

## NOTE

## Use of non-lethal procedures to detect and monitor *Aeromonas salmonicida* in potentially endangered or threatened populations of migrating and post-spawning salmon

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**ABSTRACT:** Non-lethal assay of mucus was assessed for detection of *Aeromonas salmonicida* among feral populations of salmon returning to the Salmon River (Altmar, NY, USA). *A. salmonicida* was isolated from returns of 2 year classes of coho *Oncorhynchus kisutch* and chinook *O. tshawytscha* salmon. Data suggested that non-lethal assay of mucus was comparable to standard lethal procedures used to detect and isolate *A. salmonicida* from kidney tissues. Non-lethal procedures were also used to detect *A. salmonicida* and monitor the effectiveness of antibiotic therapy for the control of furunculosis among valuable Atlantic salmon *Salmo salar* broodstock maintained at the Richard Cronin National Salmon Station (Sunderland, MA, USA).

**KEY WORDS:** *Aeromonas salmonicida* · Furunculosis · Non-lethal detection · Mucus · Kidneys · *Salmo salar* · *Oncorhynchus kisutch* · *O. tshawytscha*

Typical and atypical variants of *Aeromonas salmonicida* can infect an extensive range of freshwater, estuarine, and marine fishes (Evelyn 1971, McCarthy & Roberts 1980, Ezura et al. 1984, Pedersen et al. 1994). Although the pathogen is usually associated with disease among cultured fish, feral populations are also affected. Probably the most notable outbreaks of furunculosis within the natural environment occurred among salmonids in northern Europe in the early 1900s. The severity of these epizootics caused Great Britain to form the Furunculosis Committee to study the epizootiological significance of such outbreaks (Mackie et al. 1930, 1933, 1935). Although *A. salmonicida* has not caused such widespread problems since that time (Bucke 1993), the bacterium has been inter-

mittently shown to either infect or cause disease in wild fish. For example, Rabb & McDermott (1962) detected *A. salmonicida* in brook trout *Salvelinus fontinalis* from the Beaver River (Ontario, Canada) while Weber & Zwicker (1979) reported its occurrence among Atlantic salmon *Salmo salar* in the Restigouche River (New Brunswick, Canada). Nomura & Kimura (1981) detected *A. salmonicida* in kidneys of chum *Oncorhynchus keta*, pink *O. gorbuscha*, masu *O. masou*, and kokanee salmon *O. nerka* from 18 rivers and 1 lake in Japan. Hastein et al. (1978) diagnosed the bacterium as the cause of an atypical furunculosis mortality among minnows *Phoxinus phoxinus* in a Norwegian lake and Wiklund & Bylund (1993) described its role in the etiology of dermal ulcerations of flounders *Platichthys flesus* in the Baltic Sea. More recently, McArdle et al. (1993) implicated the disease as a contributing factor to the decline of sea trout *Salmo trutta* from several rivers in Ireland, and the spread of the disease among feral fish may be at least partially attributed to the escapement of carrier fish from contaminated farms (Johnsen & Jensen 1994).

Because *Aeromonas salmonicida* can be isolated from internal organs of freshly dead, moribund, and even asymptomatic fish, diagnostic examinations generally involve necropsy (Thoeson 1994). Although lethal inspections may not present problems when stocks of fish are plentiful, lethal sampling is inappropriate when applied to stocks that are threatened, endangered, or involved in restoration programs. The restoration of Atlantic salmon to the northeastern United States is one such program where adult returns to New England rivers are small and each sexually

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mature, adult fish represents a genetically valuable commodity.

Cipriano et al. (1992) investigated non-lethal sampling techniques for use with restoration programs and reported on the culture of *Aeromonas salmonicida* from dermal mucus of salmonids either clinically or subclinically affected with furunculosis. Non-lethal isolation was then compared with systemic recovery by repeated sampling of asymptomatic lake trout *Salvelinus namaycush*, and the comparisons confirmed that mucus was indeed a valid site for detection of the pathogen (Cipriano et al. 1994). Those studies, however, dealt only with intensively cultured, captive populations of fish. The current study was conducted to evaluate the application of non-lethal detection of *A. salmonicida* among feral salmon. The study also assessed the use of such techniques to monitor the pathogen after antibiotic treatment of valuable broodstock.

**Materials and methods. Isolation of *Aeromonas salmonicida* from feral salmon:** Each autumn, sexually mature coho *Oncorhynchus tshawytscha* and chinook *O. kisutch* salmon, which migrate from Lake Ontario into the Salmon River, enter the fish ladder at the Salmon River State Fish Hatchery in Altmar, New York, USA. Mature fish navigate the ladder and enter a sorting pond where they are held, briefly. All fish used in this study were sampled on site, as they were removed from the sorting pond. At the height of each return, approximately 100 fish of each species were processed in October 1992 and 1993, to evaluate mucus as a valid sample for assay of *A. salmonicida* in feral fish. Mucus and kidney samples were collected from each fish and processed as described by Cipriano et al. (1992). Samples were individually diluted 1:10 (weight/volume) in sterile phosphate buffered saline (pH = 7.2). Serial log<sub>10</sub> dilutions of each sample were prepared in phosphate buffer and 0.01 ml aliquots of each dilution were plated onto Coomassie Brilliant Blue (CBB) agar (Tryptic Soy Agar, Difco Laboratories, Detroit, MI, USA, supplemented with 0.1% Coomassie Brilliant Blue, R-250, Bio-Rad Laboratories, Richmond, CA, USA). Inoculated CBB agar plates were incubated for at least 72 h at ambient temperature (18 to 20°C) until colonies were visible. Bacteria were quantified in those dilutions yielding 10 to 30 colony forming units (cfu) per plate. Each of these colonies was subcultured onto Tryptic Soy Agar and identified according to microbiological procedures and taxonomic schemes described by MacFaddin (1980). In addition, all dark blue colonies were plated on Tryptic Soy Agar and confirmed as *A. salmonicida* subsp. *salmonicida* if resultant bacteria were non-motile bacilli that yielded an alkaline over acid reaction in Triple Sugar Iron agar (Difco Laboratories), fermented glucose, were cyto-

chrome oxidase positive, produced a brown diffusing pigment, liquified gelatin, but failed to produce indole or ornithine decarboxylase.

**Application of non-lethal sampling to monitor furunculosis:** Approximately 600 two-year-old Atlantic salmon were held as broodstock in 4 outdoor raceways at the Richard Cronin National Salmon Station (Sunderland, MA, USA) to produce progeny for the Connecticut River restoration program. During the spawning cycle from October through November of 1993, fish were handled twice each week. Hatchery biologists noted that some fish were lethargic, had darkened in color, and that several had developed dermal lesions indicative of furunculosis. The presence of *Aeromonas salmonicida* in the kidneys of several dead salmon was confirmed by U.S. Fish and Wildlife Service biologists at the Fish Health Unit (Lamar, PA, USA). Because mortality persisted throughout the spawning period (5 to 10 fish per week), a more complete bacteriologic examination was conducted by non-lethal assay. Mucus from a sample of 27 fish in each of 2 raceways was collected, processed, and plated onto CBB agar, as previously described. Following confirmation of *A. salmonicida*, all salmon were injected intraperitoneally with 2.4 mg of oxolinic acid per kg of fish under an Investigational New Animal Drug permit issued to the U.S. Fish and Wildlife Service by the U.S. Food and Drug Administration for use on Atlantic salmon. Mucus samples from 27 fish per raceway were again taken at 21 d after injection. Bacteria in the mucus samples were quantified and identified, as described previously.

**Results. Isolation of *Aeromonas salmonicida* from feral salmon:** *A. salmonicida* subsp. *salmonicida* was isolated from both year classes of chinook and coho salmon that were sampled upon their return to the Salmon River. In the 1992 returns, the pathogen was detected from the mucus and kidney samples of 15 chinook salmon; 3 additional fish were positive for the bacterium in the kidney but not in the mucus. Regardless of whether mucus or kidney was assayed, bacterial concentrations ranged from  $3.0 \times 10^3$  to  $3.0 \times 10^7$  cfu of *A. salmonicida* per gram of sample. In 1993, fewer chinook salmon were infected than in the previous year. The bacterium was only detected from the mucus of 6 fish and concentrations ranged from  $1.3 \times 10^5$  to  $1.8 \times 10^7$  cfu of *A. salmonicida* per gram of mucus. The kidneys of 4 of these fish were also *A. salmonicida*-positive, the concentration of the bacterium ranging from  $1.0 \times 10^3$  to  $2.2 \times 10^3$  cfu per gram of kidney.

In October of 1992, *Aeromonas salmonicida* was detected in mucus samples from 38 coho salmon, 28 of which were also infected in the kidneys. Bacterial concentrations ranged from  $1.0 \times 10^3$  to  $3.0 \times 10^7$  cfu of *A. salmonicida* per g of sample regardless of the source

of sample. In the 1993 return, 17 coho salmon had bacterial concentrations ranging from  $1.1 \times 10^3$  to  $1.8 \times 10^7$  cfu of *A. salmonicida* per gram of mucus. The kidneys of 9 of these fish were also infected, the bacterium occurring in concentrations ranging from  $3.3 \times 10^3$  to  $1.4 \times 10^7$  cfu of *A. salmonicida* per gram of kidney.

**Application of non-lethal sampling to monitor furunculosis:** When Atlantic salmon were initially examined at the Richard Cronin National Salmon Station, *Aeromonas salmonicida* subsp. *salmonicida* was detected in most of the fish sampled. The pathogen was detected in the mucus of 23 out of 27 fish in raceway 3, and 25 out of 27 fish in raceway 4. Bacterial concentrations were as high as  $8.8 \times 10^8$  cfu of *A. salmonicida* per gram of mucus (Fig. 1). Prior to treatment, total mortality was estimated at 5 to 10 fish  $\text{wk}^{-1}$ . At 21 d

after treatment, non-lethal sampling indicated that a single injection of oxolinic acid had proven very effective. *A. salmonicida* was not isolated from the mucus of any of the fish sampled from either raceway. Mortality was followed for 55 d after treatment during which time only 5 fish died; 3 from fungal infections, 1 as a result of predation, and the other from jumping out of the raceway.

**Discussion.** Results of the present study indicated that non-lethal procedures developed to detect *Aeromonas salmonicida* from mucus of intensively cultured, domesticated salmonids (Cipriano et al. 1992, 1994) were readily adapted to detect the pathogen among feral salmon returns. This was conclusively demonstrated in the 1992 and 1993 returns of chinook and coho salmon in the Salmon River where it was possible to sacrifice the fish to compare the rates of *A. salmonicida* detection in kidney and mucus samples. In one case (the 1992 chinook sample), mucus was a slightly less effective source of sample (by 3 fish) than kidney material. However, for the 1993 chinook salmon return and among both the 1992 and 1993 coho salmon returns, mucus was actually a better source of sample than kidneys. These data indicated that non-lethal methods could be used to detect and isolate *A. salmonicida* from feral fish. Such information was extremely important for application in the Atlantic salmon restoration effort and it allowed us to use mucus sampling as a means for monitoring the effectiveness of chemotherapy used on Atlantic salmon at the Richard Cronin National Salmon Station.

Because feral Atlantic salmon brood stock for Connecticut River restoration are such a valuable commodity, we reiterate that routine lethal sampling of the population to evaluate general health status is not allowed by state and federal agencies. Until now, disease control measures were instituted only after the appearance of mortalities and clinical signs of disease, thus limiting management options at the hatchery. Once mortality was confirmed, oral treatment with ROMET (a potentiated sulphonamide) was normally administered even though fish by this stage usually displayed a degree of inappetence. Under such conditions, the efficiency of conveying a therapeutic dose for a sufficient amount of time was questionable (Inglis & Richards 1992).

Although a systemic diagnosis of the etiological agent causing mortality among the Atlantic salmon was accomplished before the initial examination by non-lethal means, such diagnosis did not ascertain the severity or prevalence of the pathogen within the population. The ability to assess bacterial prevalence by non-lethal mucus assay allowed hatchery biologists to quantify the severity of infection and to monitor the efficacy of treatment. The information provided indi-

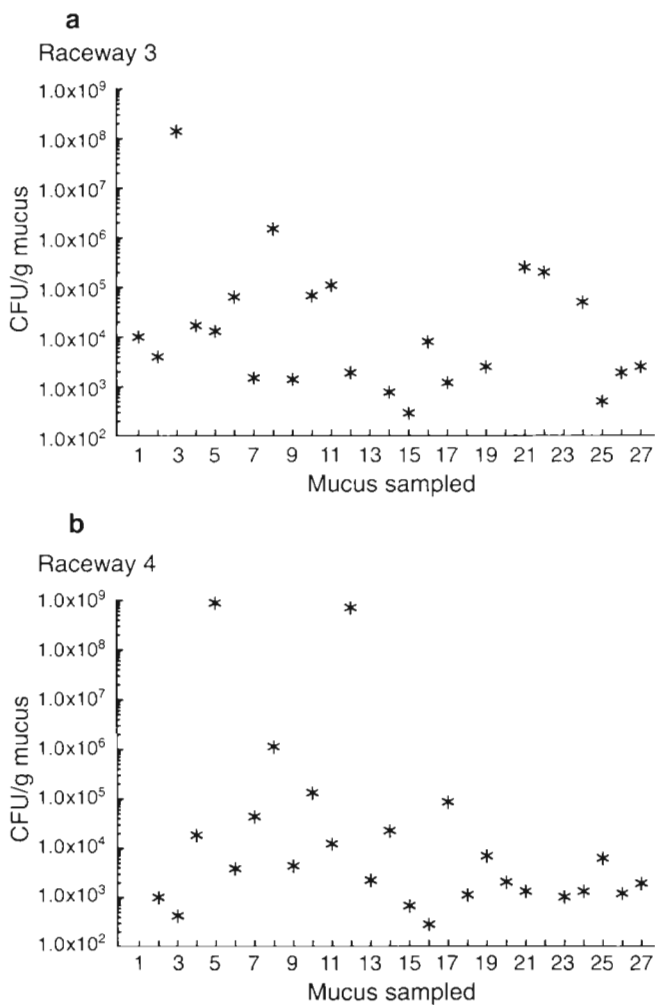


Fig. 1. Concentrations of *Aeromonas salmonicida* expressed as colony forming units (cfu) per gram of mucus on post-spawning Atlantic salmon *Salmo salar* maintained in raceways (a) 3 and (b) 4 at the Richard Cronin National Salmon Station (Sunderland, MA, USA), before treatment with oxolinic acid

cated that almost all fish sampled had relatively high concentrations of the pathogen on their external mucus and an injection of oxolinic acid seemed to eliminate the infection. In fact, additional sampling (not presented here) was later conducted to monitor pathogen levels and adapt management strategies to prevent further epizootics. Our results, therefore, suggest that relatively safe, non-lethal culture of mucus has direct and immediate application for the monitoring of *Aeromonas salmonicida* infections in extremely valuable populations of fish.

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