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Control of Furunculosis and Enteric Redmouth Disease in Sea-Run Atlantic Salmon Broodstock in the Connecticut and Merrimack Rivers

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Abstract.—Adult sea-run Atlantic salmon *Salmo salar* captured and transported to Richard Cronin National Salmon Station (Sunderland, Massachusetts), Nashua National Fish Hatchery (Nashua, New Hampshire), and Whittemore State Fish Hatchery (Waterford, Connecticut) during 1986–1992 were treated with oxolinic acid and a bacterin. The bacterin was developed against furunculosis and enteric redmouth disease. Among the 2,552 fish that were treated since 1986, 362 died and 65 (18%) of those fish had furunculosis. Among 206 untreated fish that were maintained as controls, 109 died and 63 (57.8%) had furunculosis. The reduction in mortality could not be attributed to either vaccine or antibiotic alone without further study. A 3-year study was designed to investigate if adult Atlantic salmon, undergoing the stress of migration, handling, and spawning, could mount a protective humoral immune response. Although the salmon were able to produce an agglutinin response, evidence was not found for production of a protective humoral response by these vaccinated Atlantic salmon.

The infectious bacterial disease furunculosis (causative agent, *Aeromonas salmonicida*), causes losses among sea-run Atlantic salmon *Salmo salar* that are captured from the Merrimack and Connecticut rivers and held for spawning. Enteric redmouth disease (ERM), caused by *Yersinia ruckeri*, has been a bacterial pathogen of lesser concern to managers of sea-run broodstock holding facilities throughout New England. Early attempts at treatment for furunculosis involved antibiotic baths and injections, antiserum injections (1979–1980), as well as vaccination with an *A. salmonicida* bacterin. These actions, however, were never completely satisfactory at controlling infections and subsequent mortality of the valuable broodstock. Since enhanced numbers of fish have been returning to southern New England rivers, furunculosis has become an increasingly important cause of mortality.

In attempts to increase survival of hatchery-released Atlantic salmon and subsequently the number of adult returns, previous investigations have assessed the efficacy of vaccinating parr and smolts. For example, Baum et al. (1982) vaccinated 2-year-old Atlantic salmon smolts with a trivalent *Vibrio* bacterin. These fish were not artificially challenged but were reared in a commercial net-pen operation. Survival among vaccinated and nonvaccinated fish did not significantly differ. Successful vaccinations of Atlantic salmon parr against coldwater vibriosis, a disease caused by *Vibrio salmonicida* have been reported by Holm and Jorgensen (1987), Lillehaug et al. (1990) and Lillehaug (1990). Only a few studies concerning immunization of Atlantic salmon against furunculosis and ERM have been reported. Shieh (1985) reported that fingerlings immunized with extracellular protease from an avirulent *A. salmonicida* were protected against challenge from a virulent *A. salmonicida.* Bruno and Munro (1989) indicated that fry vaccinated with a *Y. ruckeri* bacterin were protected against water-borne challenge for up to 165 d. The response of broodstock to vaccination has not been extensively studied even though vaccination of adults is used as a preventive step to

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control furunculosis and ERM in Atlantic salmon restoration.

In 1985, a devastating outbreak of furunculosis occurred among sea-run Atlantic salmon broodstock at the Nashua National Fish Hatchery (Nashua NFH, Nashua, New Hampshire) and Richard C. Cronin National Salmon Station (Cronin NSS, Sunderland, Massachusetts). As a result, U.S. Fish and Wildlife Service (USFWS) hatchery biologists considered the use of oxolinic acid to prevent epizootics in future spawning populations of sea-run Atlantic salmon. Oxolinic acid is a member of the quinoline group of antibacterial compounds that is used extensively in European fish culture (Grave et al. 1990). In 1986, a protocol to prevent furunculosis and ERM was established and used until 1994. Adult Atlantic salmon returning to the Connecticut and Merrimack rivers were removed from the river, anesthetized, weighed, and measured, and scale samples were collected. Fish were then injected with oxolinic acid and a commercial bacterin prepared against furunculosis and ERM. Adult salmon reaching the hatchery facilities were not only subjected to natural stressors involved in their return to freshwater but to other stressors, such as handling and transport. Therefore, the ability of these fish to mount a protective immune response induced by the bacterin injection was questioned. The economy of vaccinating fish that may not be physiologically or immunologically responsive was questioned, as was the efficacy of oxolinic acid administration.

Methods

Injection of broodstock.—With the help of the National Fish Health Research Laboratory (NFHRL) in Leetown, West Virginia, an Investigational New Animal Drug (INAD) permit was obtained from the Food and Drug Administration (FDA) for the use of oxolinic acid in a protocol for the prevention of disease in sea-run Atlantic salmon of the Merrimack and Connecticut rivers. The protocol required that Atlantic salmon captured and held at Cronin NSS, Whittemore State Fish Hatchery (Whittemore SFH, Waterford, Connecticut), and Nashua NFH receive an intraperitoneal injection of 2.4 mg oxolinic acid/kg fish in combination with an *A. salmonicida–Y. ruckeri* bacterin at the rate of 0.5 mL/kg fish (not to exceed 2.5 mL bacterin/fish). The INAD permit also required the maintenance of control fish and prohibited release of treated fish into the wild.

Serum collection from 1990 broodstock and agglutination procedure.—Serum was collected via the caudal artery from postspawning males maintained at the Cronin NSS. Females that survive spawning are routinely reconditioned and used to produce eggs in subsequent years. Males, on the other hand, are normally sacrificed after spawning and were available for use. Individual serum samples were stored at −70°C until needed. Serum agglutinins to *A. salmonicida* and *Y. ruckeri* antigens were determined by means of standard agglutination procedures (Garvey et al. 1977) in 96 well microtiter plates. Serum from each salmon was tested against three antigens: sonicated cells of *A. salmonicida* (isolate 3.10) and formalin-treated whole cell preparations of *Y. ruckeri* (isolates 11.40 [serotype 1] and 11.29 [serotype 2]) from the bacteriological reference collection maintained at the NFHRL. Phosphate-buffered saline (PBS, pH 7.2) was used in three wells for each antigen tested to serve as the negative control. Antibody titers were expressed as the reciprocal of the last $\log_2$ dilution showing positive agglutination.

Serum collection from 1991 broodstock and agglutination procedure.—In June 1991, 30 grilse that were maintained in isolation at the Craig Brook National Fish Hatchery (East Orland, Maine) were transported to the NFHRL. These fish were maintained throughout the spawning cycle in conditions similar to those at the broodstock stations. Eighteen fish were given the bacterin injection, and nine fish were injected with saline. Biweekly serum samples were taken from each fish to determine kinetics of the agglutinin response to the bacterin. At Cronin NSS, samples were taken in November from 48 males that received the combined antibiotic and bacterin injection and from 6 salmon maintained as controls. Additionally, 28 treated fish (males and females) and 10 control fish were sampled at Nashua NFH. Serum agglutinins for all samples were determined for *A. salmonicida* and *Y. ruckeri* antigens as described previously.

Serum collection from 1992 broodstock and agglutination procedure.—The procedures described above were repeated in 1992; only sample sizes differed. The number of salmon maintained from June to November at the NFHRL consisted of 19 fish that received the bacterin and 4 control fish. At Cronin NSS, 50 treated males and 16 control fish were sampled in November. At Nashua NFH, 50 treated salmon and 4 control fish were sampled in November.

Passive immunization.—Serum samples from the 1991 and 1992 fish having titers to isolate 3.10 antigen of five or greater were pooled, and the sera from control fish were also pooled for each year.

TABLE 1.—Summary of mortality of treatment and control groups of sea-run Atlantic salmon broodstock from the Connecticut and Merrimack rivers treated with oxolinic acid and vaccinated against *Aeromonas salmonicidia* and *Yersinia ruckeri,* 1986–1992.

Year	Number of fish	Deaths (%)	Deaths positive for *A. salmonicida* (%)	Deaths positive for *Y. ruckeri* (%)
Treatment groups[a]				
1992	599	14 (2)	1 (7)	0
1991	426	95 (22)	33 (35)	0
1990	434	35 (8)	1 (3)	0
1989	170	67 (39)	30 (45)	0
1988	135	12 (9)	0	0
1987	429	37 (9)	0	0
1986[b]	359	102 (28)	0	0
All	2,552	362 (14.2)	65 (18.0)	0
Control groups[c]				
1992	43	26 (61)	7 (27)	1 (4)
1991	39	34 (87)	26 (77)	0
1990	37	11 (30)	10 (91)	0
1989	12	6 (50)	1 (17)	0
1988	11	8 (73)	6 (75)	1 (17)
1987	36	11 (31)	5 (45)	0
1986	28	13 (46)	8 (62)	1 (8)
All	206	109 (53.0)	63 (57.8)	3 (2.8)

[a] Treated groups received an intraperitoneal injection of oxolinic acid at 2.4 mg/kg fish and vaccine at 0.5 mL/kg fish (up to 2.5 mL).
[b] Received oxolinic acid at 2.4 mg/kg fish and vaccine prepared against *A. salmonicida* only at 0.5 mL/kg fish.
[c] Received no injections.

Pooled serum was used to passively immunize brook trout *Salvelinus fontinalis* according to the methods of Cipriano and Heartwell (1986). Atlantic salmon were not available for testing of the sera from adult fish in this study; therefore, brook trout were substituted. Brook trout can be injected with Atlantic salmon sera without adverse effects, and this is a reliable method for testing the ability of the sera components to protect fish from a bacterial disease.

Fish passively immunized with sera having high response to isolate 3.10 or control sera were challenged with a virulent isolate of *A. salmonicida* (isolate 3.139). For each treatment group, two replicates of 10 fish were immunized by intraperitoneal injection with 0.1 mL of the appropriate sera. Two tanks, each with 10 fish injected with 0.1 mL PBS, served as controls. A group of 5 fish that were immunized with high-titer sera from control fish or PBS were maintained throughout the experiment but were not challenged. Mortality was recorded for 14 d after challenge; dead fish were necropsied, and subsequent isolations of the challenge organism was confirmed by standard biochemical tests (Amos 1985 as updated by Thoesen 1994).

Results

Since 1986, mortalities have been reduced in groups of Atlantic salmon given the combined antibiotic and bacterin treatment compared with the noninjected control groups (Table 1). Over the 7-year period, 2,552 salmon were treated, and 362 of these died. Of these 362, only 65 (18%) died of furunculosis, and none died of ERM. Conversely, among the untreated control fish (N = 206), 109 fish died and 63 (57.8%) died due to furunculosis. Additionally, 3 fish (2.8%) died due to ERM (Table 1). Results from 1992 are presented in Table 2. As shown, mortalities did not occur at the Whittemore SFH, neither among the treated fish nor the fish maintained as controls. Deaths from furunculosis or ERM among the treated fish at Cronin NSS did not occur in 1992. However, 21 of the 29 control fish died; six of these deaths were due to furunculosis infections and one fish was positive for *Y. ruckeri* (Table 2). Similar re-

TABLE 2.—Results of 1992 treatments for control of *Aeromonas salmonicida* in sea-run Atlantic salmon broodstock from the Connecticut and Merrimack rivers. Treatments are detailed in Table 1.

Facility	Group	Number of fish	Number of deaths (%)	Number of deaths (%) attributed to *A. salmonicida*	Number of deaths (%) attributed to *Yersinia ruckeri*
Richard C. Cronin	Treated	303	8 (2.6)	0	0
National Salmon Station	Control	29	21 (72.4)	6 (28.6)	1 (4.8)
Whittemore Salmon	Treated	105	0	0	0
Holding Facility	Control	9	0	0	0
Nashua National	Treated	191	6 (3.1)	1 (17)	0
Fish Hatchery	Control	5	5 (100)	1 (20)	0
All	Treated	599	14 (2.3)	1 (7)	0
	Control	43	26 (61)	7 (27)	1 (4)

TABLE 3.—Percent of prespawn Atlantic salmon that died of fungus (*Saprolengia* spp.), furunculosis (*Aeromonas salmonicida*), and enteric redmouth disease (*Yersinia ruckeri*) at Richard C. Cronin National Salmon Station and Nashua National Fish Hatchery in 1992. Fish were treated with oxolinic acid and bacterin (vaccinates); the controls were untreated.

	Cronin		Nashua	
Cause of death	Controls	Vaccinates	Controls	Vaccinates
Fungus	48.3	0	80.0	0
Furunculosis	20.7	0	20.0	0
Enteric redmouth	3.5	0	0	0

TABLE 4.—Percentage of sampled Atlantic salmon ($N = 50$) with agglutinin responses to *Aeromonas salmonicida* 3.10 antigen by titer range.

Agglutinin titer range	Percent of salmon
<1–2.8	3
2.9–5.7	5
5.8–8.6	18
8.7–11.3	16
11.4–14.2	39
14.3–17.0	18

sults were found at Nashua NFH during the same year, except that one treated fish died from furunculosis. Fungal infections contributed extensively to mortality at both the Cronin NSS and Nashua NFH (Table 3).

Among 50 male Atlantic salmon tested in 1990, 91% had agglutinin responses over 5.7 to isolate 3.10 antigen (Table 4). In fact, 39% of these fish had agglutinin responses between 11.4 and 14.2. The highest agglutinin response to 3.10 antigen was 17.0, and the mean for the tested sample was 11.1 ± 3.75. The agglutinin responses to *Y. ruckeri* antigen were much lower for the Atlantic salmon sera sampled in this study. The mean response to serotype-1 antigen, 11.40, was 4.5 ± 2.4. Responses greater than 10 were not detected. The *Y. ruckeri* serotype-2 antigen, 11.29, evoked the lowest agglutinin responses in the sera sampled. Only 20% of the fish had responses above 3.6 when tested against 11.29 antigen, and the mean for the group was 2.7 ± 1.6 (Table 5).

In 1991, grilses obtained from Maine and injected only with bacterin had agglutinin titers to *A. salmonicida* (3.10) that reached 15.2 ± 1.6 and continued at 12 and above for the duration of the study. Control fish, also from Maine, injected with only PBS had titers that remained much lower throughout the study (Table 5). Control fish maintained at Cronin NSS (6 fish) and Nashua NFH (10 fish) were sampled in June 1991. Control fish at both broodstock facilities were not injected and had agglutinin titers of 1 or less. In November, postspawning fish were sampled at both broodstock facilities. The surviving males at Cronin (48) had a mean agglutinin titer to *A. salmonicida* (isolate 3.10) of 14.0 ± 5.6, similar to that of fish given the bacterin injection alone at the NFHRL. The Nashua fish had lower titers to the same antigen (9.9 ± 6.3) but were experiencing a fungal epizootic as well as natural exposure to *A. salmonicida* in some cases (Table 1). Titers to *Y. ruckeri* antigens, 11.40 and 11.29, were low (≤ 3) for all groups of fish (Table 5).

During the 1992 season, furunculosis was successfully controlled in the sea-run Atlantic salmon that were treated according to the protocol and were carefully quarantined from the untreated groups of fish at each broodstock facility. As in recent years, there has been no evidence of furunculosis at Whittemore SFH in either group of fish. The presence of *A. salmonicida* in Cronin NSS and Nashua NFH sea-run Atlantic salmon was confirmed in 1992 (Table 2). Untreated salmon suffered prespawn mortality between 70% and 100%. *Aeromonas salmonicida* was isolated from 20% to nearly 30% of moribund fish. *Yersinia ruckeri* was isolated from one control fish at Cronin NSS. In comparison, *A. salmonicida* was isolated from only 1 of the 191 fish treated with the protocol at Nashua NFH. This represents only 0.1% of the combined population of treated sea-run broodfish returning to both river systems (Table 2). Agglutinin titers from the salmon sampled in 1992 were similar to those found in 1990 and 1991 (Table 5).

Results from the passive immunization trials indicated that protection was not conferred to the brook trout receiving the sera with a high agglutinin titer to *A. salmonicida* antigens (Table 6). Mortalities of 50% or greater occurred in the fish receiving sera from control fish, however, 70% or greater mortality was observed in the fish receiving the high-titer sera in both 1991 and 1992.

Discussion

The immune mechanisms of Atlantic salmon are similar to those of other salmonids. Typical serum immunoglobulins (Ig) similar to IgM have been demonstrated in Atlantic salmon (Havarstein et al. 1988), and vaccinated Atlantic salmon parr and smolts have been protected from challenge to specific fish pathogens (Baum et al. 1982; Shieh 1985; Holm and Jorgensen 1987; Bruno and Munro

TABLE 5.—Atlantic salmon serum agglutinin titers to whole cell bacterial antigens (mean ± SD of the reciprocal of the last $\log_2$ dilution showing positive agglutination) at Richard C. Cronin National Salmon Station (Cronin NSS), the National Fish Health Research Laboratory (NFHRL), and the Nashua National Fish Hatchery (Nashua NFH), 1990–1992.

Year and facility	Group	Number of fish	Titer to *Aeromonas salmonicida*, isolate 3.10	Titer to *Yersinia ruckeri*	
				Isolate 11.40	Isolate 11.29
1990					
Cronin	Treated	50	11.1 ± 3.8	4.5 ± 2.4	2.7 ± 1.6
1991					
NFHRL	Vaccinates[a]	18	15.2 ± 1.6	≤1	≤1
	Controls	9	2.1 ± 4.2	≤1	≤1
Cronin	Treated	48	14.0 ± 5.6	2.8 ± 1.6	1.4 ± 1.2
	Controls	6	≤1	≤1	≤1
Nashua	Treated	28	9.9 ± 6.3	≤1	≤1
	Controls	10	≤1	≤1	≤1
1992					
NFHRL	Vaccinates[a]	19	7.1 ± 4.2	≤1	≤1
	Controls	4	2.0 ± 2.3	≤1	1.3 ± 0.9
Cronin	Treated	50	11.6 ± 4.7	1.6 ± 1.8	≤1
	Controls	16	≤1	≤1	≤1
Nashua	Treated	50	11.8 ± 4.6	≤1	≤1
	Controls	4	1.0 ± 2.0	≤1	≤1

[a] Did not receive oxolinic acid treatment.

1989; Lillehaug 1990, 1991; Lillehaug et al. 1990). The ability of adult Atlantic salmon to mount an immune response, especially upon return from ocean migration, has not been investigated.

Despite stress of transport and spawning, the data presented show that adult fish responded immunologically to the *A. salmonicida* portion of the combined bacterin. The strong agglutinin response to *A. salmonicida* is similar to Atlantic salmon responses to other pathogens such as *Vibrio* sp. (Havarstein et al. 1990; Lillehaug 1991). The duration of the response, approximately 6 months, is also within the range reported for fish that have been injected with other bacterins (Johnson et al. 1982; Lamers 1985; Thuvander et al. 1987). Additionally, salmonids exposed to *A. salmonicida* produce serum agglutinins predominantly against the bacterial O-antigen (Cipriano and Pyle 1985). Atlantic salmon sampled in this study responded not only to the O-antigen portion of *A. salmonicida* lipopolysaccharide (LPS) but also to other antigens as demonstrated by an agglutinin response detected to *A. salmonicida* isolate 3.64 (data not shown). The O-antigen portion of LPS is not produced by 3.64 but A-layer is produced by this isolate (Cipriano and Blanch 1989); therefore, agglutinins not only directed against *A. salmonicida* LPS but to A-layer and other antigens were induced in fish injected with the bacterin.

TABLE 6.—Total mortalities (%) for brook trout challenged with *Aeromonas salmonicida* after injection with pooled sera from vaccinated or nonvaccinated Atlantic salmon (10 fish/replicate) at Nashua National Fish Hatchery, Richard C. Cronin National Salmon Station, and the National Fish Health Research Laboratory (NFHRL), 1991–1992.

Year and replicate	Sera from vaccinates			Sera from controls		
	Nashua	Cronin	NFHRL	Nashua	Cronin	NFHRL
1991						
Replicate 1	80	100	80	80	90	80
Replicate 2	70	90	100	70	50	50
1992						
Replicate 1	90	70	80	70	50	100
Replicate 2	100	9	90	80	70	90
Replicate 3	90	70	100	90	90	70

An increase in agglutinin (or antibody) production in fish has rarely been correlated with increased protection after challenge by the respective pathogen (Cossarini-Dunier 1986; Cipriano and Ruppenthal 1987). Successful passive transfer of immunity and correlation of antibody titer with protection, however, have been reported for some fish (Harrell 1979; Viele et al. 1980; Cipriano 1982; Vinitnantharat and Plumb 1993). In this study, protective immunity was not passively transferred to naive brook trout from Atlantic salmon sera having a high titer to *A. salmonicida* antigen. The bacterin, therefore, did not confer protection to the salmon by eliciting a humoral response. Nonspecific immune reactions or cel-

lular responses to the bacterin could contribute, in part, to the success of the treatment for the broodstock.

The salmon had intermediate to nondetectable agglutinin responses to both *Y. ruckeri* serotype-1 and serotype-2 antigens. The salmon mounted an agglutinin response to *Y. ruckeri* serotype-1, but the response was not as high as for *A. salmonicida.* The agglutinin response to *Y. ruckeri* serotype-2 was the lowest among the antigens tested; however, serotype-2 was not a component of the bacterin used to vaccinate the fish. A low response, therefore, was expected. *Yersinia ruckeri* was isolated from the kidney of one fish included in this study. At least some of the fish had been exposed naturally to *Y. ruckeri* antigens in addition to the bacterin. Lack of an agglutinin response, in this case, does not reflect failure of the salmon to mount an immune response because they did produce agglutinins to *A. salmonicida.* Instead, salmon may not respond to *Y. ruckeri* in the same manner as they do to *A. salmonicida.* Other investigators have considered that the cellular response, not the humoral response, may play a primary role in the immune response of salmonids to *Y. ruckeri* (Cossarini-Dunier 1986; Cipriano and Ruppenthal 1987).

As evidenced by the similar titer to *A. salmonicida* antigens among the fish held at NFHRL, Cronin NSS, and Nashua NFH, oxolinic acid did not affect the ability of Atlantic salmon to produce an agglutinin response to the bacterin. The success of the antibiotic–bacterin injection for the broodstock, therefore, is probably attributed to both components. Even high levels of agglutinins may not be protective, but the same bacterin injection used to produce the elevated antibody response may evoke nonspecific immune reactions that were not measured in this study. Although challenge studies with the Atlantic salmon broodstock could not be conducted, data suggest that protection against *A. salmonicida* is not conferred by immunization alone but that antibiotic prophylaxis is important. Alternative antibiotics should be evaluated as replacements for oxolinic acid. Data gathered since 1986 support the fact that furunculosis infections can be kept to a minimum with the continued use of oxolinic acid–bacterin injection (Table 1). The efficacy of this protocol for controlling furunculosis appears successful, but epizootics among groups of treated fish are still a strong possibility. The following combined factors may contribute to prespawn mortality caused by bacterial infection: (1) inadequate immune response in sea-run Atlantic salmon due to the physiological stress brought on by spawning season and (2) consequential exposure of the broodstock to sources of *A. salmonicida,* such as the untreated control fish that often suffer severe outbreaks of furunculosis, near the onset of spawning season. It is believed that these were the two main factors contributing to the 1989 and 1991 epizootics at Nashua NFH.

Another devastating health factor has been identified. Lethal fungal infections (*Saprolegnia* spp.) are a constant threat to Atlantic salmon broodstock. These infections were previously controlled with malachite green under an INAD permit. The permit, however, has been permanently suspended by the FDA. An INAD for the extended use of formalin as a fungal control in broodstock was successfully established for the 1992 salmon run at Nashua NFH and Cronin NSS. The same group of fish set aside as controls for the injection protocol were also used as an untreated control group for the formalin INAD. Records indicate that the majority of control fish succumbed to fungus (Table 3) and that mortality occurred within 2 months of capture. Fungus was controlled in the groups treated prophylactically with formalin. Unless the FDA-approved label is extended to allow use of formalin as a fungicide in species and life stages other than those already indicated (Schnick 1989), fungal infections in Atlantic salmon broodstock will continue to be prevented with formalin under the INAD protocol provisions. Unfortunately, unless these stocks of fish are declared threatened, untreated control groups may continue to be a necessary part of the INAD process.

There is little hope, however, that oxolinic acid will achieve approval by the FDA. Recently, the FDA has banned all veterinary use of the entire class of quinolines. It is necessary, therefore, to now consider other options for fish health management.

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