NATIONAL BIOLOGICAL SERVICE
U.S. DEPARTMENT OF THE INTERIOR

NBS INFORMATION BULLETIN

No. 40 1995

Nonlethal Procedures for Detecting and Monitoring Furunculosis in Salmon

Aeromonas salmonicida, the etiologic cause of furunculosis, infects many freshwater, estuarine, and marine fish. The bacterium is readily isolated by necropsy from internal organs of freshly dead, dying, and apparently healthy fish. Statistical verification requires sacrificing a subsample of fish from each population to be examined. Lethal samples are not appropriate where fishery resources are limited. For example, the return of Atlantic salmon to streams of the northeastern United States are limited, and each sexually mature adult fish is valuable. Because *Aeromonas salmonicida* adversely affects the health of feral and cultured Atlantic salmon (*Salmo salar*) volved in the restoration program, the development of nlethal procedures for detecting and monitoring the athogen is important.

We reported earlier that *A. salmonicida* could be cultured from the mucus of infected salmonids. However, those studies were of intensively cultured, captive populations. We have extended the work to evaluate the application of nonlethal assay to feral salmon and to assess the pathogen prevalences after antibiotic treatment of valuable broodstock. The ability to detect the pathogen in each population by mucus assay was reliable and alleviates the need for destructive sampling.

Aeromonas salmonicida Is Isolated From Mucus Samples of Feral Salmon

Each fall, sexually mature coho salmon (*Oncorhynchus kisutch*) and chinook salmon (*Oncorhynchus tshawytscha*) migrate from Lake Ontario into the Salmon River near Altmar, New York. At the peak of each return, 100 fish from each species were processed to assay mucus for *A. salmonicida* in feral fish. We collected mucus and kidney samples after spawning. The sample was diluted 1:10 (weight per volume) in sterile phosphate-buffered saline (pH = 7.2). Serial $\log_{(10)}$ dilutions of samples were prepared in phosphate buffer, and 0.01 mL aliquots of each dilution were plated onto Coomassie Brilliant Blue agar. Inoculated CBB agar plates were incubated as long as 72 h at ambient temperature until colonies were visible. Bacteria were quantified in dilutions containing 10 to 30 colonies. Individual colonies were subcultured onto Tryptic Soy Agar and identified.

In the 1992 return, the pathogen was detected from mucus and from kidney samples of 15 chinook salmon; 3 other fish were positive by kidney alone. The assays showed a bacterial prevalence of 3.0×10^3 to 3.0×10^7 cfu of *A. salmonicida* per gram of sample. In 1993, fewer chinook salmon were infected. The bacterium was only recovered from the mucus of six fish with concentrations of 1.3×10^5 to 1.8×10^7 cfu of *A. salmonicida* per gram of mucus. Four of these fish were infected systemically and bacterial prevalence was 1.0×10^3 to 2.2×10^3 cfu per gram of kidney.

In October 1992, *A. salmonicida* was detected in samples of mucus from 38 coho salmon—28 were also infected in the kidneys. Bacterial prevalence ranged was 1.0×10^3 to 3.0×10^7 cfu of *A. salmonicida*, regardless of the source of sample. Among 1993 coho returns, 17 salmon sustained bacterial prevalences of 1.1×10^3 to 1.8×10^7 cfu of *A. salmonicida* per gram of mucus. Nine fish were infected systemically with concentrations of 3.3×10^3 to 1.4×10^7 cfu of *A. salmonicida* per gram of kidney.

Nonlethal Sampling Monitors Control of Furunculosis After Treatment

About 600 2-year-old Atlantic salmon were held as broodstock in raceways at the Richard Cronin National Salmon Station (Sunderland, Massachusetts). During the October–November 1993 spawn, fish were handled twice

weekly. Biologists noted that some fish were lethargic, had darkened in color, and several had dermal lesions indicative of furunculosis. Mortality persisted throughout spawning (5–10 fish per week), and a more complete bacteriologic examination was conducted by nonlethal assay of the mucus from each of 27 fish in two raceways. The pathogen was detected from mucus of 23 fish in one raceway (68% of the total bacteria) and 25 fish in another (78% of total bacteria). Prevalence was as high as 8.8×10^8 cfu of *A. salmonicida* per gram of mucus.

After confirmation of *A. salmonicida*, all salmon were injected intraperitoneally with 2.4 mg of oxolinic acid per killigram of fish under an Investigational New Animal Drug permit issued to the U.S. Fish and Wildlife Service by the Food and Drug Administration for use on Atlantic salmon. Mucus samples from 27 fish per raceway were again sampled at 21 days after injection. No *A. salmonicida* was isolated from the mucus of any of the fish sampled from either raceway, and the microflora was more indicative of bacteria commonly associated with the skin of healthy fish. Only five fish died during another 55 days after treatment—three from fungal infections, one from predation, and another by jumping from the raceway.

Nonlethal Assay for *A. salmonicida* Is an Effective Management Tool for Valuable Stocks

Nonlethal assays discussed here were developed to provide hatchery personnel with the ability to assess and monitor the health of live fish. Nonlethal culture of mucus has immediate application for monitoring, preventing, and controlling furunculosis within environments where *A. salmonicida* is enzootic.

For further information contact

Rocco C. Cipriano, L. A. Ford, and J. D. Teska
National Fish Health Research Laboratory
1700 Leetown Road
Kearneysville, West Virginia 25430
(304)725-8462