Starliper 2001

Journal of Aquatic Animal Health 13:56–62, 2001

The Effect of Depuration on Transmission of *Aeromonas salmonicida* between the Freshwater Bivalve *Amblema plicata* and Arctic Char

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Abstract.—A model system was used to study bacterial fish pathogen transmission between the freshwater bivalve *Amblema plicata* and two strains (Nauyuk and Labrador) of Arctic char *Salvelinus alpinus*. *Aeromonas salmonicida*, the cause of fish furunculosis, was readily transmitted from Arctic char to *A. plicata* and vice versa via simple cohabitation. Clinical furunculosis was artificially established in Nauyuk Arctic char via horizontal exposure to Labrador Arctic char that received intraperitoneal injections of *A. salmonicida*. After the Nauyuk Arctic char began to die, *A. plicata* were placed in the tank with the fish. After 33 d of cohabitation, a group of 10 *A. plicata* was cultured, and *A. salmonicida* was isolated from all 10. The remaining *A. plicata* were transferred to other tanks being supplied with specific-pathogen-free water. At 1, 5, 15, and 30 d after transfer, 60 uninfected Labrador Arctic char were cohabitated with the *A. plicata*. Transmission of *A. salmonicida* from *A. plicata* to the Arctic char was evaluated via fish mortality and bacterial culture after 3–4 weeks of exposure. Mortality to *A. salmonicida* occurred in groups exposed to *A. plicata* after 1 and 5 d of depuration but not in groups exposed after 15 and 30 d. The bacterium was not isolated from either the *A. plicata* or the Arctic char in the 15- and 30-d groups. Results indicate that the current minimum 30-d quarantine of freshwater bivalves destined for relocation to prevent spread of zebra mussels *Dreissena polymorpha* is sufficient to allow depuration of a fish pathogen and, thus, to prevent the spread of disease.

There are approximately 300 species and subspecies of freshwater bivalves that are native to the waters of North America. Williams et al. (1993) reported that greater than 70% of the species and subspecies are categorized as endangered, threatened, or of special concern. The survival of freshwater bivalves depends on the quality of their environment. Furthermore, they utilize fish in part of their life cycle for development of glochidia; thus adverse effects placed on the intermediate fish hosts directly impact development of the bivalve larvae and subsequent numbers of juvenile animals. Examples of adverse effects include siltation from runoff due to agriculture or construction (Ellis 1936; Kat 1982) and waterway construction or activities such as dredging and dam building (Fuller 1974; Keller and Zam 1990). The combined effects of these activities with the inadvertent introduction of zebra mussels *Dreissena polymorpha* have caused concern for the continued survival of large-river native species (Hebert et al. 1991; Nalepa 1994).

The U.S. Fish and Wildlife Service along with other federal and state agencies initiated efforts in the mid-1990s aimed at conserving native freshwater bivalves in selected large-river systems despite the advancing spread of zebra mussels. One such effort was to collect native animals and transfer them to safe locations. The goal is to maintain and propagate them and to reintroduce them at an appropriate future date when impacts caused by zebra mussels are of lesser concern. Sites to maintain the native bivalves include salmonid-rearing hatcheries.

The concerns for preventing and the consequences of the transmission of fish pathogens via transfer of fish or fish eggs between facilities are well documented (Piper et al. 1982). This is particularly relevant to hatcheries because they typically rear fish under intensive culture, which may render the fish more susceptible to diseases (Wedemeyer 1996; 1997). Fish health policies include guidelines and restrictions based on the premise of preventing pathogen transfer to naïve facilities and fish. The primary means for prevention is via fish health inspections and assigned health certifications. The potential for transfer of fish pathogens also is relevant to relocation of freshwater bivalves from natural river waters to hatcheries. Bivalves, being filter feeders, could be exposed to fish pathogens in their environment and acquire them via filtering. The potential for pathogen transfer with the relocated bivalves is not known, nor is an effective time that is needed for bivalve

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Received April 24, 2000; accepted October 13, 2000

depuration of infectious agents. This study was done in an attempt to address these questions.

A model system was used to evaluate the transmission of pathogens between fish and bivalves. In a study by Starliper and Morrison (2000), groups of bivalves were exposed by waterborne challenge to various fish pathogenic and nonpathogenic bacteria previously isolated from fish. No mortality or other obvious adverse effects to the bivalves were noted after the challenge. However, one group that was exposed to *Aeromonas salmonicida,* the cause of furunculosis, followed by a 24-h period of depuration and subsequent cohabitation with a susceptible fish host resulted in disease and death to the fish. From this it was determined that this experimental situation could be used as a model to study the potential for pathogen transmission.

Methods

Amblema plicata were collected by brailing during the June 1999-collecting season from the Ohio River (river mile 175), near Muskingum Island and adjacent to Wood County, West Virginia. This region is not easily accessible for public use, and the mussel beds are protected by the Ohio River Islands National Wildlife Refuge. Harvesting and collection are only allowed by permit for scientific and management purposes. The *A. plicata* were quarantined for 60 d at an enclosed holding facility. Zebra mussel veligers were detected after the first 30 d; thus the *A. plicata* spent another 30 d in quarantine, at which time they were determined to be free of zebra mussels (Chaffee 1997). After quarantine, the *A. plicata* were transported by live well (truck) to the Leetown Science Center where they were placed in 566-L aquaria (density = 21.4 kg/m^3) supplied with 24°C reservoir water until used for experimental purposes. A substrate (20 cm deep) consisting of sand and small gravel covered the bottom of each aquarium; dissolved oxygen was between 8 and 9 mg/L, and the water flow was about 30 L/min. The *A. plicata* received food via the water that was comprised primarily of chlorophytes and diatoms at a density ranging from 2.00×10^3 to 2.90×10^5 cells/mL (R. Villella, U.S. Geological Survey, personal communication).

Two strains of Arctic char *Salvelinus alpinus* were used, provided by the Conservation Fund's Freshwater Institute, Shepherdstown, West Virginia. The Labrador strain averaged 250 g each, and the Nauyuk strain averaged about 500 g each. The fish were originally received as eggs from broodstock that were certified as specific-pathogen free (Office of the Federal Register 1999). The fish were held indoors, in 1,130-L circular tanks at a density of less than or equal to 70 kg/m^3 that were fed with flow-through spring water at 40 L/min (12°C; pH 7.0–7.2; dissolved oxygen was 10–12 mg/L). The artificial lighting was adjusted to conform to the natural day/night cycle. The fish were fed 1.5% of their body weight per day with a 38% protein diet (Aquaculture Food; Zeigler Brothers, Inc., Gardners, Pennsylvania).

The strain of *A. salmonicida* (AS-98-1) was originally isolated from a population of Atlantic salmon *Salmo salar* used for research at the Leetown Science Center in 1998. The isolate conformed biochemically to criteria typical of the species (Hiney and Olivier 1999). The bacteria were grown in 100 mL of tryptic soy (TS) broth (Difco Laboratories, Detroit, Michigan) at 20°C on a 100-rpm shaker for 48 h. Tenfold dilutions of the cells were prepared in TS broth for challenge and for determination of viable cell numbers. Drops (0.025 mL) of each dilution were placed on the surface of TS agar with 0.01% Coomassie brilliant blue (CBB medium; Bio-Rad Laboratories, Hercules, California) (Cipriano and Bertolini 1988). The resulting bacterial colonies were enumerated, and the number was converted to colony-forming units (CFU) injected per fish.

Isolation of *A. salmonicida* from fish was done by inoculation of kidney and mucus onto CBB plates by the use of one of two methods. One was a viable cell quantification technique; tissues were collected aseptically and placed in preweighed tubes, the tubes were reweighed, and the tissues were diluted 1:10 and homogenized in sterile 0.1% peptone and 0.05% yeast extract (pep-ye; Difco). Additional 10-fold dilutions were prepared for each sample in pep-ye and used to inoculate CBB as previously described. Mucus was collected as described by Cipriano et al. (1992). Numbers of *A. salmonicida* were reported as CFU per gram of kidney or mucus. Cell counts of total bacteria were also determined on the same CBB plates; this equaled *A. salmonicida* plus all other bacteria that grew. Streak plating was the other technique. Two plates (to enhance isolation probability) were inoculated for each tissue and from each fish. All plates were incubated at 20°C for 48 h. Presumptive, blue colonies were transferred to fresh medium for biochemical characterization of *A. salmonicida* by the use of standard identification methods (MacFaddin 1980; Koneman et al. 1983; Hiney and Olivier 1999).

Bacteria were isolated from *A. plicata* by use of the techniques of Starliper et al. (1998) with modifications. Physical data (weight, length, and width) were recorded from each animal. Before opening the valves, the external surface of the shells was gently scrubbed clean by use of a brush and 200 mg of sodium hypochlorite/L and then allowed to air dry. Aseptic technique was used for removing the internal soft tissues. The shells were pried apart, the abductor muscles were cut, and the liquid inside of the shell but outside of the soft tissues (termed "fluid") was collected and quantified. Tenfold dilutions were then prepared in pep-ye, and 0.025-mL drops were placed on CBB plates. The soft tissues were removed and portioned as two samples. One sample, labeled "Gut," contained primarily digestive tract, and the other, labeled "OT," comprised the remaining soft tissues. To evaluate the presence of *A. salmonicida* in the soft tissues without influence from residual bacteria on their external surfaces or fluid, each soft tissue sample was surface disinfected by dipping in 200 mg of sodium hypochlorite/L for 30 s followed by rinsing in sterile pep-ye. Tissues were placed in preweighed laboratory bags (Tekmar, Cincinnati, Ohio); the tissues were weighed and diluted 1:2 (w/v) in pep-ye and homogenized for 2 min in a stomacher (model 80 Laboratory Blender; Seward Medical, London, United Kingdom). About 2 mL of the resulting liquid homogenate was transferred to a sterile tube for ease of handling, and from this, a series of three 10-fold dilutions were prepared in pep-ye. Drops (0.025 mL) from each dilution were placed onto the surface of CBB plates. The plates inoculated from the fluid, Gut, and OT dilutions were incubated at 20°C for 48 h. Presumptive and blue *A. salmonicida* were enumerated and picked for biochemical characterization to confirm their identity. Total bacterial colony numbers were also determined.

The presence of *A. salmonicida* in the tank was determined by collecting 1 L of water effluent; this was centrifuged for 30 min at 4°C at 5,000 × gravity (Sorvall RC2-B, HS-4 rotor). The supernatant was discarded, and the pellet was suspended in 5 mL of pep-ye. A series of 10-fold dilutions was prepared, and 0.025-mL drops were placed on the surface of CBB plates. *Aeromonas salmonicida* and total bacteria were reported as CFU per milliliter of tank water. The blue colonies were confirmed as *A. salmonicida* as described previously.

An artificially induced clinical case of furunculosis in Nauyuk Arctic char served as the source of *A. salmonicida* for the *A. plicata*. Two groups of 60 Labrador Arctic char were exposed to 2.26×10^2 and 2.26×10^0 CFU/fish, respectively, by intraperitoneal injection. These fish were placed in a 900-L tank along with about 300 Nauyuk char. The challenge doses to the Labrador char were selected on the basis of a previous determination of a dose lethal to 50% of the fish (Reed and Muench 1938) of 5.12×10^0 CFU/fish for the isolate used. Susceptibilities of both Arctic char strains to *A. salmonicida* were similar (data not presented). The two sizes of Arctic char were used to distinguish injected fish from those that were not. Cell concentrations were chosen to produce mortality in the Labrador char at a level sufficient to provide a horizontal exposure with *A. salmonicida* and subsequent development of furunculosis in the Nauyuk char. *Amblema plicata* ($N = 127$) were placed in the bottom of the fish tank when the first Nauyuk char died due to *A. salmonicida*. By this time, nearly all of the injected Labradors had died.

Before adding the *A. plicata* to the tank, 10 were sampled to determine their baseline levels of bacteria and to ensure *A. salmonicida* was not present by bacterial culture of Gut, OT, and fluid samples as previously described. The *A. plicata* were transferred to their respective clean tanks after a desired prevalence of *A. salmonicida* of greater than 50% was achieved. Prevalence was determined by the use of 10 *A. plicata* examined after 17 and 33 d of cohabitation. The remaining *A. plicata* ($n = 107$) were transferred to four previously disinfected tanks (152 L). Sixty uninfected Labrador char were added to each tank on days 1, 5, 15, and 30 after the *A. plicata* were introduced. Immediately before adding the char, depuration of *A. salmonicida* was evaluated by bacterial culture of tank water effluent. Also, 10 and 7 *A. plicata* from the 1- and 5-d groups, respectively, were cultured for *A. salmonicida* as previously described.

All transmission studies of *A. salmonicida* were conducted in tanks fed with spring water as previously described. The 900-L tank received 31.1 L/min, which equaled 2.1 turnovers/h. The four 152-L tanks were regulated to an equal rate of 5.83 L/min or about 2.3 turnovers/h. *Amblema plicata* were allowed to acclimate over a period of 2 d from reservoir water to spring water before cohabitation with the Nauyuk char infected with *A. salmonicida*.

Transmission of *A. salmonicida* from *A. plicata* to the Labrador char was presumptively determined via development of disease and mortality and confirmed by biochemical characterization of

TABLE 1.—Prevalence and viable cell counts of *Aeromonas salmonicida* (AS) from *Amblema plicata* after 17 and 33 d cohabitation with the diseased Nauyuk Arctic char and following relocation after 1 and 5 d depuration in pathogen-free water. Also included are the mean viable cell counts for all (total) bacteria that grew on the CBB[a] primary isolation plates. Abbreviations are as follows: OT = all other soft tissues, P:S = ratio of number of positive tissues to number sampled, CFU = colony-forming units.

Statistic	Gut[b]	OT	Fluid[c]
Prevalence after 17 d			
AS P:S	1:10	2:10	3:10
AS CFU/g or mL	2.64×10^4	1.32×10^5	1.09×10^3
Range		2.64×10^2–2.64×10^5	5.20×10^2–1.44×10^3
All bacteria, mean (CFU/g or mL)	1.18×10^5	9.61×10^5	2.91×10^4
Prevalence after 33 d			
AS P:S	6:10	10:10	4:10
AS CFU/g or mL	4.20×10^4	1.20×10^4	2.50×10^2
Range	2.70×10^1–1.60×10^5	1.00×10^2–1.10×10^5	4.00×10^1–4.00×10^2
All bacteria, mean (CFU/g or mL)	1.60×10^5	6.40×10^4	4.63×10^3
Prevalence after 1 d of depuration			
AS P:S	3:10	1:10	1:10
AS CFU/g or mL	1.40×10^2	2.00×10^2	1.33×10^2
Range	2.00×10^1–3.00×10^2		
All bacteria, mean (CFU/g or mL)	9.52×10^4	6.62×10^4	3.77×10^4
Prevalence after 5 d of depuration			
AS P:S	1:7	4:7	4:7
AS CFU/g or mL	1.00×10^2	2.78×10^2	3.67×10^1
Range		1.60×10^1–1.00×10^3	1.33×10^1–6.67×10^1
All bacteria, mean (CFU/g or mL)	6.96×10^4	1.10×10^5	2.64×10^4

[a] Tryptic soy agar with 0.01% Coomassie brilliant blue.
[b] Gut = primarily digestive tract tissues.
[c] Fluid = liquid collected from within the valves, but outside of the soft tissues.

the bacteria from the mucus and kidney of each fish. In the cohabitation groups with no mortality after 3–4 weeks, bacterial culture of *A. plicata* and char was done to determine the presence of *A. salmonicida*.

Results

The average length of each *A. plicata* was 100.7 mm, ranging from 57 to 128 mm. The width averaged 75.6 mm and ranged from 45 to 99 mm. The mean total weight was 243.6 g (range of 51.1−493.8 g); an average of 23.0 g (9.4%) of the total weight was comprised of soft tissues. Gut samples accounted for an average of 12.5 g (range of 1.2−24.1 g; 54.1%) of the total soft tissue, whereas OT samples comprised the balance of the total soft tissues and had a mean weight of 10.6 g and weights ranging from 2.3 to 18.4 g/sample. There was an average of 21.4 mL (3.5−47 mL) of pallial fluid collected from each *A. plicata*.

The first intraperitoneal-injected Labrador Arctic char died 7 d after challenge. The first Nauyuk Arctic char died 17 d after the initial challenge. The cause of death was confirmed biochemically as *A. salmonicida*. Regardless of the route of exposure, the counts of *A. salmonicida* from kidney tissues were similar: 1.22×10^9 CFU/g for the injected Labradors and 1.10×10^9 CFU/g for the Nauyuk char. Total bacterial counts from the Labrador char kidneys were similar suggesting that *A. salmonicida* was the predominate bacterium in these fish. Likewise, total counts from the Nauyuk char were also similar to the counts of *A. salmonicida* (average, 1.12×10^9 CFU/g). The mean total bacterial counts from mucus were only slightly higher than the cell counts of *A. salmonicida*. However, higher numbers of *A. salmonicida* were isolated from the mucus of horizontally infected fish, 4.39×10^7 CFU/g compared with 1.71×10^7 CFU/g from the injected-Labrador mortality.

Aeromonas salmonicida was not isolated from the 10 *A. plicata* examined to ensure the pathogen was absent before cohabitation. Tissues and fluids from all *A. plicata* resulted in bacterial growth on CBB. Mean cell counts for Gut and OT were 1.84×10^4 and 1.88×10^5 CFU/g, respectively. The average number of bacteria isolated from the fluids was 2.04×10^4 CFU/mL.

After 17 d of cohabitation, *A. salmonicida* was cultured from 3 of 10 (30%) *A. plicata*, from 1 Gut sample, and from 2 OT samples (Table 1). The cell count for the positive Gut sample was 2.64×10^4

TABLE 2.—Prevalence and viable cell counts of 1-d and 5-d groups of *Aeromonas salmonicida* (AS) isolated from *Amblema plicata* after 23 and 26 d, respectively, of cohabitation with Labrador Arctic char. The *A. plicata* were the source of *A. salmonicida* to the Arctic char; after becoming infected, the Arctic char would then serve to reinfect the bivalves. Abbreviations are as follows: OT = all other soft tissues, P:S = ratio of number of positive tissues to number sampled, CFU = colony-forming units.

Statistic	Gut[a]	OT	Fluid[b]
	1-d group		
P:S	2:10	5:10	4:10
AS CFU/g or mL	6.25×10^4	2.48×10^4	3.55×10^3
Range	6.10×10^4–6.40×10^4	1.00×10^2–5.40×10^4	1.33×10^1–1.07×10^4
	5-d group		
P:S	8:10	7:10	6:10
AS CFU/g or mL	3.09×10^3	8.39×10^3	1.40×10^3
Range	1.00×10^1–2.20×10^4	1.00×10^2–4.80×10^4	1.33×10^2–4.00×10^3

[a] Gut = primarily digestive tract tissues.
[b] Fluid = liquid collected from within the valves but outside of the soft tissues.

CFU/g. Counts for the positive OT samples were 2.64×10^2 and 2.64×10^5 CFU/g. The bacterium was isolated from three fluids at concentrations ranging from 5.20×10^2 to 1.44×10^3 CFU/mL. A second evaluation done on day 33 of 10 *A. plicata* revealed 100% *A. salmonicida* (10 OT, 6 Gut, and 4 pallial fluid; Table 1). Viable *A. salmonicida* from OT ranged from 1.00×10^2 to 1.10×10^5 CFU/g with a mean of 1.20×10^4 CFU/g. By day 33, mortality in the Nauyuk char had subsided to three to six fish per week, and total mortality in the population of fish was about 40%. At the time the *A. plicata* were transferred, kidney and mucous tissues of 60 fish were cultured on CBB for isolation of *A. salmonicida.* Kidneys from four fish were positive (mean of 6.73×10^6 CFU/g), and 55 of the mucous tissues were positive and had a mean of 5.87×10^5 CFU/g.

Results of depuration of *A. salmonicida* by *A. plicata* after 1 and 5 d are presented in Table 1. After 1 d of depuration, prevalence of the bacterium decreased from 6 to 3 of the 10 Gut samples (mean of 1.40×10^2 CFU/g) and from 10 to 1 of the OT and from 4 to 1 of the fluid samples. Also, 5.99×10^1 CFU of *A. salmonicida*/mL was isolated from the tank water effluent. Prevalence of *A. salmonicida* in the *A. plicata* was higher when evaluated after 5 d of depuration; positives were detected from four of seven OT samples and four of seven fluids, including two that were paired with the OT samples of the same bivalve. One Gut tissue was positive. However, *A. salmonicida* was not isolated from the tank water effluent. When the three samples from each animal were combined, *A. salmonicida* was isolated from 6 of 7 *A. plicata* after 5 d of depuration, whereas 3 of 10 were positive after 1 d of depuration. *Aeromonas salmonicida* was not isolated from tank water effluent from either of the groups after 15 or 30 d of depuration.

No *A. salmonicida* was detected in the Labrador char exposed to *A. plicata* 15 or 30 d after their removal from the Nauyuk char. In both the 1- and 5-d groups, disease and mortality to the fish resulted. The first dead fish in each group was recorded on the 14th day after the clean char were added. *Aeromonas salmonicida* was biochemically confirmed from kidneys and mucous samples from the dead fish in both the 1- and 5-d groups. The 1-d group was terminated 23 d after cohabitation was initiated. Within this time, mortality to the fish totaled 29 of 60 (48%). *Aeromonas salmonicida* was isolated from 8 of 31 kidneys and 11 of 21 mucous samples from the surviving fish. The 5-d group was terminated after 27 d; 34 of 60 (57%) char died, and *A. salmonicida* was isolated from 3 kidneys and 19 mucous samples of the fish that survived through 27 d. In both the 1- and 5-d groups, *A. salmonicida* increased in prevalence and concentration in the *A. plicata* (Table 2), relative to the levels in *A. plicata* evaluated after depuration alone at 1 and 5 d (Table 1).

The 15- and 30-d groups were terminated after 29 and 22 d, respectively, of cohabitation. No mortality to the Labrador char occurred in either of these groups. No *A. salmonicida* was isolated from the char or the *A. plicata* after conclusion of the observation periods.

Discussion

It is encouraging to fishery and bivalve resource managers that in the worst-case scenario, *A. salmonicida* was not experimentally transmitted to Arctic char at some time less than 15 d, which is

only one-half of the current minimum-recommended quarantine to prevent spread of zebra mussels. The model system used in this study provides strong evidence of the potential for *A. salmonicida* to be spread by Arctic char and the freshwater bivalve *A. plicata*. The *A. plicata* appears capable of acquiring and harboring *A. salmonicida* and disseminating viable cells such that disease and mortality occur to susceptible hosts. *Aeromonas salmonicida* provides an excellent bacterial pathogen to study transmission between bivalves and fish. The vectoring animal *A. plicata* readily acquired the pathogen from water, and the bacterium is highly pathogenic to many salmonid hosts.

In this study, what could be considered a worst-case scenario was established by using clinically diseased and dying Arctic char as the source of *A. salmonicida* for the *A. plicata*. Furthermore, transfer of *A. plicata* to clean tanks was not done until the prevalence of *A. salmonicida* reached 100%. Therefore, the *A. plicata* harbored a near-maximum CFU of *A. salmonicida* that could be anticipated by the use of infected fish as the source of the pathogen. Considering these experimental criteria, it would be very unlikely that bivalves would be exposed to this type of challenge of viable cells in large rivers because of the volume and dilution potential of the flowing water.

The number of *A. plicata* positive for *A. salmonicida* decreased from 100% to 30% after 24 h of depuration. This rapid depuration is also encouraging. There was a negligible change in the prevalence of *A. salmonicida* in *A. plicata* assayed at 5 d of depuration compared with the level in the 1-d group. Likewise, the mean number of CFU of *A. salmonicida* per gram was similar for the two groups. Of particular interest would be the mechanisms involved in depuration (i.e., displacement by other bacteria or active ingestion or phagocytosis).

Depuration does not appear to have a direct correlation with time after transfer away from the source of *A. salmonicida*. In a study by Starliper et al. (1998), the fish pathogen *Flavobacterium columnare* was isolated from a single *A. plicata* sampled from the Ohio River. Other bivalves collected at the same site and time were quarantined to allow depuration. When some of these were examined for bacteria after only 1 d of depuration, *F. columnare* was not isolated. These bivalves were naturally infected with the pathogen, and the prevalence was 7% compared with the 100% prevalence of *A. salmonicida* in the present study.

Depuration of *A. salmonicida* by *A. plicata* in the present study contrasts with depuration of viruses by marine bivalves (Canzonier 1971; Mortensen et al. 1992). Mortensen et al. (1992) exposed *Pecten maximus* to infectious pancreatic necrosis virus (IPNV) by injection and bath challenge methods. Tissues from the infected scallops were examined for IPNV, and the virus was detected at least 11 months after challenge in injected animals and 50 d in the waterborne-exposed animals. Although IPNV titers only decreased after exposure, the authors surmised that the persistence of the virus may have been due to viral replication. The persistence of IPNV relative to that for *A. salmonicida* might be related to other factors such as the high challenge dose of virus, the bivalve species, or the methods of exposure. Also, the persistence could be explained by the virus being sequestered in digestive tissues and, thus, avoiding depuration (Canzonier 1971; Hay and Scotti 1986).

Canzonier (1971) exposed hard clams *Mercenaria mercenaria* to low levels of *Escherichia coli* and coliphage S-13. In 16°C seawater, the clams depurated *E. coli* from an initial concentration of 1.0×10^5 CFU/mL of digestive tissue homogenate to less than 1 CFU/mL within 48 h. In contrast, clams were less efficient at phage depuration. After 6 d, the phage titer in digestive tissues was less than 1 log unit below the initial titer. Phage depuration by *M. mercenaria* was related to water temperature and was accelerated above 16°C (Canzonier 1971).

Future plans to initiate health inspections of freshwater bivalves in a manner similar to those done routinely on captive fish populations should follow mandatory quarantine procedures for the bivalve. In previous studies by Starliper et al. (1998), it was shown that the distribution of the bacterial flora of bivalves changes rapidly after a change in their aquatic environment and this included the ridding of the pathogen *F. columnare*. The quarantine would allow the bivalves an opportunity to depurate, and then an inspection for fish pathogens would likely yield a more accurate account of the bacteria that might be relocated to a hatchery concurrent with the relocated bivalves.

Acknowledgments

Appreciation is extended to Patricia Morrison at the U.S. Fish and Wildlife Service's Ohio River Islands National Wildlife Refuge, Parkersburg, West Virginia, for collection of the *A. plicata* and to the Conservation Fund's Freshwater Institute, Shepherdstown, West Virginia, for providing the

fish. Thanks to the West Virginia Division of Natural Resources for their support and effort throughout the scientific collection permit process. Thanks to Jennifer Hickerson and Erin McKee, two undergraduate students from Shepherd College, Shepherdstown, West Virginia, who completed their senior intern projects within this study. Thanks to Rita Villella, Pete Bullock, and Emmett Shotts for their reviews.

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