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QUARANTINE OF *AEROMONAS SALMONICIDA*-HARBORING EBONYSHELL MUSSELS (*FUSCONAIA EBENA*) PREVENTS TRANSMISSION OF THE PATHOGEN TO BROOK TROUT (*SALVELINUS FONTINALIS*)

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ABSTRACT Furunculosis, caused by the bacterium *Aeromonas salmonicida*, was artificially induced in brook trout (*Salvelinus fontinalis*) in an experimental tank. Ebonyshells (*Fusconaia ebena*) were placed to cohabit with these fish to acquire the pathogen through siphoning. After 2 wk of cohabitation, 10 of the mussels were assayed by bacterial culture and all were found to harbor *A. salmonicida*. The mean cell count from soft tissue homogenates was 1.84×10^5 cfu/g, which comprised an average 14.41% of the total bacteria isolated from tissues. From the fluids, a mean of 2.84×10^5 *A. salmonicida* cfu/mL was isolated, which comprised an average of 17.29% of the total bacterial flora. The mussels were removed from the cohabitation tank and distributed equally among five previously disinfected tanks, 35 per tank. The *F. ebena* in each tank were allowed to depurate *A. salmonicida* for various durations: 1, 5, 10, 15 or 30 days. After each group had depurated for their assigned time, 10 were assayed for bacteria, tank water was tested, and 20 pathogen-free bioindicator brook trout were added to cohabit with the remaining mussels. Depuration was considered successful if *A. salmonicida* was not isolated from tank water or the mussels, and there was no infection or mortality to bioindicator fish. After 1 day of depuration, *A. salmonicida* was not isolated from the soft tissues; however, it was isolated from one of the paired fluids (10% prevalence). The tank water tested positive, and the bioindicator fish became infected and died. From the 5-day depuration group, *A. salmonicida* was not isolated from soft tissues, but was isolated from three fluids (30%; mean = 1.56×10^2 cfu/mL). Tank water from the 5-day group was negative, and there was no mortality among the bioindicator fish. However, *A. salmonicida* was isolated from 2 of 20 fish at the end of the 14-day observation period. One *F. ebena* fluid sample was positive for *A. salmonicida* from the 10-day depuration group, but none of the soft tissue homogenates. The pathogen was not isolated from 10-day tank water, but there was a 30% cumulative mortality to the bioindicator fish. *Aeromonas salmonicida* was not isolated from any of the soft tissue homogenates, fluids or tank water from the 15 day or 30 day depuration groups, and the bioindicator fish remained pathogen- and disease-free. Study results showed that the *F. ebena* were harboring a high *A. salmonicida* cell load going into depuration, but at 15 days and beyond, the pathogen had been depurated to the extent that the mussels did not serve as pathogen vectors.

KEY WORDS: pathogen, transmission, vector, quarantine, depuration, freshwater mussels

INTRODUCTION

Conservation of freshwater mussels is a priority objective for several federal and state natural resource agencies in the United States. A significant number of the species or populations that are native to North American waters are imperiled or at-risk (Williams et al. 1993). There are several contributing factors to the declines. Work is being done on various topics; protecting natural habitats, status monitoring and identification of fish hosts and population augmentation with captive-reared animals. One conservation effort underway is relocation of imperiled mussels from threatening environments to refuges for maintenance and propagation. Most of these species included in this effort have never been maintained or reared in captivity, so this presents difficult challenges for aquaculturists, such as meeting the mussels' environmental and nutritional requirements. Notwithstanding are the potential problems associated with pathogens and diseases among captive-reared animals in refugia, particularly because these mussels originate from natural, open waters (e.g., rivers). Refugia include several federal and state fish hatcheries that rear a suite of important sport and restoration fish species. When wild fish are moved to hatcheries or when hatchery fish are moved among facilities, there is the risk for introduction and transmission of pathogens and diseases (Piper et al. 1982). A similar risk can be predicted for relocation of mussels to refugia; namely, introduction of pathogens via the mussels to hatchery resident fish and to mussels already in captivity. Although a case of mussels acting to "vector" a pathogen has yet to be documented, laboratory studies have demonstrated that mussels can act to transmit a bacterial pathogen from diseased to disease-free fish (Starliper & Morrison 2000, Starliper 2001). Perhaps more importantly, there are pathogen introduction risks that hatchery propagated mussels pose to wild mussel populations when they are placed into streams as part of population augmentation or restoration efforts. Thus, there is a need to address issues related to the biology of pathogen movement among mussels. This information is pivotal for health and disease management strategies, because disease and mortality prevention is critical for successful conservation of imperiled species.

Mussels collected for relocation to hatcheries must first undergo a quarantine to ensure that zebra mussels (*Dreissena polymorpha*) are not attached and disseminated (Chaffee 1997, Gatenby et al. 1998). Using the model developed to study pathogen transmission (Starliper 2001), it was shown that threeridge (*Amblema plicata*) depurated the fish pathogen *Aeromonas salmonicida* in less than 15 days. Successful depuration was defined by not reisolating *A. salmonicida* from the mussels or their tank water, and cohabited, susceptible Arctic char *Salvelinus alpinus* remained pathogen- and disease-free. This study was a first indication that if mussels were maintained in pathogen-free water, the duration of quarantine for zebra mussels would suffice for depuration of pathogens to the extent that vectoring did not occur. This initial study was completed using *A. plicata*, a species common in the Ohio River drainage that is not imperiled. Also, the study was conducted at 12°C, which is the ambient temperature of the laboratory water supply. The objective of this study was to repeat the *A. salmonicida* depuration study of Starliper (2001) but with se-

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lected changes to be more representative of actual quarantine parameters. This study used *Fusconaia ebena*; a surrogate mussel species selected to represent imperiled species being relocated for captive-rearing (Dr. Richard Neves, pers. comm.) and was done at 20°C, a water temperature more indicative of zebra mussel quarantine temperatures.

MATERIALS AND METHODS

The *Fusconaia ebena* were collected in October 2002 from Kentucky Lake (35°29′44″N; 88°00′27″W), Tennessee River mile 102.0, Humphreys County, Tennessee. Water depth at the collection site was between 4.5 and 9 m, and the substrate consisted of mud, clay and gravel. The mussels were shipped overnight to the National Fish Health Research Laboratory, Leetown, West Virginia and placed into holding tanks for acclimation to laboratory water. The tanks were 158 L (1 m diameter, circular) and supplied with flow-through, specific fish pathogen-free spring water with a total hardness of about 280 mg/L. The dissolved oxygen of incoming water averaged 10 mg/L, and the flow was about 11.4 L/min or ~4.3 water turnovers per hour. The water was heated from the ambient temperature of 12°C to 20°C, the temperature at which all studies were conducted. The mussels were not directly fed a mussel-specific (e.g., algal) diet throughout the study. Instead, their food came from two sources. The incoming spring water contained low levels of coliform and noncoliform bacteria. Indirect feeding also occurred during the bacterial challenge and depuration periods, when the fish were fed at a rate of about 0.5% body weight per day.

The fish used were the "Nashua" strain of brook trout (*Salvelinus fontinalis*) averaging 80–100 g each. This species was chosen because it is highly susceptible to *A. salmonicida*, the causative agent for furunculosis, and thus would serve as the source of the pathogen to mussels and as an excellent bioindicator to evaluate *F. ebena* depuration of *A. salmonicida*. These are the same criteria used by Starliper (2001) in selecting Arctic char (*S. alpinus*) as a donor and bioindicator.

Ten *A. salmonicida* isolates that were maintained in the (–70°C) collection at the National Fish Health Research Laboratory were passed through brook trout to recover and maximize virulence factors that may have been compromised during storage. Passage through fish was done by growing each bacterial isolate in tryptic soy (TS; Becton, Dickinson and Company, Sparks, Maryland) broth for 48 h; 0.1 mL was intraperitoneal (IP) injected into each of 5 brook trout. As the fish succumbed, the bacterium was recovered from kidneys by streak-plate culturing onto CBB agar plates (TS agar with 0.01% coomassie brilliant blue; Bio-Rad Laboratories, Hercules, California; Cipriano & Bertolini 1988). Blue and suspect *A. salmonicida* colonies were biochemically confirmed using standard techniques (Koneman et al. 1992, MacFaddin 2000). The isolate (3.137) that resulted in the highest mortality to fish in the fewest number of days was selected to produce *A. salmonicida* donor brook trout. To determine the *A salmonicida* concentration to be used in the donor fish, a culture of 3.137 was produced by growing in TS broth and serial 10-fold dilutions were prepared in sterile TS broth through a 1×10^{-8} dilution. Groups of 10 brook trout were IP injected with 0.1 mL of a cell dilution; a control group was injected with sterile TS broth. For comparison, a set of injection groups was done at laboratory-ambient water temperature in addition to a set at 20°C. For all studies involving brook trout at 20°C, the fish were acclimated to this temperature by gradually increasing from ambient over a period of several days. Concentration dependent, *A. salmonicida*-specific, mortality was monitored to determine the desired concentration.

The procedure used to expose the *F. ebena* to *A. salmonicida* was similar to the model previously used by Starliper (2001) to infect *A. plicata*. Initially, 10 *F. ebena* were subjected to bacterial culture to ensure that they were not harboring *A. salmonicida* prior to their exposure; similarly, 20 brook trout also were examined to ensure that the laboratory population was negative. One hundred brook trout were injected IP and placed in a 1,245 L tank with 18 Lpm flow. The fish were injected with 48-h cultured cells of 3.137 diluted in sterile TS broth; each of 50 fish received 2×10^1 colony forming units (cfu), and another 50 fish received 2×10^0 cfu each. Once these injected fish began to die and *A. salmonicida* was confirmed as the cause (see later), 100 pathogen-free brook trout were added to the tank to become horizontally exposed (i.e., naturally by contact exposure) and infected with the pathogen. As the naturally infected fish began to die and *A. salmonicida* was confirmed from primary isolations, 185 *F. ebena* were added to this tank. The mussels acquired *A. salmonicida* from tank water, which contained the diseased fish. After 2 wk of cohabitation, 10 *F. ebena* were sampled for total bacteria and for 100% prevalence of *A. salmonicida* (isolated from 10 of 10 mussels). Presence of *A. salmonicida* in the cohabitation tank water was determined. The remaining mussels were removed from the cohabitation tank and distributed equally among five previously disinfected 158 L tanks, 35 animals per tank. Flow-through to the 158-L tanks was 5.83 L per min yielding about 2.2 turnovers (i.e., complete water changes) per hour. The *F. ebena* in each tank were given the opportunity to depurate *A. salmonicida* for various durations; one group depurated for 1 day and the other groups depurated for 5, 10, 15 or 30 days. After each group of *F. ebena* had depurated for their assigned time, 10 were assayed for total bacteria and *A. salmonicida*; tank water was evaluated for presence of the pathogen, (see later) and 20 pathogen-free bioindicator brook trout were added to cohabit with the remaining mussels. Vaughn et al. (2004) showed that ecosystem processes done by freshwater mussels are linearly related to biomass, so the 25 *F. ebena* to cohabit with the fish were chosen to achieve a total mussel biomass (~4 kg) in the tank, similar to that for *A. plicata* in a previous study (Starliper 2001). The bioindicator fish in each group cohabited with the pathogen-harboring mussels for up to 2 wk, or less if there was mortality among the fish. At the completion of the cohabitation, 10 of the mussels and all of the fish were subjected to primary culture for *A. salmonicida*. Depuration of *A. salmonicida* by *F. ebena* was considered successful if: (1) *A. salmonicida* was not isolated from tank water or the mussels; (2) there was no mortality among the bioindicator fish and (3) *A. salmonicida* was not isolated from the bioindicator fish.

Primary isolation of *A. salmonicida* from the brook trout was done as previously described (Cipriano et al. 1992, Starliper 2001); CBB plates were used and incubation was at 20°C for 48 h The presence of viable *A. salmonicida* in *F. ebena* was determined following the techniques of Starliper et al. (1998) and Starliper (2001), with several modifications. Physical data were recorded from each mussel; the volume (area) inside the valves was estimated by quantifying the amount of water it took to fill each valve (1 mL = 1cc). The external shell surface was gently scrubbed with a solution of 200 mg/L sodium hypochlorite; the valves were opened after cutting the adductor muscles and the liquid inside (termed "fluid") was caught in a sterile Petri dish and measured. The fluid presumably consisted of water and pallial fluid. All of

the soft tissues were excised and treated as one sample. The soft tissues were surface disinfected by gently swirling in a solution of 200 mg/L sodium hypochlorite for 30 sec and rinsed in sterile pep-ye (0.1% peptone, 0.05% yeast extract; Becton, Dickinson and Company, Sparks, Maryland). Tissues were homogenized in an equal amount (w/v; 1:2 dilution) of sterile pep-ye for 1–2 min using a Model 80 Laboratory Blender (Seward Medical, London, United Kingdom). Serial 10-fold dilutions were prepared from the fluid and tissue homogenate from each animal in pep-ye and were used to drop inoculate CBB plates. The dilution series for the fluids and tissue homogenates were carried through the 1×10^{-3} dilution, and these yielded single, isolated bacterial colonies. Plates were incubated at 20°C for 48 h; total and blue, suspect *A. salmonicida* colonies were enumerated. Suspect *A. salmonicida* colonies were biochemically confirmed as previously described. The presence of viable *A. salmonicida* cells in the water of the cohabitation (challenge and depuration) tanks was determined by bacterial culture as previously described (Starliper 2001).

RESULTS

In all, 100 *F. ebena* were assayed; the physical data collected from the animals prior to depuration and throughout the 30-day depuration period following bacterial challenge are presented in Table 1. The mean total weight (for $n = 100$) for the animals was 153.4 g, ranging in weight from 82.8 g up to 212.2 g. The overall mean length, width and depth dimensions were 74.3 mm, 64.4 mm and 43.3 mm, respectively. The average volume inside the valves was 32.5 mL. It may be noted from Table 1 that the measured physical data for the groups of mussels were similar. The mean weight of the excised soft tissues was 18.3 g per animal, which comprised an average 12.0% of their total weight. The average amount of fluid collected from each *F. ebena* was 15.3 mL or 9.9% (v/w) of the total weight.

Challenges to brook trout with *A. salmonicida* 3.137 resulted in 100% mortality at 12°C and 20°C. At the highest 10-fold dilution injected, a calculated 0.26 cfu was delivered per fish, indicative of a highly virulent pathogen to the fish. At the cooler water temperature, the mean days to 100% mortality was 7.2 days and only 6.1 days at 20°C.

Prior to the introduction of *A. salmonicida* and initiation of the pathogen transmission model, 10 *F. ebena* were examined to ensure absence of the pathogen. All of the mussels possessed a bacterial flora in their soft tissues and in their fluids; however, *A. salmonicida* was not isolated. The mean for total bacteria present in the total soft tissues homogenates was 3.64×10^5 cfu/g of tissue, and bacterial cell counts ranged between the least 6.67×10^3 to the highest of 1.87×10^6 cfu/g. The mean for total bacteria in fluids was nearly 10-fold higher than that for the tissues, at 2.36×10^6 cfu/mL. Fluid bacterial counts ranged from 8.93×10^3 to 1.33×10^7 cfu/mL. Bacterial isolation data for *F. ebena,* brook trout, and tank water throughout the study are presented in Table 2. Up to 1.30×10^3 and 1.05×10^6 cfu/mL of total bacteria was isolated from the tank water.

As for the establishment of the furunculosis epizootic, 2 days after the brook trout were injected, six of the fish had died, and the etiologic cause of their deaths was confirmed to be *A. salmonicida.* The pathogen-free fish were introduced at this time. After 6 days of cohabitation, the noninjected fish began to die; the *F. ebena* were introduced and by then nearly 100% of the injected fish had succumbed (8 days post IP injection). During the course of the next 2 wk, an additional 163 pathogen-free fish (at three different times) were acclimated to 20°C and introduced to the cohabitation tank. This was done to maintain mortality and maximize shedding of viable cells by the fish into the water column for presentation to the mussels. Total, cumulative mortality among the horizontally exposed fish was 67% during this 2-wk period.

A predetermined level of infectivity in *F. ebena* was established for this study; a 100% prevalence of *A. salmonicida* was to be achieved prior to moving mussels to the tanks for depuration, thus ensuring a maximum pathogen carryover into depuration. Primary isolation from 10 *F. ebena* showed that *A. salmonicida*

TABLE 1.

Means and ranges in physical measurements of ebonyshells *Fusconaia ebena* from Kentucky Lake.

	Pre[a] (20[b])	Day 1 (10)	Day 5 n = 20	Day 10 n = 10	Day 15 n = 20	Day 30 n = 20
Mean total weight (g)	164.1	145.0	144.2	162.5	152.5	152.4
Range in total weight (g)	92.7–201.6	111.2–179.3	106.7–185.4	110.6–210.2	82.8–194.3	117.8–212.2
Mean length (mm)	76.9	70.8	72.4	76.5	75.0	73.7
Range in length (mm)	61.0–89.0	65.0–75.0	67.0–77.0	64.0–84.0	62.0–86.0	66.0–83.0
Mean width (mm)	66.1	61.9	63.3	65.1	64.4	64.5
Range in width (mm)	55.0–73.0	57.0–66.0	54.0–68.0	60.0–75.0	56.0–73.0	57.0–72.0
Mean height (mm)	44.0	43.2	43.6	42.2	42.8	43.5
Range in height (mm)	36.0–50.0	37.0–55.0	39.0–50.0	39.0–45.0	33.0–50.0	38.0–50.0
Mean volume inside valves (cc)	32.0	29.7	28.9	32.7	35.6	35.0
Range in volume inside valves (cc)	19.0–41.0	20.0–42.0	19.0–35.0	23.0–39.0	22.0–47.0	24.0–55.0
Mean %: soft tissues of total (w/w)	12.1[c]	11.4	12.5	11.4	11.8	12.1
Rnage in %: soft tissues of total (w/w)	10.2–14.6	10.6–13.4	10.0–15.2	9.1–14.2	10.0–13.4	10.0–14.4
Mean %: fluid of total (v/w)	9.5	9.5	9.7	10.3	9.9	10.6
Range in %: fluid of total (v/w)	7.0–12.5	8.4–10.9	7.0–14.8	6.9–12.3	7.5–12.7	8.6–13.0

[a] Pre are the *F. ebena* samples for *Aeromonas salmonicida* before depuration was initiated; Days 1 through 30 are the duration of depuration in flow-through, pathogen-free water.

[b] The number of *F. ebena* assayed for primary isolation of *A. salmonicida.* "Pre" includes 10 to ensure the animals were negative for the pathogen prior to the start of the study and subsequently, 10 to determine the prevalence of *A. salmonicida* infection. On the other days, only 10 *F. ebena* were assayed if there was mortality among the brook trout placed to cohabit.

[c] As an example, the weight of their soft tissues comprised 12.1% (the mean for the 20) of the total weight of the animal.

TABLE 2.

Transmission of *Aeromonas salmonicida* from brook trout *Salvelinus fontinalis* to ebonyshells *Fusconaia ebena*, subsequent depuration of the pathogen by the mussels, and isolation of the bacterium from cohabitation tank water and pathogen-source or bioindicator brook trout.

	F. ebena Soft Tissues (cfu/g)[a]			*F. ebena* Fluids (cfu/mL)[a]			Water[b]	BKT[c]
	No Pos[e]	Mean	Range	No Pos	Mean	Range	cfu/mL	No Pos
Pre-Expt[d]								
Total bact	10	3.64×10^5	6.67×10^5–1.87×10^6	10	2.36×10^6	8.93×10^3–1.33×10^7	N/R	N/D
A. salmon	0	N/R	N/R	0	N/R	N/R	N/R	0/20
at 100%								
Total bact	10	1.70×10^6	1.18×10^5–5.92×10^6	10	1.57×10^6	1.47×10^5–6.97×10^6	1.05×10^6	N/D
A. salmon	10	1.84×10^5	1.46×10^4–5.74×10^5	10	2.84×10^5	7.32×10^3–2.13×10^6	2.40×10^5	67% ≤2wk
After 1d								
Total bact	10	2.28×10^6	1.92×10^5–1.51×10^7	10	9.71×10^5	2.80×10^5–3.40×10^6	2.13×10^5	N/D
A. salmon	0	N/R	N/R	1	6.80×10^3	N/R	5.80×10^4	55% ≤14d
After 5d								
Total bact	10	5.08×10^4	1.46×10^3–1.85×10^5	10	1.76×10^5	7.60×10^3–9.48×10^5	1.30×10^3	N/D
A. salmon	0	N/R	N/R	3	1.56×10^2	6.70×10^1–2.68×10^2	0	23/20 at 14d
After 10d								
Total bact	10	2.22×10^5	9.36×10^2–1.35×10^6	10	1.24×10^5	3.07×10^3–1.00×10^6	1.19×10^5	N/D
A. salmon	0	N/R	N/R	1	1.32×10^2	N/R	0	30% ≤14d
After 15d								
Total bact	10	7.65×10^5	6.40×10^3–5.74×10^6	10	2.98×10^4	6.00×10^3–6.91×10^4	2.76×10^4	N/D
A. salmon	0	N/R	N/R	0	N/R	N/R	0	0/20
After 30d								
Total bact	10	5.48×10^5	3.20×10^3–4.66×10^6	10	4.72×10^4	3.53×10^3–2.20×10^5	5.12×10^5	N/D
A. salmon	0	N/R	N/R	0	N/R	N/R	0	0/20

[a] cfu = bacterial colony forming units.
[b] Water from the *F. ebena* and brook trout cohabitation tank.
[c] BKT = brook trout; cumulative percent mortality within the specified time; if no mortality, the number of fish positive for *A. salmonicida*.
[d] Pre-Expt = *F. ebena* and brook trout prior to their exposure to *A. salmonicida;* at 100% = 100% prevalence of *A. salmonicida* from 10 *F. ebena;* after 1, 5, 10, 15 or 30 days of depuration by the *F. ebena;* Total bact = all bacteria isolated with the exception of *A. salmonicida; A. salmon* = *A. salmonicida*.
[e] No Pos = number of *F. ebena* (out of 10) that bacteria or *A. salmonicida* was isolated; N/R = not relevant, for example, if *A. salmonicida* was not isolated from any, there is no mean cfu; N/D = not done.

was isolated from the tissues and fluid from each animal. The average of *A. salmonicida* from tissues was 1.84×10^5 cfu/g, whereas the mean for the total bacterial flora was 1.70×10^6 cfu/g. The *A. salmonicida* cfu/g comprised an average 14.41% of the total bacterial cfu/g in tissues; *A. salmonicida* cell counts ranged from 1.46×10^4 to 5.74×10^5 cfu/g of soft tissues. From the fluids, a mean of 2.84×10^5 *A. salmonicida* cfu/mL was isolated, which comprised an average of 17.29% of the mean total bacterial flora, 1.57×10^6 cfu/mL. Cell counts of *A. salmonicida* in fluids ranged between 7.32×10^3 and 2.13×10^6 cfu/mL. The total bacteria count from the tank water was 1.05×10^6 cfu/mL and of this, *A. salmonicida* accounted for 22.86% or 2.40×10^5 cfu/mL. Isolation of this pathogen from tank water and the cumulative mortality (67%) in the pathogen-source fish were indicative of an active epizootic that presented the *F. ebena* with an opportunity for uptake of a high number of pathogenic cells.

After 1 day of depuration, *A. salmonicida* was not isolated from the soft tissues of 10 *F. ebena*. However, a bacterial flora was present in all animals, and the mean cell count for total bacteria was 2.28×10^6 cfu/g, with a range in counts from 1.92×10^5 to 1.51×10^7 cfu/g. From the fluid samples of these 10, the mean for total bacteria was 9.71×10^5 cfu/g, with cell counts ranging between 2.80×10^5 and 3.40×10^6 cfu/g. *Aeromonas salmonicida* was isolated from the fluid of one *F. ebena*; 6.80×10^3 cfu/mL was isolated or 0.61% of the total bacteria (1.12×10^6 cfu/g) from that specimen. Therefore, the prevalence of *A. salmonicida* in the 1-day group was 10%. The water was positive for *A. salmonicida*, because 5.80×10^4 cfu/mL was isolated. The bioindicator brook trout introduced to cohabit with the 1-day group of *F. ebena* became infected with *A. salmonicida*, and there was a 55% (11/20) mortality among the brook trout within the 14-day observation period.

From the 5-day depuration group, *A. salmonicida* was not isolated from any of the homogenates of soft tissues; however, the pathogen was isolated from 3 of the 10 paired fluid samples, and the mean for these three was 1.56×10^2 cfu *A. salmonicida* per mL of fluid. The range in *A. salmonicida* cell counts from the three positives was 6.70×10^1 to 2.68×10^2 cfu/mL, which comprised an average of 0.21% of the total bacteria isolated. Tank water from the 5-day group was negative for *A. salmonicida*, and there was no mortality among the brook trout during the 14-day observation period. However, *A. salmonicida* was isolated from 2 (10%) of the 20 fish at the end of the observation period, with one positive mucus sample and one positive kidney tissue streak from another fish.

One *F. ebena* fluid sample was positive for *A. salmonicida* from the 10-day depuration group; the cell count was 1.32×10^2

cfu/mL, which comprised 1.43% of the total from that sample (9.20×10^3 cfu/mL). The average cell count for total bacteria from the 10-day fluids was 1.24×10^5 cfu/mL. *Aeromonas salmonicida* was not isolated from any of the 10 soft tissue homogenates of the 10-day group; the average total bacterial cell count was 2.22×10^5 cfu/g. The pathogen was not isolated from 10-day tank water, but there was a 30% cumulative mortality among the brook trout within the 14-day observation period.

Aeromonas salmonicida was not isolated from any of the soft tissue homogenates or fluid samples from any of the 10 *F. ebena* from either the 15-day or 30-day depuration groups, although each of the 40 samples contained a bacterial flora. Means for total bacteria from soft tissues were 7.65×10^5 and 5.48×10^5 cfu/g for 15-day and 30-day groups, respectively. Means from fluids were 2.98×10^4 and 4.72×10^4 cfu/mL for 15-day and 30-day groups, respectively. The pathogen was not isolated from tank water from either the 15-day or 30-day depuration groups, and the bioindicator fish did not incur mortality. An *A. salmonicida* infection was not established, based on negative primary isolation results at the end of both of these observation periods.

DISCUSSION

The mussels used in this study were not fed algae during the course of the experiments because it was believed that they would filter a greater number of the challenge bacteria from the water, thereby, creating maximum uptake of *A. salmonicida*. This would result in a worst-case scenario; create the opportunity for the selected depuration durations to fail allowing the bacterium to continue to be isolated and/or transmitted to susceptible brook trout. The *A. salmonicida*-exposed mussels were likely harboring more viable and infectious *A. salmonicida* cells than would be anticipated to be carried by mussels collected from feral waters and yet, depuration of the pathogen was successful at some point beyond 10 days, but <15 days. This overall result is not unlike that seen in the previous study with *A. plicata* and Arctic char at 12°C (Starliper 2001). That study evaluated depuration at 1, 5, 15 and 30 days and did not include a 10-day group. In that study, *A. salmonicida* depuration was not complete by day 5; however, it was by 15 days. The current study included the 10-day group to better define an endpoint for depuration by *F. ebena*.

Although the primary result of the two studies was similar, there were differences relative to the performance of the pathogen transmission model. In general, the *F. ebena* acquired *A. salmonicida* (to 100% prevalence) more quickly, and they tended to rid the pathogen at a faster rate than did the *A. plicata*. These differences could perhaps be explained by the differences in size or species of the mussels used and other contributing factors, such as the elevated water temperature resulting in increased filtering by the mussels. Changes in respiration and filtering relative to water temperature differences have been noted in certain marine bivalves (Dame 1996, Gosling 2003). However, in mussels held at like temperatures, clearance activities among freshwater stream-species are similar and vary proportionally with biomass (Silverman et al. 1997, Vaughn et al. 2004). The brook trout could have provided more *A. salmonicida* cells in the water than did the Arctic char even though both hosts had similar susceptibilities to this pathogen. Within the time that the *F. ebena* were exposed to *A. salmonicida* to the 100% prevalence of infection, mortality to the pathogen-source brook trout was 67%, whereas mortality in Arctic char that were the pathogen source to *A. plicata* was 40%. It took ≤2 wk for the *F. ebena* to reach 100% prevalence, whereas at a similar time (17 days) the prevalence in *A. plicata* was at only 30%. Even at 100% prevalence, *A. salmonicida* was isolated from all of the *A. plicata* tissues (OT), but from only 4 of 10 (40%) of the paired fluid samples. All 10 of both the tissues and fluids from the *F. ebena* were positive for the bacterium. The mean of *A. salmonicida* cell counts in *A. plicata* fluids was 2.50×10^2 cfu/mL, and the mean of the (OT) tissue homogenates was 1.20×10^4 cfu/g. The mean cell counts from *F. ebena* were considerably greater, 2.84×10^5 cfu/mL and 1.84×10^5 cfu/g from fluids and tissues, respectively.

The virulence testing with 3.137 demonstrated how highly susceptible the brook trout were to the pathogen. This host-pathogen bioindicator model proved to be highly sensitive for detection of an endpoint for successful depuration to have been completed. In the biology of furunculosis, the disease caused by *A. salmonicida*, mucus plays an important role in the pathogenesis of disease as an attachment or entry point for the bacterium into fish. If *A. salmonicida* were shed or purged by the *F. ebena*, the cohabited brook trout would likely stand a good probability of becoming infected, which would demonstrate the presence of the pathogen in the water. Even though infections to fish may not have resulted in mortality, the infection could have led to disease if left untreated.

After 1 day of depuration, the *A. salmonicida* cell count from tank water of the *A. plicata* at 12°C was 5.99×10^1 cfu/mL (Starliper 2001) and from the *F. ebena* tank water at 20°C, the cell count was 5.80×10^4 cfu/mL. The higher cfu/mL in water from the *F. ebena* after 1 day of depuration corresponded with higher mean *A. salmonicida* cell counts when the mussels were determined to be at 100% prevalence, that is, just prior to when the mussels were placed into the tanks to begin depuration. The higher starting cell load in the *F. ebena* at initiation of depuration and the higher cfu/mL in their tank water after 1 day seems to indicate a more active siphoning and ridding of the pathogen by the mussels, relative to that of the *A. plicata* at 12°C. The quicker rate of depuration was also reflected in the prevalence of *A. salmonicida* positives after 1 day. The prevalence in *A. plicata* (Gut) tissues was 30%, and 1 of the 10 (OT) tissue homogenates was positive; *A. salmonicida* cell counts from these tissues ranged between 2.00×10^1 and 3.00×10^2 cfu/g. In contrast, the pathogen was not isolated from any of the *F. ebena* tissue homogenates. From the paired fluid samples, 1 of 10 from each mussel species was positive for *A. salmonicida*, with cell counts of 1.33×10^2 cfu/mL from *A. plicata* and 6.80×10^3 cfu/mL from *F. ebena*.

When comparing the prevalence of *A. salmonicida*-positive mussels after 1 day of depuration to that after 5 days of depuration, the prevalence after 5 days was higher in the current study on *F. ebena* than in the previous study with *A. plicata* (Starliper 2001). An explanation for this could be that the *A. salmonicida* cells being shed by the mussels up to the day 5 sampling may have been resiphoned and acquired (i.e., recycled). The increased prevalence at 5 days was particularly noteworthy for *A. plicata*, as 6 of 7 were positive compared with 3 of 10 after 1 day. Another potential contributing factor to the increase in prevalence at 5 days, which was not specifically evaluated in either study, was the possibility for multiplication of *A. salmonicida* cells within mussel tissues or fluids.

Aeromonas salmonicida was not isolated from water samples after 5 days in either study at 12°C or 20°C, yet in both studies, infections were established in the bioindicator fish. This demonstrated that the fish proved to be a sensitive indicator for presence of the pathogenic bacterial cells. The fish were placed to cohabit with the mussels, which in essence provided a long-term exposure

of the fish to cells shed into the water. The mussels could have been shedding pathogenic bacterial cells for most or all of the time leading up to the 10-day depuration sampling. Although the mean *A. salmonicida* cell counts (1.56×10^2 cfu/mL) from the three positive *F. ebena* fluids at the 5 day sampling comprised only 0.21% of the total bacteria isolated, there was an average of 13.8 mL (9.5% of the total animal weight; Table 1) of fluid volume measured from each mussel in this tank of 25 mussels. Therefore, it is estimated that 30% of the 25 (or approximately 8) were positive for *A. salmonicida* and that each mussel contained an average of 2.15×10^3 cfu. Considering the cohabitation in this way, the *F. ebena* were providing a significant pathogen exposure to the brook trout. On the other hand, the water samples were a "point-in-time" assessment. Perhaps, isolation of *A. salmonicida* from water would have been successful if water samples were collected and analyzed at multiple times (e.g., hourly) after 5 days of depuration.

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