing the fish to carbon dioxide-laden water, followed by a blow to the head (Eissa, 2005). Following gamete collection, fish were visually inspected for any external skin lesions pathognomonic for Furunculosis and the abdominal cavity was cut open to examine individual internal organs for signs associated with BKD, followed by the collection of approximately one gram of tissue from anterior, posterior and middle kidney sections. Cross contamination was avoided by replacing dissecting tools with sterile tools following the dissection of each fish. Eggs were collected in clean disinfected buckets from mature ripened female salmon then mixed with milt stripped from mature ripened male salmon (milt were collected directly from the middle stream). The egg-milt mixture was diluted with chilled water in a ratio of 1/3 egg-milt / 2/3 water then left for two hours till transported to the state hatchery in secured clean vehicles (Eissa, 2005). In the hatchery the eggs were egg hardened in 80 mg/L povidone iodine for 30 minutes. Fertilized eggs (eyed eggs) were incubated in thoroughly disinfected egg trays of an incubator with constant chilled water flow that covers fertilized eggs all the time of incubation.

### 2.2 Sampling and sample processing

Swabs from the skin lesions and kidney samples representing the anterior, posterior, and middle sections of the kidney were transferred in sterile 7.5 cm × 18.5 cm Whirl Pak® bags (Nasco, Forte Atkinson, and WI), kept on ice, and were softened as much as possible through multiple cycles of physical pressure. The homogenized skin and kidney tissues were diluted in 1:4 (w/v) Hank's Balanced Salt Solution (HBSS, Sigma Chemical Co, St. Louis, MO, USA) and then stomached for 2 minutes at high-speed using the Biomaster Stomacher-80 (Wolf Laboratories Limited, Pocklington, York, UK) (Eissa, 2005). In the case of fertilized eggs, a random amount of fertilized eggs were divided into two equal halves then one half was diluted with HBSS in a ratio of 1 whole egg mixture: 2 HBSS (v/

v) then stomached for 2 minutes at high-speed till mixture got completely homogenized. The second half of fertilized eggs were clean washed several times with sterile HBSS to get rid of any surface contaminant then dissected using a clean dissecting tools under dissecting microscope was previously placed under the laminar flow. Embryos were retrieved from the dissected eggs then similarly treated using the same method adopted for homogenization of eggs (Eissa, 2005).

### 2.3 Culture

Isolation of *A. salmonicida* was performed according to Cipriano and Bullock 2001: One hundred microliter (100 µl) aliquots of stomached skin tissue were spread onto tryptic soy agar (TSA, Remel, Lenexa, Kansas, USA) and Coomassie brilliant blue agar (CBBA) (Cipriano and Bertolini, 1988). Coomassie brilliant blue agar was prepared by adding 0.1 g Coomassie brilliant blue (Sigma-Aldrich) powder to 1 liter of TSA agar. Inoculated TSA and CBBA plates were incubated at 22 °C for 24 – 48 h. Representative colonies were selected and re-streaked for isolation on TSA and again incubated at 22oC for 24 – 48 h. Identification tests were subsequently performed on these pure isolates.

Isolation of *R. salmoninarum* was performed according to Eissa 2005: One hundred microliter of the suspension was added to one end of the Selective Kidney Diseases Medium (SKDM) (Austin *et al*, 1983) in the 2nd cycle and Modified Kidney Disease Medium (MKDM) (Eissa, 2005) plate during the third cycle and then spread over the surface using a sterile bacteriological loop. Inoculated plates were incubated at 15 °C for up to 2 weeks and were checked periodically for growth using an inverted dissecting microscope, thus allowing the detection of early colonial growth.

### 2.4 Isolate confirmation

*R. salmoninarum* isolates were confirmed using the biochemical criteria reported in Sander and Fryer (1980) and Austin and Austin (1999). A number of biochemical tests on each isolate were performed including motility, using motility test medium (DIFCO-BD and Company Sparks, MD, USA), cytochrome oxidase with Pathotec strips (Remel), catalase test with 3% hydrogen peroxide, hydrolysis of esculin using bile esculin agar (Remel), and DNAse test using DNAse test medium (Remel). Carbohydrate utilization was performed using basal media (DIFCO-BD). Each one of the following sugars was added individually to the basal medium to test for the utilization of each sugar: arabinose, glucose, lactose, maltose, rhamnose, salicin, sucrose, sorbitol, xylose. All sugars were from Sigma. Results of biochemical tests were matched against standard *R. salmoninarum* biochemical characters described by Bruno and Munro (1986a).

*A. salmonicida* isolates were presumptively identified using conventional biochemical tests including; catalase with 3% hydrogen peroxide solution, cytochrome oxidase with Pathotec oxidase strips (Remel), motility in motility test medium (BD and Company Sparks, MD, USA), citrate utilization using Simmons Citrate (Remel), sugar utilization using triple sugar iron (TSI, Remel) , oxidation and fermentation of glucose using OF Basal media with glucose as soul carbohydrate source (BD Diagnostics, Sparks, MD, USA), esculin hydrolysis using bile esculin agar (Remel), presence of poly A layer using uptake of coomassie blue on Coomossie Brilliant Blue Agar and presence of brown diffusible pigment after 48 h of growth on TSA. Further biochemical testing was performed using API20E and 20NE tests (BioMerieux Inc, Durham, NC) which incubated at 22 °C and interpreted at 48 – 72 hours according to manufacturer's instructions.

### 2.5 Nested PCR

Single *R. salmoninarum* colonies were identified using highly specific oligonucleotide primers designed by Pascho *et al* (1998), which amplify a region of the gene encoding the *R. salmoninarum* p57 antigen in a nested polymerase chain reaction (nPCR). The nPCR assay using these primers is considered the method of choice to confirm *R. salmoninarum* isolates (Pascho and Elliott, 2004). The DNA extraction method is the method used by Chase and Pascho (1998) and modified by Eissa (2005).

Single *A. salmonicida* colonies were identified using highly specific oligonucleotide primers designed by Miyata *et al* (1996). The extracted DNA was amplified using oligonucleotide primer set specific for *A. salmonicida* sub sp. *salmonicida* (Miyata *et al*, 1996). The controls consisted of a PCR mixture without DNA template (negative control) and with DNA extracted from known *A. salmonicida* sub sp. *salmonicida* (positive control). The PCR products were electrophoresed in 2% agarose gel (Invitrogen Corporation), stained with ethidium bromide, visualized with ultra violet light and photographed using Kodak EDAS System (Eastman Kodak Company, Rochester, NY). Samples were considered positive when a 512 bp band was detected.

### 2.6 Povidone iodine

A buffered 1% Iodine solution which contains 10% Povidone-Iodine complex was used in the current experiment (Ovadine, Western Chemical Incorporation-Ferndale, Washington).

## 3 Results

### 3.1 Returning spawners

A total number of 650 chinook salmon spawners were tested for the *A. salmonicida* and *R. salmoninarum* infection during the period covering three successive spawning cycles. The average prevalence of *R. salmoninarum* in chinook salmon returning to the Little Manistee Weir (LMRW) were 51.6% compared to 79% as an average for *A. salmonicida* infection in the same group.

In the first spawning cycle, the *A. salmonicida* pathogen was isolated from a total of 210 from 300 spawner fish skin with prevalence of 70%. The average colony forming units (CFU) procured from the CBBA and TSA cultured plates was $1.7 \times 10^3$ CFU/plate. On the other hand, *R. salmoninarum* were isolated from a total number of 246 spawner fish kidney with prevalence of 82%. The average colony forming units (CFU) counted on MKDM cultured plates was $0.6 \times 10^2$ CFU/plate (Table 1).

In the second cycle the prevalence of *A. salmonicida* noticeably decreased to 50% and the average CFU procured from the CBBA and TSA cultured plates was $2.5 \times 10^2$ CFU/plate. The *R. salmoninarum* were isolated from a total number of 150 spawner fish kidney with prevalence of 75%. The average CFU counted on MKDM cultured plates were $0.5 \times 10^2$ CFU/plate (Table 1).

In the third cycle the prevalence of *A. salmonicida* gradualy decreased to 35% and the average CFU gained from the CBBA and TSA cultured plates was $1.2 \times 10^3$ CFU/plate. The *R. salmoninarum* were isolated from a total number of 150 spawner fish kidney with prevalence of 80%. The average CFU counted on MKDM cultured plates were $1.2 \times 10^2$ CFU/plate (Table 1).

**Table 1.** Prevalence of *A. salmonicida* and *R. salmoninarum* infection in three successive spawning cycles from Chinook salmon

| Cycle number | | Cycle 1 | Cycle 2 | Cycle 3 |
|---|---|---|---|---|
| Number of fish/cycle | | 300 | 200 | 150 |
| *A. salmonicida* | % of fish infected | 70 | 50 | 35 |
| | Average CFU*/plate ($\times 10^3$) | 1.7 | 0.25 | 1.2 |
| *R. salmoninarum* | % of fish infected | 82 | 75 | 80 |
| | Average CFU*/plate ($\times 10^2$) | 0.6 | 0.5 | 1.2 |

### 3.2 Fertilized eggs and embryos

Isolation of *A. salmonicida* from the fertilized egg homogenates was only performed during the third pre-rinsing spawning cycle with an average prevalence of 35%. The total colony count obtained on the cultured plates was $0.3 \times 10^2$ CFU. Representative samples from the fertilized eggs have been dissected under the inverted dissecting microscope, then homogenized and the homogenate was tested for the infection with *A. salmonicida*. The results indicated that the embryos were well protected from being infected with the pathogen (Table 2).

**Table 2.** Prevalence of *A. salmonicida* and *R. salmoninarum* infection in eggs collected during three successive spawning cycles from Chinook salmon

| Cycle number | | Cycle 1 | Cycle 2 | Cycle 3 |
|---|---|---|---|---|
| *A. salmonicida* | % of eggs infected | ND | ND | 35 |
| | Average CFU*/plate ($\times 10^2$) | | | 0.3 |
| | % of embryos infected | | | 0 |
| | Average CFU*/plate ($\times 10^2$) | | | 0 |
| *R. salmoninarum* | % of eggs infected | 20 | 10 | 15 |
| | Average CFU*/plate ($\times 10^2$) | 0.4 | 0.1 | 0.2 |
| | % of embryos infected | 9 | 6 | 6.5 |
| | Average CFU*/plate ($\times 10^2$) | 0.1 | 0.3 | 0.25 |

Note: ND: Not Done; *CFU: Colony Forming Unit

The case was different when the intracellular pathogen *R. salmoninarum* considered. In the first cycle: 20% of the fertilized eggs were infected with the pathogen, regardless the infection being intra-ovum or on the egg shell. The colony count of the recovered bacterial colonies was slightly higher than that of *A. salmonicida* from the same fertilized eggs ($0.4 \times 10^2$ CFU). Moreover, embryos procured from the same eggs exhibited an average of 9% infection with total colony count of $0.1 \times 10^2$ CFU/plate. The pathogen was isolated from the fertilized eggs with an average prevalence of 10%, 15% for the 2nd and 3rd cycles successively while the total colony count increased from $0.1 \times 10^2$ CFU to $0.2 \times 10^2$ CFU/plate. The embryos showed slightly lesser prevalence of infection presented by 6% and 6.5% for 2nd and 3rd cycles successively. The total colony count was $0.3 \times 10^2$ CFU and $0.25 \times 10^2$ CFU/plate successively (Table 4).

Isolation trials from equal number of post rinsing spawner Chinook salmon and fertilized eggs indicated a

sharp decline in bacterial colonies number per plate (25 colonies per plate for *A. salmonicida* and 10 colonies/ plate for *R. salmoninarum*) for spawners and 30 CFU/ plate and 20 CFU/plate (for *A. salmonicida* and *R. salmoninarum* respectively) for eggs (Tables 3 and 4). The achieved post-rinsing results are highly indicative for the efficacy of povidone iodine as efficient disinfectant for both fish and eggs.

**Table 3.** Spawning cycle 4: Pre-rinsing and post-rinsing results of povidone iodine application on spawners Chinook salmon

| Pathogen | Prevalence | Pre-rinsing | Post-rinsing |
|---|---|---|---|
| *A. salmonicida* | % of fish infected | 60 | 51 |
| | Average CFU*/plate ($\times 10^3$) | 1.3 | 0.025 |
| *R. salmoninarum* | % of fish infected | 78 | 79 |
| | Average CFU*/plate ($\times 10^2$) | 2 | 0.1 |

**Table 4.** Spawning cycle 4: Pre-rinsing and post-rinsing results of povidone iodine application on Chinook salmon whole eggs and embryos

| Prevalence of *A. salmonicida* and *R. salmoninarum* | | Pre-rinsing | | Post-rinsing | |
|---|---|---|---|---|---|
| | | Eggs | Embryos | Eggs | Embryos |
| *A. salmonicida* | % of eggs infected | 25 | 0 | 10 | ND |
| | Average CFU*/ plate ($\times 10^2$) | 2 | 0 | 0.3 | 0 |
| *R. salmoninarum* | % of eggs infected | 10 | 2 | 79 | ND |
| | Average CFU*/ plate ($\times 10^2$) | 1 | 0.1 | 0.2 | 0.1 |

Note: ND: Not Done; *CFU: Colony Forming Unit

## 4 Discussion

Sanitation is one of the cornerstones of fish health management in modern aquaculture. A number of infectious diseases, particularly external bacterial infections, may be directly attributed to accumulation of organic debris in the aquaculture facility. Ponds may be disinfected between groups of fish by draining, drying, and in some instances by use of chemical disinfectants such as hydrated lime. For smaller systems such as tanks and aquaria, debris should routinely be removed from the system by siphon hose and equipments should be disinfected between aquaculture facilities. Chemical disinfectants should be used to disinfect fish facility between groups of fish. On the other side iodophors are routinely used to disinfect both broodstocks and fertilized eggs to control vertical as well as horizontal transmission of fish pathogens. Office of International Epizootics (OIE) and most of the international fish health organizations policies require that eggs be disinfected in 50 mg/L of iodophors for 30 minutes at the facility where those eggs are fertilized. If the same eggs are shipped to a second facility, policy further states that those eggs must undergo a second disinfection in 100 mg/L of iodophors for 10 minutes at the receiving station.

The results of the current experiment demonstrated that the specific fish pathogens *A. salmonicida* and *R. salmoninarum* associated with typical clinical picture have been isolated from examined salmon broodstocks before gamete collection throughout the three successive spawning cycles with different prevalence and intensity of infection. The proximity of infection approached 51% and 79% for *A. salmonicida* and *R. salmoninarum* respectively. The isolation of the above mentioned pathogens from both broodstocks and fertilized eggs during the entire period confirms the existence of an ideal cause for enzoocity of both diseases in salmonids from the Great Lakes basin which coincide with those described by (Hnath and Faisal, 2005; Eissa, 2005) for the *R. salmoninarum* and (Cipriano and Bullock, 2001) for *A. salmonicida* infections. Meanwhile, the isolation of both organisms from the homogenate of the fertilized eggs support the fact that such pathogens can be vertically transmitted regardless being inside or outside the egg (Evelyn *et al*, 1984; Evelyn *et al*, 1986a,b; Eissa, 2005; Cipriano and Bullock, 2001). However, the failure to isolate *A. salmonicida* from the embryos dissected from the eyed eggs at any of the three successive spawning cycles strictly confirms the fact that the pathogen can be transmitted by egg-shell contamination and not by the intra-ovum route which is in complete agreement with those reported by McCarthy (1977) and Bullock and Stuckey (1987).

On the contrary, *R. salmoninarum* was isolated from both whole egg- homogenate and embryos dissected from eyed eggs at average prevalence 15% for eyed eggs and 7% for embryos ideally strengthen the fact that such obligate intracellular pathogen can be vertically transmitted by both egg-shell contamination and via the intra-ovum route as documented by several authors during the past three decades (Eissa, 2005; Evelyn *et al*, 1984; Bruno and Munro, 1986b).

The sharp decrease of prevalence of *R. salmoninarum*

infection among chinook salmon spawners could be due to the culling procedures adopted in weirs which could shared in minimizing the possibility of vertical transmission through infected females to their progeny. Rinsing the spawner fish in povidone iodine in concentrations of 60 mg/L for 30 minutes as initial dose followed by a maintenance dose of 70 mg/L for 10 minutes before collecting eggs or milt might resulted in sequential minimization of total colony forming unit of *R. salmoninarum* retrieved from the spawners throughout the three spawning cycle which in part coincides with Ross and Smith (1972) and Maule *et al* (1996). The 10% Povidone-Iodine complex solution in such concentration would minimize the bacterial load covering the fish surfaces and in turn minimized the expected descend of the pathogen on eggshell and in milt. Although the internationally approved 10% povidone iodine is considered among the most efficient and safe disinfectants due to its organic nature, its effective concentration was moderately affected by the presence of excessive organic matter and fish mucus which enforced us to continuously replace the rinsing solution every now and then.

The marked decline of prevalence of *A. salmonicida* infection among the spawners Chinook salmon from 70% in the first spawning cycle to 35% in the third cycle with concurrent decline in bacterial load explains the proposed efficacy of the aforementioned povidone iodine dose as potent disinfectant for broodstocks before egg and milt collection during spawning process. This result runs parallel with that reported by Cipriano *et al* (2001) who obtained similar results after applying the povidone iodine on both ♂ and ♀ Atlantic salmon broodstocks in six successive spawning cycles.

Although the *R. salmoninarum* was isolated from the fertilized eggs with an average prevalence of 10%, 15% for the 2nd and 3rd cycles successively, the total colony count increased from $0.1 \times 10^2$ CFU to $0.2 \times 10^2$ CFU/plate. Using MKDM isolation medium during third cycle enhanced the bacterial growth on the isolation plates and in turn increased the CFU/plate in the third cycle (Eissa, 2005). The higher prevalence (35%) of *A. salmonicida* among the same examined fertilized eggs denotes the abundance (ubiquity) of such pathogen on both fish surfaces and water compared to the *R. salmoninarum* (Cipriano and Bullock, 2001). Despite the fact that *A. salmonicida* was isolated in a prevalence rate (35%) higher than that of *R. salmoninarum* (20%), the total CFU/plate of *R. salmoninarum* was slightly higher than that of *A. salmonicida* retrieved from the same fertilized eggs. This support the fact that *R. salmoninarum* can be transmitted by both egg-shell contamination as well as intra-ovum route (Evelyn *et al*, 1986a,b; Eissa, 2005), while *A. salmonicida* can only be transmitted by egg-shell contamination (Cipriano and Bullock, 2001) which implicitly translated into higher *R. salmoninarum* load in egg homogenate than do *A. salmonicida*. Also, these results might explain the marked ability of iodophores to reduce bacterial load on the egg surface with lesser effect on the intraovum infection which agrees in part with that reported by Evelyn *et al* (1984).

Isolation trials from equal number of post rinsing spawners and fertilized eggs during the fourth spawning cycle indicated a sharp decline in bacterial colonies number per plate (25 colonies per plate for *A. salmonicida* and 10 colonies / plate for *R. salmoninarum*) for spawners and 30 CFU/plate and 20 CFU/plate (for *A. salmonicida* and *R. salmoninarum* respectively) for eggs. The results obtained in the fourth post-rinsing cycle are considered as an ultimate fate for the preceded three successive cycles.

Finally, the results came in this study are rich subject for future research about the use of environmentally and biologically safe disinfectants that can efficiently minimizes disease occurrence among fish population with lesser effects on different stages of fish production as well as their enclosing environment .

## Acknowledgment

We are grateful to the managerial section of the Michigan DNR. This work could not come to light without their generous support.

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