



# *Bulletin*

of the Aquaculture Association of Canada

Proceedings  
BCMAFF Furunculosis Workshop

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# MANAGING FURUNCULOSIS IN THE '90s

SECOND BCMAFF WORKSHOP ON FURUNCULOSIS, 14-15 FEBRUARY 1995

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## **Calendar**

• **Canadian Conference for Fisheries Research (CCFFR)**, 4-6 January, 1996. Major themes: 1) behavioural ecology and fisheries science; 2) recent advances in the science of aquaculture; and 3) a joint CCFFR/SCL session on the land-water interface. Papers on these major themes are particularly encouraged, but submissions on other areas related to fisheries and aquatic habitat are also welcome. Information: Dr. Jim Grant, Department of Biology, Concordia University, 1455 de Maisonneuve Blvd. West, Montreal, Quebec H3G 1M8 (tel 514 848-2431; fax 514 848-2882; e-mail grant@vax2.concordia.ca).

• **World Aquaculture 96 & the Bangkok Seafood Show**, 30 January–2 February 1996, Queen Sirikit National Convention Center, Bangkok, Thailand. Annual conference of the World Aquaculture Society is hosted by the Thailand Department of Fisheries and the Chulabhorn Research Institute. Sea Fare Expositions, 850 NW 45th Street, Seattle, WA USA 98107 (tel 206 547-6030; fax 206 548-9346).

• **Whirling Disease Workshop**, 6-8 February 1996, Denver, Colorado. Information: Eric Bergeron, (tel 970 491-5396 or fax 491-1413. Attendance is limited so respond early. Registration fee \$75 (until 31/12/95) and \$100.

• **Aquaculture America 96 Conference and Exposition**, 14-17 February 1996, Arlington, Texas, USA. Sponsored by the US Chapter of the World Aquaculture Society and hosted by the Texas Aquaculture Association. Technical sessions, producer seminars, and trade show. Tours to aquaculture facilities, reception at the Circle R Ranch. Information: Sea Fare Expositions, 850 NW 45th Street, Seattle, Washington 98107 (tel 206 547-6030; fax 206 548-9346).

• **Refrigeration & Aquaculture Colloquium**, 20-22 March 1996, Bordeaux Convention Centre, France. Applications and consequences of refrigeration in aquaculture—scientific, technological and economic aspects. Information: Bordeaux Congrès Service, Palais des Congrès, Quartier du Lac, 33 3000 Bordeaux Lac, France (fax 33 56 43 17 76).

Cover: *Salmon smolts at the Capilano Hatchery, Vancouver (William Pennell photo).*

•**Aquaculture Canada 96** — the Annual Meeting of the Aquaculture Association of Canada, 2-5 June 1996, Ottawa. Theme: Diversification. Information: Cyr Couturier, Aquaculture Unit, Fisheries and Marine Institute, Memorial University, P.O. Box 4920, St. John's, Newfoundland (tel 709 778-0609; fax 709 778-0661).

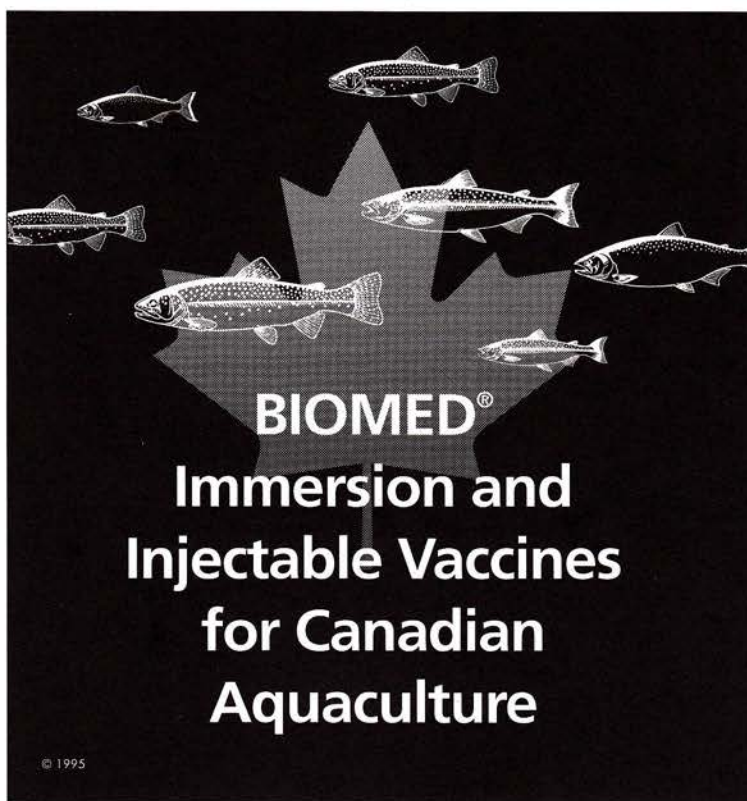
•**International Congress on the Biology of Fishes**, 14-18 July, 1996, San Francisco State University. Meeting combines several established meetings (GUTSHOP, Amazonian Fishes, High Performance Fish, Pacific Biotech, Smolt Workshop, Fish Larvae/Eggs, Anadromous and Catadromous Fish, Fish Stress). Themes: Metabolic Performance, Tropical Fish Biology, Biotechnology Applications, Functional Anatomy, Feeding Ecology & Diet, Contaminant Impacts, Environmental Adaptation, Species Specific Symposia. Deadlines: Abstracts 15 February 1996. Contact: Don MacKinlay, Fisheries and Oceans, 555 West Hastings Street, Vancouver, BC V6B 5G3 (tel 604 666-3520; fax 604 666-3540).

•**Second World Fisheries Congress**, 28 July – 2 August 1996, Brisbane Convention and Exhibition Centre, Brisbane, Australia. Theme: Developing and Sustaining World Fisheries Resources, the

State of Science and Management. Hosted by the Australian Society for Fish Biology. Information: Second World Fisheries Congress, P.O. Box 1280, Milton, Queensland 4064, Australia [tel 617 369 0477; fax 617 369 1512].

•**International Astacology Association**, 11th Symposium, 11-16 August 1996, Lakehead University, Thunder Bay, Ontario. Includes paper and poster sessions on all aspects of crayfish science — culture, physiology, management, taxonomy, zoogeography, and ecology. There will be field trips to visit crayfish habitats in the Thunder Bay region involving travel through northern Ontario's coniferous forest adjacent to Lake Superior. Information: Dr. W. Momot, Dept. Biology, Lakehead University, 955 Oliver Road, Thunder Bay, Ontario P7B 5E1 Canada (tel 807 343-8277; fax 807 343 8023; e-mail Walter.Momot@lakeheadu.ca).

•**Coastal Zone Canada 96**, 12-17 August 1996, Rimouski, Québec. Theme: Integrated Management and Sustainable Development in Coastal Zones. Deadline for abstracts 1 February. Information: CZC '96 Secretariat, Group de recherche en environnement côtier, Université du Québec, 310 allée des Ursulines, Rimouski, Québec G5L 3A1 (tel 418 724-1707; fax 418 724-1842).



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# PROCEEDINGS of THE SECOND BCMAFF Workshop on Furunculosis "MANAGING FURUNCULOSIS IN THE '90s"

Campbell River, 14-15 February 1995

If the success of a workshop is best measured by the participation and attendance of the target audience, then the second workshop on furunculosis held in Campbell River, British Columbia, was definitely successful. Eighty participants, primarily from the industry, fought a freak winter snow storm to attend the meeting and discuss management of furunculosis in the 1990s.

In 1990, Atlantic salmon was quickly replacing the Pacific species as the fish of choice for farming in British Columbia. Along with the switch in species came the expectation of new disease challenges, including furunculosis which has been shown to affect Atlantic salmon more than the Pacific species. The first workshop in 1991 (published in issue 92-1 of the *AAC Bulletin*) anticipated that furunculosis would become a problem and discussions focused on the disease process, how it might impact the fish and farmer, and possible management strategies that could be applied to many of the new Atlantic salmon farming operations developing in B.C. Participants from other farming countries provided insight into the many ways this disease has been managed and how these lessons may be applied to the B.C. industry.

Three years later with Atlantic salmon farming well established in this province, farmers attending the second workshop had an opportunity to discuss the success of the management strategies proposed at the first workshop. More importantly, the attendees discussed new developments for management of this disease which have become an integral part of fish health programs.

Presentations on the first day of the workshop focused on the status of furunculosis in other countries such as Norway and Scotland, as well as on both coasts of Canada. Dr. Randolph Richards, keynote speaker, set the stage for the day's discussions on furunculosis describing its history, occurrence in most wild salmonid populations and discovery more than one hundred years ago. Participants learned that with the advent of the new generation of oil-based vaccines, Scotland has not experienced a problem with this disease for almost two years. Similarly, Norway has embraced vaccines while Eastern Canada appears to have reduced problems through a more regulatory approach.

The new "tool" in the kit of fish health managers is the oil-based furunculosis vaccine. Despite some promising results in British Columbia, there are sceptics amongst the producers—problems experienced with lost growth and excessive peritoneal adhesions were cited as potential pitfalls of vaccine use. Despite these problems, the new generation of fish vaccines is becoming the method of choice in managing furunculosis.

I would like to thank the British Columbia Ministry of Agriculture, Fisheries and Food (BCMAFF) for their assistance and support in organizing this meeting. I would especially like to thank the Aquaculture Association of Canada, Susan Waddy and Jay Parsons for their persistence and perseverance in getting this document edited for publication.

Joanne Constantine  
BCMAFF, Courtney

# Furunculosis in the British Columbia and Washington State salmon farming industries

Diane Morrison

Differences exist between the furunculosis situation in the British Columbian and Washington State salmon farming industries. The difference appears to be that Washington facilities produce carrier-free smolts, because of differences in freshwater sources. Management in B.C. must focus on producing "clean", healthy smolts for saltwater entry. Medicated feed usage for furunculosis treatment in the British Columbian industry is also discussed.

## Comparison of the situation

Is there a difference in the furunculosis situation of British Columbia and Washington State? And, if so, what is the reason for the difference? Since December 1992, I have had the opportunity to visit three of the four main saltwater Atlantic salmon production companies in Washington. During that time I have not seen a single furunculosis epizootic. Our laboratory occasionally cultures *Aeromonas salmonicida* from samples I have submitted and the occasional fish will be observed in pens exhibiting the clinical signs of furunculosis. Sometimes an antibiotic treatment of one or two pens is necessary. Interestingly, the prevalence and severity of furunculosis in farmed Atlantic salmon in Washington State are much lower than in British Columbia. However, it is incorrect to assume that every Atlantic salmon producer in B.C. has problems with furunculosis — many producers manage the disease well and have minimal losses. It is only when the two industries are compared as a whole that it becomes obvious that the furunculosis situation in British Columbia is worse than in Washington. The question that must be answered is: why does this difference exist?

To examine the problem, I broke down the production cycle into its freshwater and saltwater components and looked for differences that could affect the furunculosis situation. In the freshwater facilities, I considered the following

factors: water source, stock origin, broodstock rearing and spawning location, use of artificial lights, use of vaccines, and general management. From this, some differences became apparent. In Washington, many of the hatcheries use either ground water sources or surface water sources that do not contain anadromous fish. Many of our British Columbia hatcheries have to use surface water sources which may contain resident and/or anadromous fish. This difference may affect the producer's ability to produce a "clean" or *Aeromonas salmonicida*-free fish. Jarp et al.<sup>(1)</sup> found that the main risk factor for infection with *A. salmonicida* subsp. *salmonicida* was the migration of anadromous fish into the freshwater supply of the hatchery.

Many of the Washington producers maintain their broodstock in freshwater throughout their life, while in B.C. the majority of producers maintain their broodstock in saltwater and then move them to freshwater lens sites for spawning or else they spawn at the saltwater site. Since vertical transmission of *A. salmonicida* is not reported to occur, it is difficult to see how this could affect the furunculosis levels in the progeny. Differences in water sources and levels of *A. salmonicida* in those waters may be the most significant factor. Washington producers were one year ahead of the British Columbia producers in incorporating IP oil-based furunculosis vaccines in their freshwater facilities. We may see a further reduction in furunculosis epizootics in British Columbia with the industry-wide

use of these vaccines. Differences in general management are difficult to assay without an intimate knowledge of each facility, so I encourage all producers to compare methods and techniques.

In the saltwater phase of the cycle, I compared the following factors: saltwater entry times, use of single year classes at a site, use of fallowing, antibiotic treatments, general management, and wild stock interactions. Again, Washington producers were one year ahead of British Columbia producers in their use of early saltwater entry. Both locations now experience great success with these early entry fish. The smolts start to enter saltwater in November at a size of 70 to 110 g. These fish are well over the stress associated with saltwater entry and are more immunocompetent when water temperatures begin to rise and the risk of furunculosis increases. Due to the limited number of production sites available in both British Columbia and Washington, very few companies fallow sites. Many companies in both B.C. and Washington use single year class sites. General management and strategies on the saltwater sites are very similar. As stated earlier, little therapeutic intervention is required in Washington as compared to British Columbia. The number of wild stocks in B.C.

may be higher than in Washington and this may add to the risk of transfer of disease from wild stock to farmed stock.

## Management

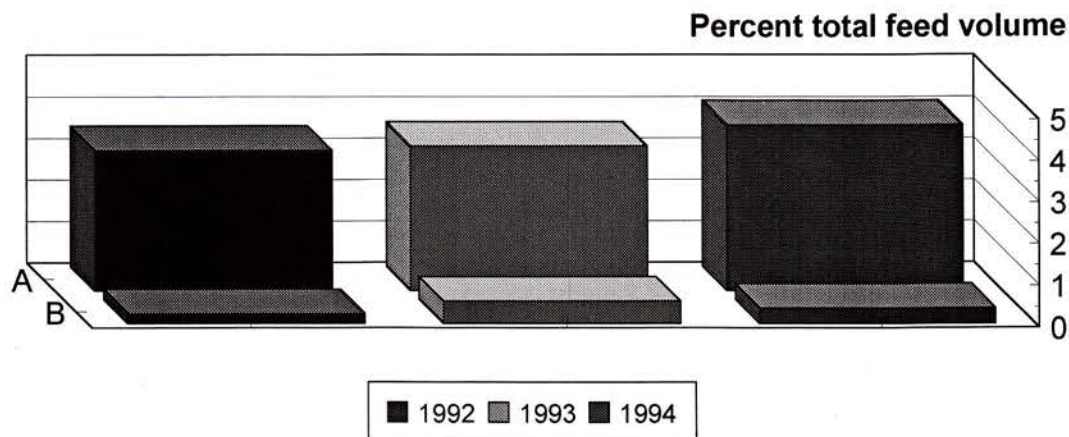
Differences that I was able to identify in management strategy occurred in the freshwater stage of production. I therefore feel that this is where British Columbia producers should focus. It is imperative for the hatchery to have a clean water supply, free of anadromous fish. The goal must be the production of large, 60 to 100 g, healthy *Aeromonas salmonicida*-free, vaccinated smolt. Early saltwater entry will help to ensure that the smolts are well acclimatized prior to increasing summer water temperatures. As new site leases become available, single year class sites and fallowing of sites between production cycles must be adopted as part of management practices.

## Review of medication usage 1992-1994

Figures 1 and 2 show the feed and medicated feed usage at these Washington and British Columbia sites for the years 1992 to 1994. The

Figure 1.

## Milled Medicated Feed Usage (as a % of total volume produced)



A = % milled med feed

B = % milled med feed Rx'd for furunculosis

volumes are expressed as percentages of the total feed volume that Moore-Clark, Vancouver, produced. During these years, the percentage of feed which was medicated has not changed significantly nor has the percentage of medicated feed prescribed for furunculosis treatments (Fig. 1). I then looked at when in the production cycle the medicated feed prescribed to treat furunculosis was used (Fig. 2). The first summer is defined as the time between smolt saltwater entry and the autumn. For example, for smolts that enter saltwater in November 1993, their first summer lasts till October 1994. The second summer is defined as the remainder of the fish production cycle until harvest. In Figure 2, trends are beginning to appear, but will require further tracking. It appears that the smolt health and saltwater entry have been improving. It would also appear that therapeutic intervention in the second summer is still necessary. This may change with the improving quality and health of the smolts and the industry wide use of IP oil-based furunculosis vaccines. It will be of interest to look at that these figures again in 2 to 3 years.

## Summary

The difference between the furunculosis situation in British Columbia and Washington may come down to one fact — Washington producers are able to produce a carrier-free or "clean" smolt for saltwater entry. This is probably due to the difference in freshwater sources. Management must focus on producing "clean", healthy, vaccinated smolts for saltwater entry. Effective furunculosis vaccines must be incorporated into each company's production plan. Total herd health management must be adopted by each company so that baseline health data can be established and proactive health management measures can be taken when necessary.

## References

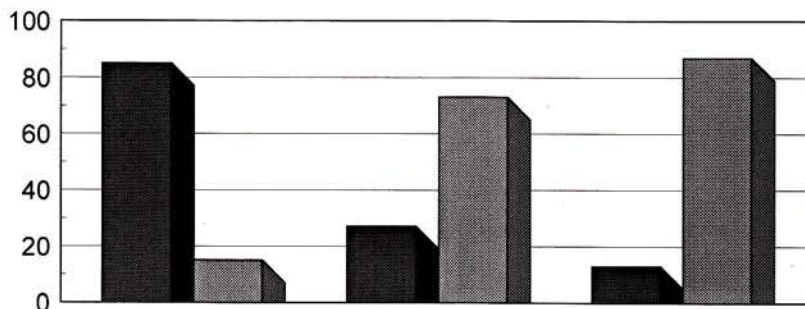
1. Jarp J, Tangen K, Willumsen FV, Djupvik HO, Tveit AM. 1993. *Dis. Aquat. Org.* 17:81-86.

*Diane Morrison, BSc, DVM, is fish health services manager with Moore-Clark Canada Inc. in Campbell River, BC.*

Figure 2.

## Medicated feed usage 1st summer Vs 2nd summer (as % of total medicated feed Rx'ed for furunculosis)

Percent



Saltwater	1992	1993	1994
1st summer	85	27	13
2nd summer	15	73	87

# An overview of furunculosis in Atlantic Canada

K. Larry Hammell<sup>(1)</sup>

Furunculosis occurs in all of the Atlantic Canadian provinces, except Prince Edward Island. *Aeromonas salmonicida* has been isolated from both wild and cultured salmonids and non-salmonids. Disease regulations which attempt to identify the carrier state in hatcheries and then prevent the transfer of positive smolt to seawater may have had some beneficial effects in the past but are now causing the disease to be under-reported.

## Introduction

Although *Aeromonas salmonicida*, the causative agent for furunculosis, has been identified for many years in wild salmonids in Atlantic Canada, statistics regarding the occurrence in cultured fish populations remain vague and under-reported. The prevalence of *A. salmonicida* infection in wild stocks in the Atlantic provinces is not known, but it does exist as an endemic disease in many watersheds. Within salmon hatcheries and farms the prevalence of clinical furunculosis is generally low, but carrier testing has identified subclinical carrier states in several hatcheries.

## Prince Edward Island

Due to the relatively shallow water coastal conditions, finfish aquaculture on Prince Edward Island is limited to freshwater culture of Atlantic salmon (*Salmo salar*), Arctic charr (*Salvelinus alpinus*), brook charr (*Salvelinus fontinalis*), and rainbow trout (*Oncorhynchus mykiss*). There are no published reports of furunculosis occurring in any of the wild or cultured fish from the Island. Furunculosis has not been suspected in any case on Prince Edward Island by veterinary clinicians since the Atlantic Veterinary College Diagnostic Services Laboratory began service in 1987.<sup>(2)</sup>

## Newfoundland

*Aeromonas salmonicida* subsp. *achromogenes* causes disease which is usually referred to as atypical furunculosis. The term atypical usually refers to either a reduced or slow colony pigmentation (i.e. brown coloration), differences in colony morphology, growth at temperatures other than routine (i.e. 22- 25°C), or aberrancies in biochemical reactions.<sup>(3,4)</sup> In the province of Newfoundland, the atypical form of furunculosis is usually the achromogenic form. The clinical presentation of atypical furunculosis can differ from the typical form in that there are very few gross external or internal lesions.<sup>(5)</sup> Histologically, both forms have similar presentations.<sup>(6)</sup>

Atypical *A. salmonicida* has been isolated in Atlantic salmon, rainbow trout, and Arctic charr from both hatcheries and seawater cages.<sup>(7)</sup> The most common clinical disease outbreak usually occurs following handling in the hatchery or at smolt transfer. Outbreaks are most often limited to the period between May and September.<sup>(7)</sup>

Since the timing of clinical outbreaks has been fairly predictable, prophylactic antibiotic therapy has been attempted in hatcheries and seawater cages for a number of years.<sup>(7)</sup> Resistance to oxytetracycline occurred in 1994 and continues in many areas. Potentiated sulfonamides are most commonly employed to treat clinical outbreaks at present.<sup>(7)</sup>

Immersion vaccination for furunculosis occurs in many Newfoundland hatcheries but with limited success. Transfer to seawater occurs at a small size for rainbow trout which makes injection vaccination difficult while fish are still in the hatchery. Due to an occurrence of high mortalities after injection vaccination in 1992, hatcheries have been reluctant to attempt injectable vaccines again.<sup>(7)</sup> However, trials with new oil-based adjuvanted vaccines are being considered.

## Nova Scotia

Atypical furunculosis has been identified in wild salmonids and non-salmonid saltwater species in Nova Scotia for many years<sup>(8,9)</sup> but typical *A. salmonicida* has not been reported.<sup>(10)</sup> However, current attitudes towards disease regulations in Nova Scotia make the interpretation of reported disease statistics difficult.

## New Brunswick

For many years, *Aeromonas salmonicida* has been known to occur in the wild fish of several watersheds in New Brunswick.<sup>(11)</sup> Some of the

# CURRENT ATTITUDES TOWARDS DISEASE REGULATIONS MAKE THE INTERPRETATION of REPORTED DISEASE STATISTICS difficult

endemic watersheds are the water sources for enhancement and commercial hatcheries. In general, the number of hatcheries with identified *A. salmonicida* infections has remained relatively constant over the past decade (see Table 1). Sea cage sites have had similar isolation patterns. Current data collection methods prevent examination of the association between the hatchery source of the smolt and occurrence in sea cages.

It is obvious from the number of isolations in sea cage sites that *A. salmonicida* has been present in seawater aquaculture for a number of years despite the general belief that stress-testing has prevented its transference. Active surveillance of fish at sea cage sites for the presence of *A. salmonicida* is not practiced and, therefore, the number of site isolations is likely under-estimated. Subclinical infections are more likely to be missed in the absence of a surveillance program. However, as clinical disease outbreaks and increased mortality rates are more likely to be investigated and positive results included in this type of summary, the number of bacterial isolations indicates that there has not been a large number of disease outbreaks attributed to *A. salmonicida* in New Brunswick marine sites.

## Screening for furunculosis in New Brunswick

Enhancement hatcheries obtain wild Atlantic salmon broodstock and spawn these fish to place progeny back into the originating river systems. Though the rearing of fish in enhancement hatcheries is not as intensive as in com-

**Table 1. Number of New Brunswick sites that have isolated *Aeromonas salmonicida* since 1984 (compiled from Department of Fisheries and Oceans data<sup>(13)</sup> and personal sources).**

Year	Hatchery Isolations	Sea Cage Site Isolations
1984	4	2
1985	4	5
1986	1	n/a
1987	1	1
1988	n/a	n/a
1989	4	3
1990	5	3
1991	1	3
1992	3	1
1993	4	2
1994	5	8

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## CURRENT DISEASE REGULATIONS OFTEN INHIBIT RATIONAL MANAGEMENT OF FURUNCULOSIS

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mercial hatcheries, the conditions are more intensive than in wild situations.

Intensive rearing places more pressure on the disease resistance of fish due to the fact that there is more frequent opportunity for contact between an infected individual and a susceptible individual and more opportunity to remain alive within the group since sick or slow individuals are not removed by predators. Pathogen transmission is more likely to occur under these conditions but clinical disease may not occur if the groups are not sufficiently stressed. This leads to subclinical infections or carrier states, conditions whereby individuals are infected with a disease organism but do not show any obvious signs of disease.<sup>(13)</sup> Logically, it is expected that the prevalence of infection with *Aeromonas salmonicida* will increase in both enhancement and commercial hatcheries since there is increased exposure between infected and susceptible individuals in both situations. The less stressful rearing conditions found in enhancement hatcheries will reduce the observed prevalence of clinical disease despite the increased prevalence of the subclinical or carrier states of *A. salmonicida* infection. However, commercial hatcheries are more likely to have clinical outbreaks during periods of stress induced by husbandry methods designed to increase overall productivity. For example, size-sorting practices are not often part of enhancement hatchery husbandry but are frequently performed at commercial hatcheries to minimize size variation and maximize growth.<sup>(14)</sup>

Stress-testing for furunculosis, a method which uses elevated temperature and injected corticosteroids, is used to identify the carrier state within populations of fish.<sup>(15)</sup> Clinicians at

the Atlantic Veterinary College of the University of Prince Edward Island have cultured kidney tissue from large numbers of apparently healthy fish from a hatchery which had clinical outbreaks of furunculosis before and after the samples were obtained, but obtained no positive cultures. This indicates that direct cultures of kidney tissues from samples of apparently healthy fish are likely to produce false negative results.

Stress-testing is required to improve the sensitivity of detection (that is, to reduce the number of false negative results). However, it is well known that many hatcheries with endemic levels of *A. salmonicida* infections have had negative results on the stress-tests. There are several possible reasons for this misclassification, including sampling error, inadequate sample size, and poor test sensitivity. Samples of 60 fish per lot (usually defined as a year class) are collected by arbitrarily selecting a small number of individuals from an assortment of tanks (eg. five fish from each of twelve tanks). The sampled fish are transported to a central facility at the Department of Fisheries and Aquaculture in St. George, New Brunswick, and injected with corticosteroid. The water temperature is gradually increased to 18°C and the fish are kept alive for two weeks. All fish which die in the two week period and all live fish at the end of the two week period are necropsied and their kidney tissue is cultured.

It is possible that a sample of sixty live fish from a positive site will not have any individuals carrying the *A. salmonicida* bacteria due to chance alone. If the test has no false negatives and the prevalence of infection is 5% or greater, then this chance error is expected to occur 5% of the time.

Sixty fish should be adequate to detect at least one infected individual if the prevalence of infection is 5% or greater. However, if the prevalence of infection is 1%, then a sample size of close to 300 individuals is necessary to detect at least one infected fish.<sup>(16)</sup>

Negative results from stress-tests for the presence of *A. salmonicida* carriers are not necessarily correct. The overall sensitivity of this method is likely less than 25%. This estimate is based upon the assumption that once a group of fish is positive, it remains positive despite antibacterial therapy or the absence of clinical disease, and also upon the assumption that if one group at a hatchery is positive, then all groups

are positive unless there are extenuating circumstances (e.g. complete isolation). If the sensitivity is truly lower than 25%, then it is possible for hatcheries to test negative when they are actually positive (i.e. false negative).

Salmon enhancement hatcheries are not obligated to perform stress tests for *A. salmonicida* carriers since the fish are being transferred to freshwater systems and not crossing any provincial boundaries. Cultures are performed on kidney tissues from a sample of 30 fish per lot, the results of which must be negative for permission to release the smolt.<sup>(17)</sup> This type of sampling and testing would be expected to identify clinically diseased fish if sick fish were included in the sample (i.e. 95% confident that the prevalence is 10% or less, if there are no positive results) but very few carrier states of *A. salmonicida* infections would be identified.

Commercial hatcheries are obligated to stress-test a sample of 60 fish per lot prior to transfer to seawater. This type of sampling and testing should identify clinically diseased fish and a limited proportion of carrier states.

Routine testing for *A. salmonicida* is usually not desired by hatchery managers due to the fact that all isolations of the bacteria must be reported. Aside from the stress-testing prior to smolt transfer, there is no mandatory testing during increased mortality rate periods. If the bacteria is endemic within a hatchery, then mortality problems are often managed without further testing. Current regulations cause mortality problems to be under-diagnosed.

This is not to say that regulations should require more testing. Rational objectives which consider the economic consequences of the disease versus industry-level control measures should be established prior to designing regulations. Attempts to control endemic disease through actions intended for eradication (e.g. quarantine) are not likely to succeed when producers do not agree with the regulations. However, current regulations may offer a false sense of security since there is a reasonable likelihood of falsely reporting stock to be disease-free.

## Summary

*Aeromonas salmonicida* has been identified in all the Atlantic provinces, except Prince Edward Island, and there have been clinical outbreaks of furunculosis at both freshwater and seawater sites. Furunculosis is a threat which must be

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## RATIONAL OBJECTIVES WHICH CONSIDER THE ECONOMIC CONSEQUENCES OF THE DISEASE VERSUS INDUSTRY-LEVEL CONTROL MEASURES SHOULD BE ESTABLISHED PRIOR TO DESIGNING REGULATIONS

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managed carefully to minimize its impact on productivity. Current disease regulations often inhibit rational management of furunculosis.

## Notes and references

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# Development of resistance in *Aeromonas salmonicida* in Scotland

R. H. Richards

Analysis of data on the development of resistance to antibiotics shows a transfer of resistance from freshwater to marine sites. Fallowing of marine sites dramatically reduces bacterial numbers at a site but has had variable success in reducing the incidence of antibiograms showing resistance. In the last two years there has been a dramatic decrease in the number of isolates obtained, reflecting the decline in furunculosis outbreaks. Surprisingly, there has also been a dramatic increase in oxytetracycline resistance.

The results presented in this paper are from detailed analysis of samples collected by the diagnostic service of the Institute of Aquaculture in Stirling, Scotland. Results to 1992 are only summarized as they have been published elsewhere.<sup>(1-6)</sup> Changes in resistance patterns since 1992 are reported here and will shortly be published more extensively. Mechanisms of the development of resistance to antibiotics such as plasmid transfer and chromosomal change will not be discussed. Instead, the focus is on the development of antibiotic resistance which took place over the late 1980s and early 1990s and

the major factors involved in the development of resistance.

In the period between 1988 and 1991, antibiotic sensitivity patterns from 354 outbreaks of furunculosis among salmon in Scotland were investigated. The study involved 46 rearing units in 36 geographically separate seawater or freshwater sites located principally on the west coast of Scotland or in the Scottish islands. Repeat samples taken within a fortnight, giving bacteria with identical sensitivity patterns, were included only once in the study, resulting in 444 isolates being tested for sensitivity to commonly available antibiotics by the disc diffusion

method. Antibiotic dose per disc and the criteria used to categorise resistance are shown in Table 1. Resistance to individual antibiotics is given in Table 2.

The greatest resistance was to oxytetracycline. In 1991, only 12% of isolates were sensitive to all six antibiotics, although many showed multiple resistance (Table 3). The patterns of multiple resistance were similar in all 3 years, but there was a significant increase in resistance to all antibiotics in 1990-1991. There were 14 patterns among 122 isolates in 1988-1989, 18 among 144 isolates in 1989-

Table 1. Bacterial inhibition zone diameter and level of antibiotic sensitivity.

Antibiotic disk dose (µg/mL)	Relationship of minimum zone site (mm) to sensitivity			
Oxytetracycline 30	28	19	12	0
Oxolinic acid 2	25	17	12	0
Co-trimoxazole 25	29	20	11	0
Sulphafurazole 100	29	20	11	0
Nitrofurantoin 100	26	17	11	0
Furazolidone 50	26	17	11	0
Amoxicillin 10	29	23	14	0
Level of sensitivity	+++	+	+	0

**Table 2. Resistance of *Aeromonas salmonicida* to antibiotics between 1988 and 1991.**

Resistance to	Resistant strains		
	% in 1988-89	% in 1989-90	% in 1990-91
None	18	19	12
Oxytetracycline	55	55	50
Oxolinic acid	41	31	54
Co-trimoxazole	11	10	15
Nitrofurantoin	26	26	37
Furazolidone	26	28	34
Amoxicillin	—	0 (n=31)	2

**Table 3. Antibiotic patterns of *Aeromonas salmonicida* isolates.**

Resistance to	Isolates in:		
	1988-89	1989-90	1990-91
0	22	28	22
1	30	43	24
2	11	7	30
4	0	1	3
5	0	1	1
1,2	19	13	25
1,3	2	3	1
1,5	1	2	1
2,3	1	2	1
2,4	0	1	0
2,5	0	1	0
4,5	12	17	19
1,2,3	4	6	8
1,2,4	0	0	1
1,3,4	1	0	0
1,4,5	0	2	9
2,4,5	9	7	10
3,4,5	0	0	1
1,2,3,4	0	0	2
1,2,4,5	5	6	6
1,3,4,5	4	2	0
2,3,4,5	0	0	2
1,2,3,4,5	1	2	12
Number of strains	122	144	178

Key: 1 = oxytetracycline; 2 = oxolinic acid; 3 = co-trimoxazole; 4 = nitrofurantoin; 5 = furazolidone

1990, and 19 among 178 isolates in 1990-1991.

Isolates with a single antibiogram were found in excess of 70% of outbreaks in all years. In the remaining outbreaks, isolates with different antibiograms were found in different cages at the same site, different fish in the same cage, and in some cases from the same fish (Table 4).

What lessons can be learned from these early results? First, disc results have been compared with more sensitive MIC (minimum inhibitory concentration) values produced by the agar doubling dilution method and found to give virtually parallel results, confirming that the disc method works well in field use.

Analysis of individual results has also shown a clear transfer of resistance from freshwater to marine sites, a spread of infection between stocks in farms with multiple year classes, and often more than one antibiogram present in an individual site. The worst case was seen when individual cages contained smolts of multiple origin, often resulting from a policy of buying the cheapest smolts available — fish are often bought in small batches, resulting in individual fish containing a number of organisms with different antibiograms, making treatment difficult. Use of a particular antibiotic often resulted in increased growth of organisms with another antibiogram as no single antibiotic would treat all isolates. Multiple antibiotic therapy was not carried out.

The antibiograms of isolates from freshwater fish with infection apparently arising from adult wild fish migrating from sea to freshwater has also changed. Whilst initial isolates were generally sensitive to all antibiotics, in some areas increasing numbers of escapee fish have apparently brought resistant antibiograms to the freshwater stock.

**Table 4. Antibiotic sensitivity patterns (antibiograms) of *Aeromonas salmonicida* isolates from outbreaks of furunculosis.**

Number of antibiograms per outbreak	Number of outbreaks		
	1988-89	1989-90	1990-91
1	74%	77%	71%
2	16%	17%	20%
3	7%	6%	6%
4	3%	0	2%
5	0	0	1%

Fallowing of sites has had variable success in reducing the incidence of antibiograms showing resistance. In some cases, new fish stocks have remained clear of infection. In others, reversion to fully sensitive strains has occurred, and yet in others the original antibiogram has been maintained. The effects of removal of selection pressure have therefore been quite variable. Perhaps the most important point is that, in the absence of "diseased fish", the organisms have limited survival ability and fallowing will dramatically reduce bacterial numbers at a site.

Improvements in husbandry such as reducing stocking density and daily removal of mortalities, together with effective vaccination, tips the balance in favour of the fish so that even though *A. salmonicida* may be present, it does not cause

disease. It is necessary however to continually monitor for *A. salmonicida* in case an outbreak occurs despite good husbandry — for instance following a storm, algal bloom, or predator attack, so that rapid treatment with an appropriate antibiotic can begin. There is no time during an outbreak to await detailed bacteriology results before commencing treatment.

In the last two years there has been a dramatic decrease in isolates obtained (Table 5), reflecting the decrease in outbreaks of furunculosis. Perhaps surprisingly, there has also been a dramatic increase in oxytetracycline resistance but no increase in the low level of resistance to potentiated sulphonamides. Amoxicillin resistance increased in 1993, but was reduced again by 1994, and fortunately this was not associated

**Table 5. Comparison of 1993 and 1994 results.**

	1993	1994
Total number of "isolates"	147	69
% Fully sensitive	24%	12%
% Resistant to oxytetracycline	62%	82%
% Resistant to oxolinic acid	38%	36%
% Resistant to co-trimoxazole	29%	30%
% Resistant to Amoxicillin	9%	2%
% Resistant to oxytetracycline and oxolinic acid	28%	33%
% Resistant to oxolinic acid and co-trimoxazole	16%	17%
% Resistant to oxytetracycline and co-trimoxazole	24%	30%
% Resistant to oxytetracycline, oxolinic acid and co-trimoxazole	13%	17%
% Resistant to oxolinic acid, oxytetracycline, co-trimoxazole and Amoxicillin	0.7%	0%

Sensitivity = +++ and ++ rating

**Table 6. MICs of Amoxicillin for resistant *Aeromonas salmonicida* isolates.**

Number of isolates	Amoxicillin MIC ( $\mu\text{g/mL}$ )
2	10
6	5
3	2.5

with strains resistant to other antibiotics. Similarly, the percentage of strains resistant to three or more antibiotics has decreased — to 13% in 1993 and 17% in 1994.

The relative resistance found to the antibiotics currently in use is interesting. The most resistance was shown to oxytetracycline, but this probably reflects its use as the drug of choice in treating fully sensitive strains because of its price and lack of problems with appetite depression. Oxolinic acid, while popular for use in freshwater, has produced variable results in salt water — experimental results have demonstrated difficulties in obtaining good uptake into the serum. It has commonly been used at three times the recommended dosage to ensure effective treatment in salt water.

The least resistance was present to potentiated sulphonamides and nitrofurans. Potentiated sulphonamides are commonly used only in the face of resistance to other drugs, as appetite depression resulting in poor feeding response by day 4 or 5 of a treatment is common. Nitrofurans have never been licensed for use in fish in the United Kingdom, but have been used in treating resistant organisms following the "cascade"

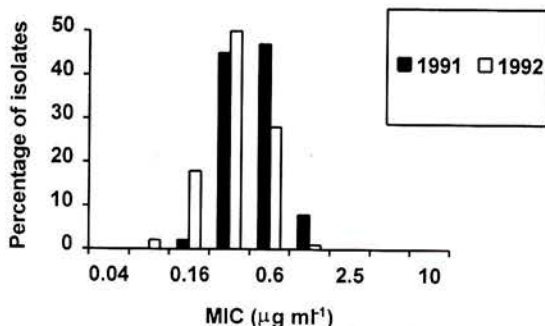
system of choice. Nitrofurans also commonly cause appetite depression and many have been banned because of their perceived toxic effects and residue risks. Furazolidone is the only remaining nitrofuran licensed for animal use in Europe and its future will be decided upon this summer.

The problem of multiple resistance also deserves special consideration. In some cases, resistance mechanisms common to different antibiotic classes may be implicated, but in others resistance may be sequentially developed. Perhaps a major implication from the point of view of monitoring is that it is necessary to carry out multiple isolations from individual fish as well as from numbers of fish during sampling programmes.

The most recent case of development of resistance is to Amoxicillin. Amoxicillin was first licensed for aquaculture use in the United Kingdom in 1990 because of lack of resistance in *Aeromonas salmonicida* and its very short withdrawal period. The two Amoxicillin products in use in 1995 have withdrawal periods of 40 degree-days and 50 to 80 degree-days, making the product especially useful in large, market-size fish.

Two hundred and ninety-five isolates from naturally occurring outbreaks of furunculosis in salmon farms in 1991 and 1992 showed MIC values between 0.09 to 1.25 mg/mL (Fig. 1). However, 11 isolates from two adjacent farm sites in September 1992 showed increasing resistance: of the isolates obtained prior to the development of "resistance", more than 90% in each year had MIC values of 0.6 mg/mL; in 1991 and 1992, 46.3% and 71% respectively had MICs of 0.3 mg/mL.

However, field results with Amoxicillin were variable and this prompted detailed analysis of serum Amoxicillin values during treatment. Analysis revealed a range of 0 to 5 mg/mL and even in successful treatments, only 70% of the population had 3 mg/mL, i.e., 30% of the population achieved sub-inhibitory or zero concentrations. Where treatment was less successful, results were worse. In most cases, this could be related to delays in commencing treatment and variable appetite in fish.



**Figure 1. MICs of Amoxicillin for isolates of *Aeromonas salmonicida* in Scotland in 1991 and 1992.**

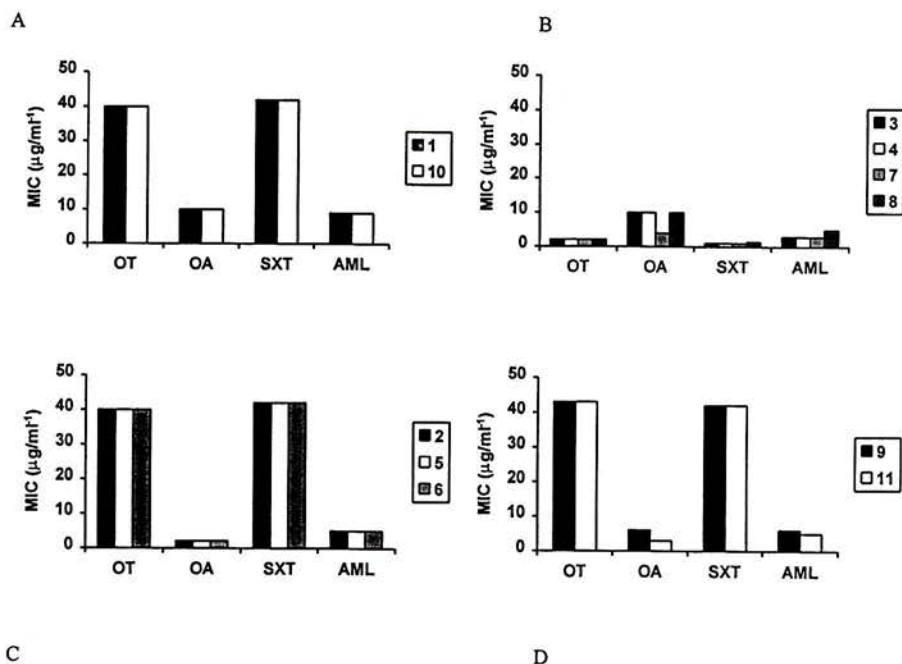


Figure 2. MICs of the four UK-licensed aquaculture antibacterial agents for the Amoxicillin resistant group of *Aeromonas salmonicida* isolated in 1992; (A) antibiogram 1, isolates 1, 10; (B) antibiogram II, isolates 3, 4, 7, 8; (C) antibiogram III, isolates 2, 5, 6; and (D) antibiogram IV, isolates 9, 11.

The resistant isolates in September 1992 provide an interesting group of bacteria; although found simultaneously in two closely related but separate locations, the eleven isolates produced a range of antibiograms (Figure 2).

It was not possible to trace the origin of this resistant group of isolates from their sensitivity patterns alone and it is not possible to even decide whether resistance was introduced from several sources or whether resistance had spread within the farms concerned. Amoxicillin had not been used in these sites prior to finding the resistant strains or even in the vicinity, so no selection pressure had been applied in the fish farms concerned. Though unlikely, because of the siting of the farms, it was possible that resistance resulted from R-plasmid transfer resulting from human or veterinary use. Detailed analyses of this possibility in Japan<sup>(7)</sup> suggest that this does not occur even in the proximity of resistance of human or animal origin.

This Amoxicillin study has been reported by Inglis and Richards<sup>(2)</sup> and Inglis *et al.*<sup>(6)</sup>

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# Comparative cost of treating furunculosis in Atlantic salmon

John Brocklebank

Furunculosis is caused by a gram-negative bacterium, *Aeromonas salmonicida*. In British Columbia, furunculosis can be treated with three approved, commercially available antibiotics. One of these is an oxytetracycline (Terramycin Aqua<sup>®</sup> from Pfizer), and two are potentiated sulphonamides (Romet<sup>®</sup> from Hoffman-LaRoche, and Tribriksen<sup>®</sup> from Mallinkrot, formerly Cooper's Agropharm). In addition, two other antibiotics (Aquaflor<sup>®</sup> or florphenicol from Schering-Plough and Erythromycin phosphate, 75% activity from Sanofi) can be made available, under exceptional circumstances, to veterinarians via an Emergency Drug Release (EDR) from the Bureau of Veterinary Drugs (BVD).

The top part of Table 1 lists the medicants available. It names the drug and its percent activity, the dose on an activity basis, treatment duration, dose of medicant incorporated into the feed when fed at a rate of 1% body weight per day and, most importantly, the cost of the drug to a veterinarian on a premix basis. It should be noted that for Terramycin Aqua<sup>®</sup> (oxytetracycline, OTC) there are two doses. Under the Feeds Act, a fish farmer can medicate fish with oxytetracycline (OTC), without a veterinarian's prescription, either by top-dressing regular feed or with milled feed, at a dose of 75 mg activity per kilogram of fish for ten consecutive days as described on the bag label. This is referred to in the Feeds Act as the Manufacturers Ingredients Brochure (MIB) level. If OTC is used at any other dose, duration, or clinical condition, a veterinarian's prescription (R<sub>x</sub>) is required.

Table 2 gives the cost of the medicant for the amount of medicated feed required for the fish biomass being treated. Several points are worth noting:

- For each medicant, including oxytetracycline at the two doses given (MIB and R<sub>x</sub>), the cost of medicant per fish and per

kilogram of fish does not change with the increase in fish biomass.

- The total cost of medicant increases with increasing fish biomass.
- For market weight fish of 3.5 kg, the lowest cost treatment is OTC at MIB level. This is followed by Romet<sup>®</sup>, Tribriksen<sup>®</sup>, OTC at R<sub>x</sub> level, Aquaflor<sup>®</sup>, and finally Erythromycin phosphate (these costs are calculated on a zero mark-up basis).
- Treating 3.5 kilograms of fish with EDR antibiotics is very expensive. A cost-benefit analysis should always be done to determine whether treatment, harvesting, husbandry, or a combination thereof is the best way to manage furunculosis. A cost-benefit analysis for each medicant based on the number of fish saved may be of less value in the decision making process than a cost-benefit analysis based on the difference in biomass saved for each treatment. The latter analysis takes into account additional production costs, delayed harvesting and processing costs, and the market price of salmon, including the risk of delayed harvest due to prescribed antibiotic withdrawal times. The exception to the cost-benefit analysis approach is broodstock that, based on their genetics, are immensely valuable.

## Antimicrobial strategy for treating furunculosis in the 1990s

The common practice to date has been to medicate fish with oxytetracycline whether they become infected with furunculosis in the hatchery, the freshwater lens, or the saltwater pen. This is based primarily on four reasons: palatability, cost, ease of administration, and an assumed sensitivity by *Aeromonas salmonicida* to oxytetracycline. As the fish biomass increases

**Table 1. Medicants available for treating furunculosis.**

Drug	Terramycin Aqua® (44% active)	Romet® (33% active)	Tribriksen® (40% active)	AquaFlor® (50% active)	Erythromycin PO4 (75% active)
Dose	75 mg/kg MIB to 100 mg/kg (Rx)	50 mg/kg (Rx)	30 mg/kg (Rx)	10 mg/kg EDR (Rx)	100 mg/kg EDR (Rx)
Duration	10 days	5 days	8 days	10 days	10 days
Quantity for feeding rate of 1%	7.5 kg/ton	5 kg/ton	3 kg/ton	1 kg/ton	10 kg/ton
Cost of premix	\$17.25/kg	\$36.40/kg	\$57.75/kg	\$750.00/kg	\$448.00/kg

**Table 2. Cost of medicants.**

Biomass	Cost of Terramycin Aqua®	Cost of Romet®	Cost of Tribriksen®	Cost of AquaFlor®	Cost of Erythromycin PO4
100,000 fish @ 75 g = 7,500 kg	\$221 \$294	\$228	\$260	\$1,125	\$4,489
100,000 fish @ 250 g = 25,000 kg	\$735 \$980	\$758	\$866	\$3,750	\$14,933
100,000 fish @ 1.5 kg = 150,000 kg	\$4,411 \$5,881	\$910	\$5,198	\$22,500	\$89,600
100,000 fish @ 3.5 kg = 350,000 kg	\$10,291 \$13,722	\$10,617	\$12,128	\$52,500	\$209,067
Cost per fish	\$0.10 \$0.14	\$0.11	\$0.12	\$0.53	\$2.09
Cost per kilogram of fish	\$0.029 \$0.039	\$0.03	\$0.34	\$0.15	\$0.59

and/or with recurrent infections, *A. salmonicida* may lose its sensitivity to oxytetracycline. Consequently, potentiated sulphonamides are employed. Frequently, Tribriksen® or low doses of Romet® (2 mg activity per kg fish) are used for the same reasons as cited for oxytetracycline.

Hopefully, the fish will remain sensitive to one of these three antibiotics throughout the remainder of the production cycle if further treatment is required. Increasingly, however, there have been reports of furunculosis developing resistance to all three antibiotics (so-called "triple

resistance"). Consequently, the fish farmer is left with EDR antibiotics that are exceedingly expensive and sometimes, depending upon the ambient water temperature, necessitate a prolonged antibiotic withdrawal period.

For the 1990s and beyond, costs of production data dictate that antibiotic management of furunculosis be based on a least cost basis. In addition, it is imperative for fish culturists to take into account the withdrawal periods prescribed for each antibiotic and the sensitivity of *Aeromonas salmonicida*, either on a site or per pen basis, to the three available approved antibiotics. Based on the cost of medicants versus biomass in Table 2, as well as my own experience, I would strongly urge fish farmers to avoid using OTC for treating furunculosis before the second year in saltwater. The main exception would be fish concurrently infected with both furunculosis and bacterial kidney disease (BKD). Treatment with OTC at the MIB level is the least expensive and in my own experience it is very efficacious. It has the shortest antibiotic withdrawal times (40 days above 10°C and 80 days below 10°C) of all the antibiotics mentioned. Treatment with OTC at R<sub>x</sub> levels appear to result in marginally better treatment response; however, the withdrawal period above 10°C is 60 days and the total cost of medicant is proportionately greater. Where antibiotic resistance is encountered or may be expected, OTC should be rotated with Romet® (50 mg activity per kg fish for 5 days). Although this dose may result in appetite suppression or feed refusal, it is very effective when dosing fish on a 2 days "on", 1 day "off", 2 days "on", etc., regime. In addition, it has a 42 day withdrawal time when fish are treated at an ambient water temperature above 10°C. Tribissen®, because it is very palatable, should be used in the hatchery, freshwater lens, or within the first year in seawater for furunculosis. Tribissen® has an 80 day withdrawal period at temperatures above 10°C.

Aquaflor® and Erythromycin-phosphate, based on their respective costs of treatment, can only be justified for treatment of furunculosis in broodstock and fish of low biomass (i.e., selected pens or fish of low mean body weight). It is essential, however, that these drugs become commercially available or at least remain available under an EDR. Aquaflor® is very effective against furunculosis. It is exceedingly palatable even to sick fish and the withdrawal time above

10°C is 60 days. Erythromycin phosphate, although more expensive than Aquaflor® as a treatment, is also required by industry for the treatment of fish of low biomass infected with both furunculosis and BKD. Its withdrawal time above 10°C is 105 days.

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# Management of furunculosis in Norway

Leiv Aarflot

Furunculosis was first introduced to Norwegian fish farms in 1985 and was a major disease problem in Atlantic salmon for many years. Although it had previously been described in wild salmon in Norway, the major outbreaks in salmon farms in 1985 resulted from imported smolt from Scotland. Furunculosis caused by *Aeromonas salm.* var. *salm.* is a group B disease under the Norwegian Fish Disease Act. Today the disease is hardly present at all in Norwegian fish farms, mainly due to strict regulations, improved husbandry and efficient oil-based vaccines. This can be visualised by the fact that the quantity of antibiotics used has dropped from 36 mt in 1990 to 1 mt in 1994, while salmon production has risen from 155,000 mt to 205,000 mt.

## Introduction

When furunculosis was first introduced to Norwegian aquaculture from imported Scottish smolts in 1985, the problem was confined to 20 farms in one area and seemed to remain localized after the fish were slaughtered and the sites emptied. However, the disease reappeared in 1989, spread along the coast, and rapidly became endemic.

There was considerable discussion as to how to handle the furunculosis situation as almost every sea site and a number of hatcheries were affected. According to Norwegian legislation for contagious diseases, furunculosis meets the criteria for a group B-disease. Thus it was illegal to sell smolt from a hatchery where the pathogen had been found and bankruptcy became a threat, and indeed a fact, for many fish farmers.

I will describe the strategies used to control furunculosis over the past few years for the various phases of the salmon culture business.

## Broodstock farms

Even though desirable, it was soon recognized that it would be impossible to have all broodstock come from sea sites free from furunculosis. Instead, regulations require that very strict hygiene precautions be followed on the farms and that the water supply to the farm be free of

anadromous fish upstream from the water intake.

Brood fish are normally transferred to freshwater in the early autumn. After stripping, each fish is autopsied, examined by a veterinarian, and bacteriological samples are taken from the kidney, eggs and semen. Only batches of eggs from fish with negative test results are retained. Each batch of fertilized eggs is disinfected in an iodine bath (10 mL Buffodine® per liter of water for 10 minutes) and labelled with the identification of the male and female parents. After delivery to a hatchery, the eggs are disinfected again.

For several years the breeding program has selected for fish resistant to furunculosis and there are high expectations for this program.

## Hatchery

Hatcheries must buy their eggs from a broodstock farm using the furunculosis control mechanisms described above. Incoming eggs must be kept separated from the rest of the biomass in the hatchery.

Very important to the hatchery is a water supply free from anadromous fish. If necessary, there are several kinds of fences available to prevent the passage of fish. However, their use often leads to conflicts with the authorities or with other interests in or around the river.

If the hatchery uses seawater, an approved

disinfectant unit (UV-radiation unit) must be installed. There are different types available, both low-pressure (Unique) and high-pressure (Aqua-care, Katadyn), and in my region, 7 out of 14 hatcheries have such equipment. Tests must be taken monthly, analyzing for UV-transmission and presence of seawater vibrios before and after radiation..

Birds can transport contagious material over long distances so outdoor tanks must be under a net roof.

Each smolt is vaccinated against furunculosis, usually with a triple oil-based injected vaccine. A veterinarian must visit the hatchery regularly — a minimum of 12 inspections per year is required — and at least 500 fish must be autopsied each year with 300 or more being examined in the three months prior to delivery of fish.

Most fish are transported by boat, although quite a number are shipped by truck. Some years ago helicopters were used, but it was expensive and many of the shipments were unsuccessful. Transportation of fish must be by an approved shipper. All equipment must be washed and disinfected between shipments.

A certificate of health and origin must accompany each shipment of smolt, fry, or eggs. The certificate provides information about the fish (origin, mean weight, number), the shipper, and the receiver. The certificate also describes how the authorities are trying to prevent the spread of contagious diseases in Norway. Before signing the certificate, the veterinarian must confirm that:

1. There are no restrictions on the hatchery because of contagious diseases.
2. There are no test results or information suggesting the presence of such diseases (except for IPN without clinical signs of any kind).
3. Regular veterinary inspections (at least 12) have been conducted during the last year and the last inspection was not more than 7 days prior to signing the certificate (which is valid for 14 days).
4. The farmer's records of daily mortality and other signs of disease in the hatchery have been inspected.
5. Postmortems of at least 500 fish were conducted over the past year and a minimum of 300 postmortems were done in the past 3 months.
6. The group of fish covered by the certificate were under health control for at least the

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**THERE SHOULD NEVER  
AGAIN BE A SINGLE SMOLT  
PUT TO SEA IN NORWAY  
WITHOUT BEING VACCINATED  
WITH THE BEST VACCINE  
AVAILABLE. AND LET THIS BE  
MY ADVICE TO THE FARMERS  
IN BRITISH COLUMBIA TOO.**

---

past 9 months (smolts).

7. The farmer has information about group A and group B diseases from the government veterinarian's office.
8. There are no anadromous fish in the water supply above the water intake.
9. There has been no intake of broodstock to the hatchery for stripping.
10. There is no intake of seawater and the fish have not been exposed to seawater. If there is an intake of seawater, a dispensation can be provided if the water is disinfected using approved equipment and the efficacy of the system is proven with monthly samples.

In hatcheries that have furunculosis, all the fish must be destroyed, the entire hatchery must be disinfected and any new fish must be tested for *Aeromonas salm.* var. *salm.* by the Latent Carrier Test (LCT) before shipments are permitted. Currently, if the hygiene routines used in the hatchery indicate that the disease has not spread within the hatchery, it is possible to keep some of the hatchery biomass after destroying the diseased groups. In any case, deliveries of fish will not be permitted until an LCT is done.

The use of oil-based vaccines, however, make it difficult to find any latent carriers of the pathogen, so probably the strategy will have to be modified. In any case, it is a principle not to ship fish when *Aeromonas salm.* var. *salm.* has been found.

### **Seawater sites**

The most important principle for the fish

farmer to remember is to buy healthy smolt. This seems obvious, but has not always been so. Today, the farmers usually get smolt from the same 3 or 4 local hatcheries each year.

When furunculosis seemed to be getting the best of Norwegian salmon culture in 1989-1990, something had to be done to prevent the smolt from becoming infected after they were moved into the sea. The industry began to stock sites with a single year-class and ever since only single-generation sites have been used. Before the smolts are stocked, the site must have been empty for 6-10 months, the nets and other equipment disinfected, and, in theory, there shall be no contact between the site and ones with older fish.

I feel that optimal conditions are very important to the control of furunculosis and that salmon lice must be under control. Recent investigations have also shown the importance of the sites themselves — current conditions as well as bottom and other topographic conditions.

The authorities demand daily removal of mortalities in the summertime and every second day during the winter; safe disposal of the waste; no more than the approved maximum densities of fish (kg fish per m<sup>3</sup> official volume); and regular veterinary inspections of fish (6 to 10 inspections per site per year, autopsy of dead fish and, if necessary, bacteriological, virological and histological examination).

If there are furunculosis pathogens in fish from the site, the fish farm will be restricted by the veterinary authorities and would need permission to move fish to or from the site or to slaughter fish. With these regulations, it has been possible for the authorities to use all the strategies available for prevention of contagious diseases.

We all know that earlier problems are easily forgotten. As long as the fish farmers have to make a living, good sound practices could be sacrificed for profit. This is true in every business and aquaculture is no exception. In my mind there is no doubt that regulations are needed to maintain the very good results we have achieved in Norway.

All of the strategies I have mentioned are important. But the fact is, without oil-based vaccines, furunculosis would still be a serious problem in Norway. The positive results in 1993 were directly related to the massive revaccination of fish in sea cages that same spring. In

1994, the smolt had already been vaccinated with oil-based vaccines (instead of water-based ones), therefore revaccination was not necessary. I will end by saying there should never again be a single smolt put to sea in Norway without being vaccinated with the best vaccine available. And let this be my advice to the fish farmers in British Columbia too.

## Current status

Since 1989, furunculosis has been the main reason for the use of antibiotics in aquaculture in Norway. There is therefore a very clear connection between the quantity of antibiotics used and the status of furunculosis.

Only small portions of the antibiotics have been used to treat diseases like vibriosis and Hitra disease (cold water vibriosis). From the figures in the table below, it is obvious that furunculosis is now under control in Norway and causes only minimal losses, if any at all, to the farmers. The only problem is that the disease still exists in the environment and would soon become a problem again if we are careless.

Year	Salmon produced (mt)	Antibiotics used (mt)
1989	115,000	19
1990	155,000	38
1991	150,000	27
1992	140,000	28
1993	170,000	6
1994	205,000	1

As far as I know, the only outbreak of furunculosis in 1994 was in a group of broodstock in their third summer in the sea without having been revaccinated. We have also had a few isolates from rainbow trout that had not been vaccinated.

Just a few years ago furunculosis cost the salmon industry as much as 4 to 500 million NOK per year. No doubt this is the main reason why the production cost per kilogram of salmon has dropped from NOK 28.21 in 1991 and 28.11 in 1992 to 23.25 in 1993. We have no figures yet for 1994, but my guess is that it will be close to NOK 20.

## Vaccinating

Every smolt in Norway is vaccinated intraperitoneally with an oil-based vaccine — 0.2 mL per fish, usually 6 weeks or more before it is put to sea. With some 60–70 million smolts, Norway is a large market for the vaccine producers. The vaccination cost per smolt is about 1 krone. The job is done either by the farmers themselves or by professional vaccinating teams. When done by farmers, machines are often used. Three different types of vaccinating machines are on the Norwegian market and they do a good job provided they are operated correctly.

The most difficult part is the handling and anesthetizing of the fish. Normally, benzocaine in propylenic glycol is used. It is very vital that the fish is quickly anesthetized (30–60 sec) and transferred back to freshwater after injection. New data have demonstrated the efficacy of vaccinating at low temperatures (2°C) and oil-based vaccines offer good protection over a long period of time. A major portion of the smolts are now vaccinated in the autumn if they are ordinary 1- or 2-year-old smolt, or in the late summer/early autumn if they are “autumn smolt”. This spreads the work over a longer period of time and it is easy to avoid interference with smoltification. Still, there is an upcoming problem with the fast growing 0+ smolts because smoltification starts so early that it can be a problem to find the right time to vaccinate — between the fish reaching 30 g and the time for delivery, taking into account the 6 weeks needed for development of immunity. Also, 0+ smolts are often on a controlled light regime making it even more complicated to find the time needed for this operation.

## Use of antibiotics

Antibiotics can only be ordered by a veterinarian on an official prescription form. It is forbidden to sell antibiotics as pure substances, so they are prescribed as medicated feed or special products like the “Aqualett”. The Aquaveterinarian Society provides the following guidelines for prescribing antibiotics:

1. There must be an increasing rate of mortality of more than 0.15% per day. Of the fish autopsied, more than 50% should have died because of a specific infectious bacterial disease.
2. The pathogen must be isolated. If the situation is acute, treatment can start immedi-

ately, but samples must be taken in advance and a clinical diagnosis should strongly support the bacteriological diagnosis.

3. The pathogen must be tested for resistance.
4. The correct antibiotic must be chosen and given to the fish in correct dosages and with the correct regimen. For instance, Oxolinic acid 25 mg/kg fish on day 1, 2, 4, 6, 8, and 10.

Copies of the prescription must be sent to the Fishery Directorate (FD) within one week. The farmer must have approval from the FD before the fish can be slaughtered (withholding time) and tests are taken to check for residues.

With the large quantities of antibiotics used in recent years, problems have developed with resistant strains of *Aeromonas salm.* var. *salm.* In some cases, it has been difficult to find a drug that works. Of the 138 strains of *Aeromonas salm.* var. *salm.* sent to the National Veterinary Institute in 1992, 30% were resistant to quinolons, 18% to oxytetracycline (OTC), and 14% to trimethoprim/sulfa (T/S). Five percent were resistant to both quinolons and OTC and 5% to OTC and T/S.

Between 1987 and 1993, there has been a change in the pattern of drugs chosen:

	OTC	Quinolons	T/S	Nifurazolidon
1987	33%	36%	6%	26%
1993	3%	84%	9.5%	0.6%

The use of OTC has dropped because of the long withdrawal period required at low temperatures and the higher price per cure than with quinolons. T/S has been used to some degree when there has been resistance to quinolons, but it also has the disadvantage of a long withdrawal time as well as problems with appetite suppression. Nifurazolidon has very serious side effects and is now only used in special cases (such as against Hexamita).

Florfenicol is a new product recently registered for use against furunculosis in salmon. This drug is very efficient but also very expensive. It is therefore difficult to predict the development in choice of drugs in the future.

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# Hatchery management of furunculosis: experiences with oil adjuvanted vaccines

John Holder

## Introduction

I would like to say that our experience with vaccines has been a pleasant one, but I cannot. There have been production problems due to vaccination, although some were caused by our own stupidity. On the positive side, such problems allow you to cull and inventory your fish.

We have tried vaccines from the three manufacturers (Aquahealth, Biomed, and Micrologix) and they all produced the same effect — 3 to 4 weeks of feeding were lost and there was a consistent decline in growth rates after vaccination. Post-vaccination mortality was acceptable, averaging 0.29% after 28 days (varying from 0 to 3.5% per group). The most prevalent causes of mortalities were fungal infections and internal organ damage from improper needle placement. These results do not include one bad experience in which most of a group of 59,386 fish died after vaccination. The fish were vaccinated late in the year and 29% died within 28 days. Mortality continued and in an effort to save the fish they were shipped to our brackish water site in June. We still lost approximately 90% of the fish. Following vaccination, the fish would not eat and became infected with fungus. No causative agents could be isolated and the diagnosis was post-vaccination stress.

The real cause was that vaccination was done

too late in the year. The fish were transitional smolts, 20-25 g, destined for out S1.5 autumn entry program. The stress of smolting plus receiving a dose of 0.2 mL of heavy oil into the body cavity was apparently too much for them to handle and they died. Smaller autumn entry fish of 13-18 g did well.

From this we learned that it is important to vaccinate early in the year and not to vaccinate transitional fish. It is also important to vaccinate at water temperatures of 8°C or warmer because at lower temperatures there is no immune response. Temperatures of 8°C or higher must be maintained for 6 weeks before exposing the fish to the possibility of a furunculosis challenge.

## Growth

A growth comparison between nonvaccinated and oil adjuvant vaccinated fish was conducted. The principal vaccine we have used is Lipogen from Aquahealth. Biomed and Micrologix vaccines have also been used, but only a small number of fish received these vaccines, 25,000 and 50,000, respectively, so comparisons of growth in fish receiving different vaccines was not done. I do believe however that growth was compromised by all 3 vaccines.

After vaccination at temperatures ranging from 8 to 11°C, up to 4 weeks are required for

Table 1. Comparison of growth in vaccinated and unvaccinated Atlantic salmon (Scottish strain).

Group and treatment	Initial weight (g)	Date of vaccination	Final weight (g)	Date shipped	Number of days	Weight gain (g)	Growth rate (g/day)
A nonvaccinated	14.43	23 September 1993	53.21	25 January 1994	124	38.78	0.31
B vaccinated	27.72	19 September 1994	53.38	30 January 1995	133	25.66	0.19
C vaccinated	25.59	30, 31 August 1994	42.01	29 November 1994	105	16.42	0.16

**Table 2. Comparison of growth in nonvaccinated and vaccinated Cascades strain (Gaspé) Atlantic salmon.**

Group and treatment	Initial weight (g)	Date of vaccination	Final weight (g)	Date shipped	Number of days	Weight gain (g)	Growth (g/day)
A nonvaccinated	24.74	19 September 1993	67.66	28 December 1993	100	42.92	0.43
B vaccinated	22.49	15 September 1994	57.84	13 December 1994	90	35.35	0.39
C vaccinated	17.68	21 September 1994	73.22	10 February 1995	142	55.54	0.39

fish to resume normal feeding. We have tried flavor-enhanced feed to entice the fish to eat, but without success.

Fish were vaccinated between August and December 1994. In the previous year, vaccination was done between December 1993 and May 1994. A comparison was done between unvaccinated fish in 1993–1994 and oil adjuvant vaccinated fish in 1994–1995 (Table 1). Fish were weighed into our tanks at the Stelling Road site and all 3 groups were held on the same temperature regime and received the same husbandry with the exception of the IP injection. In the vaccinated groups, there was a 61% decrease in weight gain. This comparison was done on other groups as well and the findings with the two strains that came from Scotland were consistent.

However, results were different with the Cascades fish, a Gaspé strain (Table 2). Vaccinated fish from that strain had a weight gain only 8.4% less than fish that were not vaccinated. One explanation for the apparent difference between strains may be the physical shape of the smolt — the Cascade strain is a fatter and more squat-shaped fish than the Scottish smolt, which has the characteristic torpedo shape and less visceral fat. The lack of reserve fat in the Scottish smolt could explain the negative effect of vaccination on growth.

So what conclusion can be drawn? I believe oil adjuvant vaccines cause a decrease in growth rate that varies with the strain of Atlantic salmon used. Overall, we experienced about a 19% decrease in growth of vaccinated fish. I do not know if this growth reduction is carried over to saltwater. However, as time goes on and the fish start to smolt, their appetite and growth seems to return to normal, although they never really gain what they lost.

What do these results mean for a hatchery? We charge the vaccination costs to the purchaser — \$0.18 to 0.22 per smolt depending on the manufacturer. But the real cost is in the loss of growth. The smolts have to be kept in the hatchery for a longer period of time or more water has to be heated to compensate for the loss of growth; these both increase costs. Smolts could be put out at a smaller size, but then grow-out time is extended and costs still go up.

The effect of vaccination on growth also delays the autumn entry program; entry dates are pushed later into the winter and valuable growth is lost. The plan is for a 70 g plus smolt in early November, but oil adjuvant vaccine makes this impossible to achieve. Also, the \$1.5 smolt goes out at a later date and smaller size. We need a vaccine that is not so hard on the fish, such as an oral version or maybe a less viscous oil adjuvant. Micrologix has one and it appears to be easier on the fish but we have little experience with it.

My final point is the health of the fish after vaccination. At our hatcheries, fungus usually appears 2–3 weeks after vaccination. We treat the fish with a fungicide for 3 days after vaccination, then again 10 days later for three alternate-day treatments using fungicides such as salt, malachite green, formalin, or a combination of the latter two.

I must stress that what I have relayed to you has been my experience. We all know that what happens at one site does not necessarily happen at another.

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# Causes of antibiotic failure in the treatment of furunculosis

John Brocklebank

A question routinely asked by fish culturists of their prescribing veterinarian is "why didn't the furunculosis-infected Atlantic salmon respond to the treatment prescribed?" The following explanations may be applicable:

## 1. Fish biomass underestimated

The consequence of this is that the fish do not receive sufficient medicated feed for the prescribed treatment period.

## 2. Inappropriate feeding rate for medication

Medicated feeding rates of less than 0.5% body weight per day may result in insufficient medicated pellets being delivered. In addition, the concentration of medicated feed in the pellet increases as the percentage body weight per day of medicated feed decreases. This may result in feed refusal.

Medicated feeding rates greater than 1% body weight per day may result in medicated feed pellets with too low an antibiotic inclusion level. The fish have to receive medicated feed for an extended feeding period to ingest the correct amount of antibiotic.

## 3. Inappropriate pellet size

It is usually best to medicate fish with a pellet size slightly smaller than the regular diet being fed. This may not be possible with hatchery fish.

## 4. Uneven distribution of drug in the feed

Medicated feed prepared in a cement mixer may not result in a uniform concentration of the drug in the feed. This results in "hot-spots"

and "cold-spots" of medicated feed containing too much or too little drug. Milled feed is preferable and the associated cost and tonnage required for purchase is more than justified.

## 5. Delayed medication

Salmon should be treated when the first moribund salmon with furuncles in the skin are observed and the mortality rate begins to increase. This approach is justified in order to limit the rate of spread of the disease on-site. In addition, salmon with severe tissue damage that survive will likely require a longer antibiotic clearance period than less severely affected salmon.

## 6. Variation between *in vitro* and *in vivo* antibiotic results

Diagnostic bacterial culture and antibiotic sensitivity (C&S) testing that is performed in a laboratory is referred to as "*in vitro*". Sometimes, the *in vitro* results may be different from those conducted using living animals or fish populations (i.e., *in vivo*). I can think of two instances in the past year when during furunculosis outbreaks the laboratory *in vitro* test was sensitive to potentiated sulphonamide antibiotics, but when the fish were treated with hand-mixed Tribissen® or subsequently with milled medicated feed containing Romet®, the mortality rate did not decline — but instead increased.

It is very important to perform Kirby-Bauer antibiotic sensitivity testing on Mueller-Hinton agar with sensitivity discs containing the appropriate concentration of antibiotic. Additionally, the diameter of the Zone of Inhibition for each antibiotic disc must be carefully measured and compared to known standards (see Table 1).

**Table 1. Antibiotic sensitivity: zone of inhibition (mm).**

	Oxytetracycline	Trisulpha	SXT (Romet®)	Erythromycin phosphate (E-PO <sub>4</sub> )
Sensitive	17	17	19	18
Indeterminate	14 to 16	13 to 16	16 to 18	14 to 17
Resistant	13	12	14	13

### 7. Poor choice of antibiotic

Choose the best antibiotic based on the culture and sensitivity result and consider the dosage used. Romet® at 50 mg activity per kilogram fish for 5 days (i.e., 2 days "on", 1 day "off", 2 days "on", etc.) may be more efficacious than lower doses of Romet® or Tribissen®. New entry smolts and fish in fresh water may respond better to Tribissen® based on palatability.

### 8. Inappropriate feeding technique

For smolts, it seems it is better to saturate the pen as often as possible throughout the day with medicated feed, while for yearling and older production fish it does not seem to matter whether the medicated feed is fed-out at one feeding and then topped up with regular feed, or the medicated feed is fed-out over the whole day. This is because each farm or site within the same company varies enormously. For example, some farms hand feed whereas others may be partially to fully automated. Consistency of medicated feeding may thus be more important.

### 9. Vitamin deficiency

The feed companies add sufficient vitamins to regular feed intended for healthy fish. However, sick fish may have a higher requirement for vitamins than healthy fish — particularly vitamin C and E. Consequently, adding vitamin C alone or in conjunction with the water and fat soluble vitamins (vitamin E, especially), either with medicated feed or with the regular feed that is fed for a few days after

feeding medicated feed, may be beneficial. I can think of one farm where subclinically infected fish have not required antibiotics following seal attacks and early spring grading due to the feeding of additional vitamins with the regular feed.

### 10. Effect of high fat diets on mortality of diseased fish

Several culturists have commented that fish infected with furunculosis sometimes have a higher mortality rate on high fat feeds (30% fat) than feeds with a lower fat content. The theory suggested by some nutritionists and pathologists is that the blood meal added to the high fat feed as a palatability enhancer and a cheap protein source may contain too much iron. Excessive iron in the diet (200 to 250 ppm) may result in two problems: 1) iron may be a limiting micronutrient for the growth of many bacterial species such as furunculosis, and 2) iron when converted from Fe<sup>2+</sup> to Fe<sup>3+</sup> gives off a free radical that may result in cell membrane damage that rapidly depletes the available store of vitamin E and selenium in fish that are already compromised. Therefore, immediately placing treated fish back on high fat feeds may be self-defeating. Perhaps this is why adding double vitamin packs to the regular feed for a few days can be beneficial.

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# Management of furunculosis in sea cages

Ted Needham

Furunculosis exists wherever there are wild salmon in the sea. Farmed salmon are therefore under a constant threat from infected wild salmon, particularly from clinically infected fish that have been released from salmon enhancement hatcheries.

*Aeromonas salmonicida* surrounds our farms, whether we are in British Columbia, New Brunswick, or Scotland. Alderson of Marine Harvest International, Scotland, using antibiotic fingerprinting, found that the organism could move up to 19 kilometers between unrelated farms. And in 1993, one of our farms was infected with an easily identifiable strain derived from infected smolts at another company's farm 10 km away.

How can we protect ourselves? Of course we should vaccinate, but it is just as important to grow our fish properly in the first place. Vaccines will fail if they are used as a prop for poor husbandry. We should have two aims:

1. Prevent pathogen buildup — salmon can be infected by as little as 10 *Aeromonas salmonicida* per milliliter of seawater. The bacterium can remain infective in organic waste in seawater for up to 56 days and high pathogen levels can overwhelm the best vaccines.
2. Delay the onset of exposure to the pathogen for as long as possible by:
  - preventing the spread of the pathogen from older to younger year classes;
  - ensuring that smolts are furunculosis-free on seawater entry;
  - cooperating with other companies in the same area to keep in phase with intakes of each year class;
  - fallowing sites for 3 to 6 months between year classes.

By refusing to release more sites, the Ministry of Environment in British Columbia is com-

pounding the problem by making the use of fallowing and single year class sites prohibitively expensive. They could be contributing to a catastrophe such as an outbreak of IHN, sea lice, or even furunculosis itself.

We can prevent pathogen build up by matching husbandry strategies to the site. For exam-

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ple, Campbell River sites have good tidal flushing and moderate temperatures in the range 7.0 to 11.5°C. Accordingly, we can summer grade and use stocking densities that reach 10 kg per m<sup>3</sup> in 15 m deep pens. Less favourable sites with lower flows and wider temperature extremes

have to be treated differently. Stocking densities should be kept below 10 kg per m<sup>3</sup>; indeed 8 kg per m<sup>3</sup> is the maximum acceptable for farmed chinook if severe kidney disease is to be avoided. The same principles apply to containing furunculosis.

It is extremely dangerous to handle farmed salmon when the temperature is much above 10 °C. Hugh Mitchell found 10°C was the critical temperature for furunculosis outbreaks on the east coast of the United States and Canada. Therefore, summer grilse grading is not an option at warm sites. Instead, the fish should be graded by size in the winter and spring. An even better strategy is to use a fast growing, low grilse strain such as Mowi and — like the Norwegians — do away with grilse grading entirely.

*Aeromonas salmonicida* is carried in the gut of the fish. Heavy handling can result in damage to the gut mucosa and cause the pathogen to become systemic. Therefore, salmon have to be grown in the best possible environment with clean nets of maximum acceptable mesh size and in conditions of minimal stress. Stress can be reduced by:

- feeding to satiation several times a day or feeding over a long period at the same time each day;
- cease feeding if the oxygen drops below 5 ppm;
- use acoustic seal scarers.

Should a furunculosis outbreak occur, antibiotics have to be given a chance to work by:

- feeding vitamin packs to aid recovery;
- using krill and other appetite enhancers;
- avoiding diets high in blood meal with excess iron.

Above all, the pathogen load has to be kept down by:

- removing slow swimmers and mortalities daily;
- reducing faecal load in the most affected populations by selective starvation;
- hygienic disposal of mortalities;
- stringent disinfection of hand nets and equipment;
- providing sanitary barriers between cage groups.

In 1994, BC Packers purchased a site with two large pen groups where the furunculosis mortalities were running at an instantaneous rate of 12% per month. We treated one group with the

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---

appropriate antibiotic while the other group was left untreated because we wanted to harvest them. Mortalities dropped to less than 1% a month by the following month at both sites because we:

- starved the untreated site;
- dove and removed mortalities every 2 days instead of every 10 days;
- changed the badly fouled 1" mesh nets to clean 2" mesh nets;
- installed acoustic seal scarers.

In conclusion, we have to adapt husbandry practices to each site. We cannot follow the same rules for smolt numbers, feeding methods, growing, and grading strategies irrespective of local conditions. If each site becomes a clone of the other, none of them will function correctly.

Severe furunculosis generally occurs on poor sites, which are badly run, and that probably started with low quality smolts. We will only control furunculosis, IHN, sea lice, or whatever else the wild salmon throw at us, if we grow our fish properly. We cannot expect vaccines or antibiotics to do our work for us.

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# Choosing a furunculosis vaccine: points to consider

Hugh Mitchell

The concept of managing furunculosis by identification and manipulation of risk factors is reviewed. One risk factor, vaccination status, is discussed in detail. Vaccine performance in a population of fish is represented as a shift of the normally-distributed herd immunity or herd resistance to a particular disease. The degree of this shift is dependent on several factors, which can also be influenced by choice and method of vaccination. Farmers must ask themselves several questions before making a decision whether to vaccinate their stock against furunculosis. Eight fundamental questions are posed with discussion provided to aid in their answer and, ultimately, in the decision of choosing a furunculosis vaccine. These are: Should you vaccinate? Should vaccination be by injection? Which bacterin(s) should be used? Which adjuvant should be used? Are there independent studies to help you choose? What should be considered when judging the quality of a performance study? Who else uses furunculosis vaccines and what are they using? Who makes the vaccine? Decisions on vaccines are not permanent and will change as products and claims change. The goal of the farmer should be to make the best informed decision possible that will yield the greatest benefit to production performance.

## Introduction

An epidemiologist<sup>(1)</sup> challenges our conventional thinking about diseases and pathogens by noting that:

*"From an ecologic viewpoint, the production of diseases or death rarely favours perpetuation of the agent; thus natural selection favours less pathogenic organisms".*

This perspective of pathogens can be extended to *Aeromonas salmonicida*, the agent associated with furunculosis. The implication is that the bacterium's primary purpose is not to cause disease and this realization is important in dealing with the disease on a fish farm.

At the first *Furunculosis Workshop* in 1991, I presented the concept that the development of furunculosis involved more than just the presence of the associative agent.<sup>(2)</sup> It was suggested that in dealing with furunculosis, as with many aquaculture diseases, there is an overemphasis on clinical pathology and pathogen avoidance.

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**Table 1. Furunculosis risk factors (in order of importance).**

- 1) smolt quality
  - 2) water temperature
  - 3) density and related factors
  - 4) pathogen load
  - 5) vaccination status
  - 6) handling practices
  - 7) uniformity of grade
- 

As pork producers realized years ago, they would benefit from not only a clinical pathological approach to disease, but also a quantitative one that correlates production parameter values with risks and (or) subclinical effects of disease. The key to controlling furunculosis is to identify the controllable risk factors involved in producing disease (disease defined here as an "economic loss of production") in the presence of the bacteria, rather than putting extensive

efforts into eradicating the pathogen. In agreement with this philosophy to controlling disease, Wall<sup>(3)</sup> stated "Diagnosis and treatment are often quite straightforward but the underlying management problems need to be corrected as well". This means that when disease occurs, it is likely that we have created conditions that favour the pathogen over the fish. Unfortunately, many of these management factors have not been clearly defined for furunculosis. However, from years of dealing with this disease, it is possible to propose some of the likely risk factors. Although nonscientific, the factors proposed in Table 1 are based on empirical experience that these factors work synergistically to produce disease when the pathogen is present.

One important risk is *vaccination status*. Vaccination can be thought of as altering the relationship between the fish and the bacteria to make it more difficult for the bacteria to cause disease. Although vaccines should not be thought of as impermeable shields, as long as the cost of administration is less than the benefits from reduced mortality rates, they are a useful risk reduction practice in aquaculture. Vaccines may also be management tools that allow for increased risks in other factors affecting disease occurrence, thereby allowing production increases (e.g., higher densities, tem-

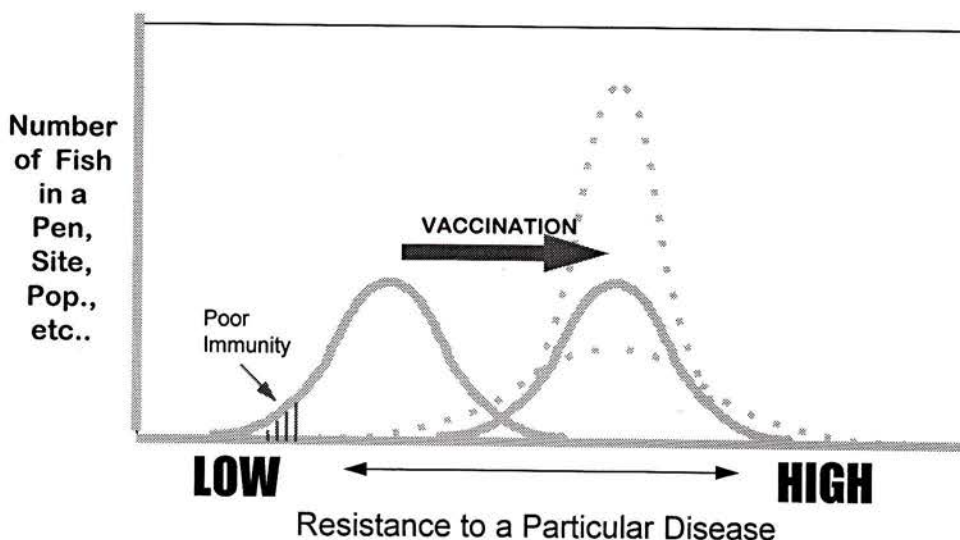
peratures, etc.) without corresponding increases in disease risk.

A critical aspect of vaccine status is the choice of vaccine. This paper examines some of the factors involved in making the choice of whether to vaccinate against furunculosis and what vaccine should be used. The approach is similar to that a fish farmer, or any consumer, should take in deciding whether to purchase and use any product: acquire an understanding of the choices available through meticulous research and comparison. The end result is the best choice to yield the greatest cost-benefit (i.e., value).

### Vaccine performance in a population

Since fish farmers are more interested in how a vaccine works at the population level than on individuals, it is important to recognize how a disease operates within a group of fish before and after vaccination. As Figure 1 illustrates, "natural" disease resistance varies among individuals within a population. This is represented in a typical "bell curve" where a small proportion of individuals have poor immunity to furunculosis, a small proportion have a high level of resistance, while the majority lie somewhere in between. Vaccination of a population shifts the curve to the right — most individuals ac-

Figure 1. Disease resistance in a population of fish.



quire greater resistance, but there is still a spread in immunity. Depending upon the disease, it is conceivable that vaccination may also increase or decrease the variation of immunity within a population (as shown with the dotted lines).

Therefore, a farmer must decide whether it is beneficial to use vaccination to increase resistance to furunculosis, keeping in mind that all fish will not respond equally and some fish will still be more susceptible than others. Furthermore, resistance imparted by vaccination can be overcome if other risk factors are not taken into account (i.e., vaccination is not absolute!).

I will outline eight fundamental questions to ask when trying to decide upon a furunculosis vaccine and provide some guidance on what should be considered in the answer.

### **Question 1: Should you vaccinate?**

The decision on whether to vaccinate or purchase vaccinated stocks will always be dynamic. The need to decrease disease risk and increase profitability is specific to the farm in terms of location, equipment, and management-style. There will also be new diseases to consider. Additionally, vaccines will continue to be modified and improved and the farmer will have to sort through all the developments and devel-

opment claims in order to make the most cost-effective decision for the operation.

One tool that should be employed is a cost-benefit analysis. This is done by some of the larger companies and may be as elaborate or as simple as a farmer is comfortable with. Advantages of complex models are that they take into account a wide assortment of factors. A disadvantage is that a lot of assumptions have to be made and these can often be compounded, making results more uncertain.

One model that can easily be applied by every farmer is that used by Lillehaug,<sup>(4)</sup> as shown in Figure 2. Although it may appear daunting at first, it is actually a simple formula that will provide an indication of the costs and benefits of vaccination. Relative percent survival (RPS) values are not standardized between vaccines and therefore are not comparable. A conservative estimate that one might want to put in the formula for furunculosis is 0.7 (0.95 for vibriosis). A computer spreadsheet can be used to manipulate the expected mortality ( $M_{no}$ ) to determine the break-even point for the number of mortalities that would have to be saved for the vaccination to pay for itself. It can also be determined how much can be saved if vaccination prevents 10%, 50%, 75%, etc. of existing mortalities. This procedure is extremely useful

**Figure 2. A cost-effectiveness model for fish vaccination (Lillehaug, 1989).**

$$\text{COSTS} = (H_{met} * W_h) + (V_{met} * P_{vac}) + C_{add}$$

$$\text{SAVINGS} = M_{no} * RPS_{met} * W_{fish} * [P_{kg} - (FCR * P_{feed})]$$

$H_{met}$  = vaccination man-hours  
 $W_h$  = hourly wage  
 $V_{met}$  = total volume of vaccine  
 $P_{vac}$  = price per liter of vaccine  
 $C_{add}$  = costs of equipment, aneth., fish lost, etc.

$M_{no}$  = expected or actual mortality  
 $RPS_{met}$  = relative % survival for vaccination method  
 $W_{fish}$  = mean wgt. at slaughter  
 $P_{kg}$  = price of fish per kg.  
 $FCR$  = feed conversion ratio  
 $P_{feed}$  = price of feed per kg.

in helping a farmer decide whether it is worth vaccinating.

### **Question 2: Should vaccination be by injection?**

It is generally accepted that injection provides superior protection to dip and oral vaccines. Against vibriosis, Lillehaug<sup>(4)</sup> concluded:

*"When the level of protection achieved by the different methods of vaccination was taken into account, it was found that injection, the method giving the best protection, was economically more beneficial than the other methods when dealing with major disease problems, in spite of it being more expensive to carry out in small fish".*

Since furunculosis vaccines do not seem to be as immunogenic as vibriosis vaccines, it is probably a safe assumption that if furunculosis is a persistent problem within a facility and (or) a farmer wishes to minimize risk as much as possible, the method that will push the "resistance bell-curve" the furthest up the scale (see Figure 1) is injection. Ultimately, the decision is based upon a combination of actual and perceived risk, the farmer's own risk-aversion, and cost-benefit. Risk is a function of the other factors listed in Table 1 and if low in some areas, dip vaccination may be adequate. As to whether a combination of dipping fry one or more times, followed by an injection booster prior to smoltification is warranted is particular to the risk of furunculosis at a hatchery. An idea of risk can be assessed by the history of furunculosis — persistent problems at the hatchery level, prior to injection, may warrant one or more "dips".

It is a popular misconception that dip vaccination is less stressful than injection. I do not know what criteria were used to determine this, but it is my experience that when an anesthetic is used for injection and the fish are handled properly, the trauma induced appears to be far less than spending a minute in a dip without sedation.

Another factor that must be taken into consideration is that injection vaccination is more labour intense than dip vaccination. This can discourage farmers. Methods have been developed that enable vaccination to be a routine part of hatchery (and even sea-cage) operations. There is no one correct way, and I am constantly amazed at the different methods that are in place. One hatchery in New Brunswick, for

example, has incorporated a "Pescalator" into its anesthetic operation so that the fish are anesthetized on the ride up the "archimedes screw" thereby giving the injectors a continuous (versus "batch") supply. As for using machines for vaccination, some people have had good luck with them and others haven't. They are a significant capital investment but they can be combined with counting and grading functions. One of their biggest drawbacks appears to be an inability to handle nongraded fish.

### **Question 3: Which bacterin(s) should be used?**

A bacterin is a vaccine that is a suspension of killed or modified bacteria that stimulates the production of antibodies against one or more diseases. It is one type of vaccine and because of the relatively small nature of the aquaculture industry will probably be the predominant type for some time because it is cost-effective to develop and produce. However, to paraphrase: "A bacterin is not a bacterin is not a bacterin."

A farmer is often faced with the choice of which furunculosis strain should be included in the vaccine. The natural tendency is to desire the "local strain". There also is a tendency to want to include as many other disease bacteria in the vaccine as are available — just in case. A brief review of some of the details and nuances of bacterin development and production is necessary in order to illustrate the importance of choosing carefully.

One fundamental aspect of a bacterin is the "antigenic mass" which is roughly the density of the bacteria in a bacterin. A general rule is that the more antigenic mass, the better the immune response (to a point). If there is no cross-protection between strains in a bacterin, then the more strains included, the less antigenic mass of each there is (unless the total amount of vaccine per dose is increased). The antigenic mass is also affected by the adjuvant (see below) and emulsifier since their type and inclusion displaces antigen mass. All this must be taken into account when a manufacturer develops a vaccine. Adding or changing strains can result in an entirely new product with different performance characteristics.

One problem that can occur in certain combinations of multi-strain ("multi-valent") vaccines is an inhibitory effect on the immune system. It should be noted, however, that bacte-

rial species can also work synergistically and actually help a vaccine work against a particular disease, even though these species do not cause the disease. This is thought to be because of stimulation of the nonspecific part of the immune system. In Biomed's Biojec 1900 (Bellevue, WA), it has been found that adding *Vibrio* sp. bacterin actually enhances protection against furunculosis! Just remember that in vaccine development and production: one plus one does not always equal two, the effect may be less or more when bacterins/strains are combined.

In terms of which strain should be included in a vaccine, the best strain of *Aeromonas salmonicida* may not always be the best one to use against the disease that strain causes. Qualities that differ between strains and should be examined before being chosen by a manufacturer include: immunostimulatory ability; cross-productivity; fermenter performance; etc. A farmer should try to determine how much research went into the selection of a particular strain(s) within a bacterin.

Since bacterins are produced by fermentation, anyone who has dabbled in beer-making knows that attention to detail and degree of quality control is extremely important and without it quality and consistency—not always apparent by looking at a finished product—can vary markedly. Bacterin fermentation—ensuring that the end product is the same as the strain that was seeded—is infinitely more complex. Although farmers are too busy to become experts in the process, they should try to understand what is involved and obtain a feel for the care and level of detail that goes into the process. Aspects that should be inquired about include: fermentation monitoring ability; type of fermentation production (e.g., batch vs. continuous); standards adhered to; background of R&D and production people, etc. If in the area, a visit to a production facility is highly recommended and will prove enlightening.

#### **Question 4: Which adjuvant should be used?**

The adjuvant is an extremely important part of a vaccine as it is a material that alters the immune response, usually enhancing both specific and (or) nonspecific parts of the response and aiding the vaccine in both strength and longevity of response. *A. salmonicida* bacterins have relatively low immunostimulatory ability so an adjuvant can be

considered essential to insure a reasonable amount of protection against furunculosis.

However, like bacterins, all adjuvants are not the same. As Midtlyng<sup>(5)</sup> demonstrated, an oil-adjuvanted injectable was clearly superior to other vaccines at the time. Biojec 1900 was the first commercially produced oil-adjuvanted combination furunculosis vaccine. Since its introduction 4 years ago, it has been used in over 133 million salmon worldwide and its success has caused other companies to develop their own versions.

The mechanism of the Biojec 1900 is twofold. The proprietary mineral oil formulation serves to produce a nonspecific immune response that results in possible visceral adhesions and pigmentation. These have been found to be essential, yet insignificant in terms of cost to the farmer. The reaction also serves to enhance the specific response to the bacterial antigen. The second property of the oil formulation is to act as a depot of antigen over time so that there can be a sustained release effect for longer protection. In-house data shows that there is some efficacy remaining 27 months post-injection.

The choice of oil formulation is also critical and it is a careful balancing act to ensure that a proper amount of nonspecific stimulation occurs without over- or under-doing it. This involves a combination of the right quality, consistency, viscosity, and immunostimulatory properties. Too much or too little can have a drastic impact on overall performance and side-effects. There are dozens of types of oils and among these types many different grades in quality. A farmer should be aware of these details and learn as much as they can about the details of research, choice, and testing involved in the selection.

Since an oil/bacterin mixture is a suspension, an emulsifier is necessary to keep a stable mixture and the choice of emulsifier is as important as oil and bacterin. The emulsifier can affect the performance of the vaccine if not chosen carefully. Although excess emulsifier may be appealing in terms of ease of injection, this can affect depot and antigen mass properties. Again, the farmer should be aware of these details and carefully consider information obtained for its reasonableness and reliability.

#### **Question 5: Are there independent studies to help you choose?**

Fish health professionals like to see inde-

pendent evaluations of a vaccine and most have a healthy suspicion of claims and studies released by the manufacturer. Unfortunately, there are not yet any independent studies comparing long-term performance of the oil-adjuvanted furunculosis vaccines. These studies are typically performed by trade associations who contract independent investigators, or by larger companies. Unfortunately, many quality investigators do not like to do research that will lead to recommending one brand over another because of the potential commercial consequences. Furthermore, as soon as the results of a study are released, they are usually dated because of the ever changing nature of vaccine refinement and development.

Nonetheless, a farmer should be concerned about the degree of independence of any performance data or comparison. As Figure 3 illustrates, there are generally three degrees of independence that can be attached to a study. The most desirable and the type the farmer should place the most confidence in is Type A — complete independence. Unfortunately, this is the rarest for reasons previously discussed. Type B is preferable; however Type C is the one a farmer usually encounters. This is not to say the information should be ignored, but there is

an obvious conflict of interest that should be incorporated into the decision-making process. The worth of the data will ultimately be based upon a judgment of trust. The study and the people behind the study should be intensely scrutinized.

**Question 6: What should be considered when judging the quality of a performance study?**

Above and beyond independence, there are qualities within a vaccine performance study which should be considered when examining data (or conducting a study of your own).

For any kind of study, it is best to have the raw data and either examine it yourself or have a fish health consultant review it. The best raw data is a photocopy of a laboratory notebook. Unfortunately, these are rarely available or divulged, and many farmers are too busy to devote their time to analyzing data for themselves.

One should always ask for the statistical analysis of a study. This is extremely important and is often misunderstood. In the rare case that a vaccine offers close to 100% protection, the effects can be quite obvious. Unfortunately, since individuals in a population respond differ-

### Figure 3. How "independent" is the study? Three broad classifications.

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- ◆ **Type A: (the most desirable) COMPLETE INDEPENDENCE**
  - conducted by a third party with no connection to vaccine company (distributorship or funding)
  - no vested interest in any particular outcome (e.g.: academic; farm association).
- ◆ **Type B: (sometimes acceptable) SOME CONNECTION**
  - conducted by third party that is funded or somehow connected with the manufacturer.
  - room for influence or "censoring"
- ◆ **Type C: (Be careful & cynical) UNDERTAKEN BY COMPANY**
  - glaring conflict of interest
  - bias can be subtle (omissions of data; exaggeration through graphical representations; etc....)
  - still important in decision-making but requires judgment in trust.

ently and most vaccines (including furunculosis) perform on a percentage basis, it is more likely that a result the farmer sees is a reduction in the level of mortalities and (or) antibiotic usage as a result of using a vaccine. Because of this, when comparing the effects of vaccines, the relative percentage saved becomes important. It therefore is extremely important to obtain as accurate and reliable information as possible from a study in order to form an opinion on the worth of a vaccine. In agriculture, cumulative incidence of a disease may only change slightly, even when efficacious vaccines are used, yet these moderate changes may still result in significant cost reductions to the farmer.<sup>(6)</sup>

The purpose of statistics is to answer one fundamental question:

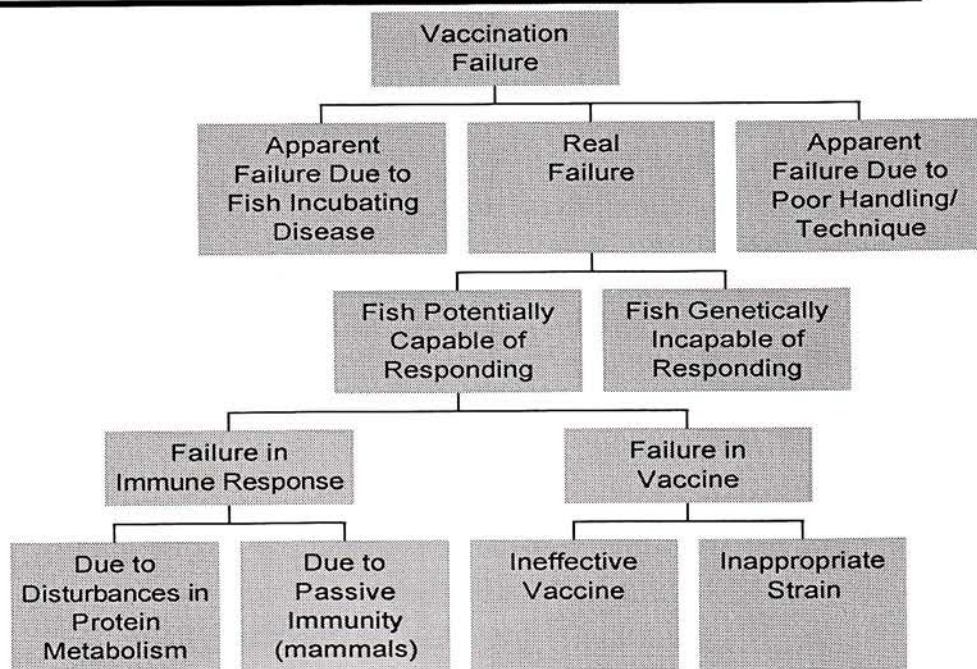
*How certain can we be that the results did not happen by chance alone?*

If you do not understand the statistics, ask someone to interpret the study. Don't accept the argument that this is production, not science, so statistics are not necessary. Statistics are a way of formalizing a study so that you are not fooled by the results. Remember that biology is fuzzy and the true effects of vaccination are not always obvious or apparent. Key elements in-

involved in a statistical design are replicates and randomness. This means there should be more than one pen of fish per similar treatment (minimum of three with the maximum depending on the variation and degree of discrimination desired) and these should have been chosen by ballot to eliminate bias. An example of a poor but common design is to compare a vaccinated fish to unvaccinated controls where the controls are chosen to be the smaller fish because the farmer feels that they are more expendable, in case the vaccine does not have an effect. The problem is that the effects of the vaccine cannot then be separated from the effects of fish size.

Caution must be taken in comparing studies conducted by different investigators, laboratories, farms, etc. Differences in timing, dosages, methods of vaccination, etc. will all affect performance so as to make comparisons meaningless. This applies when comparing RPS (Relative Percent Survival) studies. Unfortunately for fish diseases, including furunculosis, there has been no standardization of methods. The result is that RPS values for vaccine studies from different facilities are not comparable.<sup>(7)</sup> Another problem is that most studies gloss over the question of long-term protection. This is because of

Figure 4. Reasons for vaccination failure (after Tizard, 1987)



the cost involved in continuing a study over an extended period of time. Do not forget to ask for the time period for any study that you examine.

One must also note whether studies were conducted in the laboratory or the field. It is best to have both as laboratory data can bear no resemblance to field results. The degree and method of challenge can be unrealistic in laboratory studies while the inconsistency of challenge in the field may make it difficult to demonstrate the true worth of a vaccine or sort out the most superior product. S. McVey<sup>(6)</sup> has written a useful article to aid producers and fish health professionals in assessing vaccine performance in the field.

If a vaccine appears to fail in a study or in production, Figure 4<sup>(8)</sup> gives a useful flow chart as to what might have gone wrong.

#### **Question 7: Who else uses furunculosis vaccines and what are they using?**

Fish farmers are renowned for being a strong, proud, independent bunch and my personal observations seem to indicate that this admirable characteristic is shared worldwide. Unfortunately, though not always the case, the result can be that farming techniques, know-how, and information is not always shared between sites, farms, companies, and especially regions. There can also be a "holdfast" attitude that a particular farm, site, etc. warrants different strategies. While often true, it is also the case that isolated independence can result in reinvention of the proverbial wheel and costly lessons. Often the path of least resistance can be made by paying close attention to what other farmers are doing both locally and worldwide. Much can be gleaned from a near or far neighbour.

One fundamentally important aspect in selecting a vaccine is to obtain referrals. These should be from as wide a spectrum of farmers and fish health professionals as possible, both within the region and on a global basis. A good cross-section of referrals will help objectify the opinions. Producers should be asked what they use and why. Answers should be cross-referenced and farmers should come back to the vaccine manufacturers with some hard questions.

#### **Question 8: Who makes the vaccine?**

A farmer and his technical people should get to know the vaccine companies. They should go

beyond the marketers and get into the heart of the company. They meet the R&D staff, learn the history of the company and understand the mission. The people should be scrutinized for experience, qualifications, farm savvy, vision, and commitment. The degree of attention to detail that goes into the vaccine should be assessed.

## **Conclusions**

The emphasis on controlling furunculosis should be on those factors that can be manipulated. Vaccination can be used to reduce the risk of furunculosis and "buffer" the risks from other factors conducive to the development of the disease.

The decision on which furunculosis vaccine to use should not be made lightly. Do your homework — kick the tires and slam the doors. Both the investment and the returns can literally be in the millions.

Unfortunately, decisions on vaccines are not permanent ones and will change as new products and claims appear. It is not an easy task, and sorting through choices and claims will always be an effort. It will ultimately come down to a judgment call, but make it the best informed decision you can. The effort will always be worth the expenditure.

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## Furunculosis vaccines: the next generation

Julian C. Thornton

Attempts to develop effective killed cell vaccines (bacterins) for furunculosis have resulted in numerous commercial vaccines that induce only a partially protective immune response. Typically, the level of protection does not correlate with antibody titers directed to the known surface antigens of *A. salmonicida*, namely LPS and the regular surface array (A-layer). The presence of a capsule has also been postulated for certain strains of *A. salmonicida*, but to date there is no evidence linking the putative capsule to either virulence or a protective immune response.

One of the possible explanations for the lack of efficacy of the typical *A. salmonicida* bacterin preparations is that antigens important for a protective immune response are not expressed by *A. salmonicida* grown in vitro on standard media preparations. Antigen expression in vivo has been examined in very few cases, likely due to unavailable or inappropriate host model systems. However, many pathogens have been examined using fluids derived from the host as growth media and these have revealed novel antigen expression including the expression of capsule and novel protein antigens.

We examined several virulent and avirulent *A. salmonicida* strains grown inside intraperitoneal implants in rainbow trout (*Oncorhynchus mykiss*) for unique antigen expression. Western immunoblots using immune rabbit serum raised against in vivo grown cells revealed several unique antigens. With the exception of lipopolysaccharide (LPS), these novel antigens were destroyed following proteinase K treatment. The majority of these antigens were not induced in vitro in response to either iron limitation or anaerobiosis. In addition, electron microscopy demonstrated the presence of a putative capsule on in vivo grown cells. Purification and fractionation of this carbohydrate material from cells grown in carbon rich synthetic media

resulted in the isolation and separation of an antigenically distinct LPS not seen with cells grown in standard media. Antiserum raised against in vivo grown cells recognized both this LPS and the typical LPS of *A. salmonicida* apparent in in vitro grown cells. Antiserum raised against in vitro grown cells recognized only the in vitro expressed LPS. Antisera directed against in vivo grown cells was approximately 10 times more sensitive in detecting *A. salmonicida* in infected fish kidney tissue than sera directed against in vitro grown cells.

Secondly, mutants of *A. salmonicida* strains lacking either the A-protein, O-antigen, or both of these major surface antigens were tested in rainbow trout for their suitability as live vaccines (see Table 1). All of these mutants were shown to be attenuated as fish receiving  $\sim 5 \times 10^7$  cfu/mL of the respective strains showed no clinical signs of furunculosis. Immersion vaccination of fish in  $5 \times 10^7$  cfu/mL of these strains with an identical immersion dose 14 days later resulted in significant protection by all strains from challenge with a heterologous virulent strain of *A. salmonicida* five weeks after the second vaccination. The levels of protection conferred were all greater than or equal to that provided by an injected bacterin using the same vaccination schedule. With one exception, all live vaccine strains that still possessed a functional O-antigen provided protective indices (PI) 4-7 fold greater than the PI for the fish injected with bacterin. When antibody responses of vaccinated fish were compared, it was found that only vaccination by bacterin gave rise to a measurable agglutinating titer. Western immunoblots using the immune fish sera failed to reveal any major differences in antigen recognition in fish that received any of the vaccines tested. These data suggest that the immune response generated by the use of live vaccine strains is different from that generated

**Table 1. Immunity provided by various attenuated *A. salmonicida* strains compared with immunity provided by a bacterin.**

Vaccine (number of doses/route)	Phenotype <sup>1</sup>	LD <sub>50</sub> <sup>2</sup>	PI <sup>3</sup>
None (control)	na	1.4 x 10 <sup>6</sup>	1
10SR (2/immersion)	A <sup>+</sup> LPS <sup>+</sup>	>1x10 <sup>9</sup>	>714
10SR-3 (2/immersion)	A <sup>-</sup> LPS <sup>+</sup>	9.2x10 <sup>8</sup>	657
A450-3 (2/immersion)	A <sup>-</sup> LPS <sup>+</sup>	6x10 <sup>8</sup>	428
A440 (2/immersion)	A <sup>-</sup> LPS <sup>+</sup>	2.9x10 <sup>8</sup>	203
A450-1 (2/immersion)	A <sup>+</sup> LPS <sup>-</sup>	2.5x10 <sup>8</sup>	178
A450-1-3 (2/immersion)	A <sup>-</sup> LPS <sup>-</sup>	1.9x10 <sup>8</sup>	135
Bacterin (2/injection)	A <sup>+</sup> LPS <sup>+</sup>	1.6x10 <sup>8</sup>	114

1. LPS indicates the presence or absence of O-polysaccharide on the vaccine strain, and A indicates whether or not the vaccine strain produces A protein.

2. LD<sub>50</sub> values (method of Reed and Muench, 1938) were calculated after challenge with a wild type virulent strain, LD<sub>50</sub>.

3. PI = LD<sub>50</sub> vaccinates/LD<sub>50</sub> controls.

by a bacterin, and that these useful mutations may be incorporated into existing furunculosis live vaccines for further attenuation.

The construction of live bacterial vaccines has provided a potentially effective alternative to either killed whole cell, or purified subunit vaccines. For the most part, these vaccines have been directed at enteric salmonellosis, however progress has also been made in the development of live vaccines for other diseases. Many of the mutations rendering pathogens unable to persist and/or cause disease yet still retain immunogenicity, have been in genes involved in the biosynthesis of aromatic compounds (i.e. *aroA*), purine biosynthesis, galactose metabolism, adenylate synthase structure, the catabolite repression system, and mutations affecting antibiotic resistance. Some of these attenuating mutations have also been successfully introduced into strains of various fish pathogens such as *A. salmonicida*, *Vibrio anguillarum* and *Pas-*

*teurella piscicida*, all of which result in effective live vaccines.

To construct an attenuated live vaccine strain from *A. salmonicida*, a fish pathogen with relatively unknown metabolic capabilities, we recently isolated a strain of *A. salmonicida* deficient in various aspects of terminal respiration. The live vaccine strain 10SR was demonstrated to elicit protective immunity in chinook salmon (*Oncorhynchus tshawytscha*), Atlantic salmon (*Salmo salar*), and rainbow trout. It was apparent from tissue persistence data that the spread of 10SR through the host was similar to that of the wild type strain, although ultimately 10SR was cleared within 48-72 hours by the host defenses. This suggested that the live vaccine gained entry to and disseminated through the tissues in a similar manner to the virulent parental organism. Although the reasons for the clearance are unclear, it was presumably due in part to nonspecific lysis of this vaccine strain which

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**WE PROPOSE THAT THE  
INCLUSION OF THE  
MUTATIONS A<sup>-</sup> AND LPS<sup>-</sup>  
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IMPORTANT STEPS IN THE  
CONSTRUCTION OF EFFECTIVE  
LIVE VACCINES WITHOUT  
ADVERSE BIOLOGICAL AND  
ENVIRONMENTAL IMPACT.**

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is architecturally defective in its A-layer and thus more sensitive to host lytic factors. The importance of tissue entry and persistence is of obvious importance in stimulating the bacterium to express the appropriate antigens in vivo, as well as in stimulating the correct type of host immunity in the affected target tissues.

The precise mechanism of immunity to furunculosis is unclear, but both cellular and humoral immune responses have been implicated. Although levels of specific antibodies have failed to consistently correlate with protection, the requirement for the subunit of the regular surface array, A-protein, and lipopolysaccharide (LPS) to be present as antigens in bacterin preparations has been repeatedly demonstrated, suggesting that these antigens are important in stimulating some form of immune response after administration of a bacterin.

The aim of our studies was to determine which, if any, of the major surface antigens of *A. salmonicida* are necessary for the induction of immunity when live vaccines are used for the prevention of salmonid furunculosis.

The role of humoral immunity in the protection of fish from furunculosis has historically been assessed on the basis of either the passive transfer of immunity using either fish or rabbit sera raised against killed *A. salmonicida* cells, or by the examination of fish immune response

following vaccination with a bacterin. Although humoral immunity has failed to correlate well with protection when measured by serum antibody titer, limited success has been achieved using passive transfer of anti-*A. salmonicida* antibodies from either fish or rabbit sera, suggesting at least a partial role for humoral immunity in the prevention of furunculosis.

The results from vaccination trials using the various live vaccines demonstrated that effective protective immune responses are generated by live strains with or without the A-layer, and that mutants lacking even the LPS O-antigen still provided protection at least equivalent to that of an injectable bacterin. This surprising result in conjunction with the lack of agglutinating antibodies, leads to the possible conclusion that the branch(es) of the immune system stimulated by these live vaccines is at least partly different than that stimulated by a simple bacterin. Another possible explanation is that the antigens responsible for protection may differ between attenuated vaccines and simple bacterins. A series of novel, in vivo expressed antigens has been described for *A. salmonicida*; thus if the live strains persist in tissues long enough, it is probable that these novel antigens will be expressed and stimulate an immune response. It is important to indicate that these two conclusions are by no means mutually exclusive. For example, the novel antigens expressed may stimulate different branches of the fish immune system from those stimulated by antigens in bacterin preparations. The inconclusive results from Western immunoblots suggest that if humoral immunity is involved in the resistance after vaccination with live strains, it likely plays a minor role as only a weak, nonagglutinating serum response was detected.

The reduced efficacy of strains lacking the O-antigen of the LPS, compared to mutants only lacking the A-layer, is likely due to the extreme sensitivity of these strains to complement lysis, as both a functional A-layer and intact LPS are known to increase serum resistance of *A. salmonicida*. This increased sensitivity to complement likely results in highly reduced tissue persistence and thus reduced exposure of the cells of the immune system to protective antigen.

Historically it has been indicated that both A-protein and LPS, which are expressed in vivo and in vitro, are required antigens in bacterin preparations. As the levels of protection afforded by bacterin injection are minimal, it may

be that these antigens are only of secondary importance to a protective response and only live vaccines provide the novel in vivo expressed antigens for the stimulation of high level protective immunity.

The safety of the environmental release of live vaccine strains is of utmost importance — the incorporation of mutations that not only affect virulence but also the environmental persistence of the organism must be considered. It has been proposed that the presence of A-layer increases the survival of *A. salmonicida* in environments such as river beds. This is reportedly due to the charge imparted by the A-layer allowing for the interaction of A-layer possessing cells (A+) with amino acids associated with the humic acid coating of silt and sand. Also, the hydrophobic nature of A+ cells has been recently implicated in increasing the apparent concentration of *A. salmonicida* at the air-water interface, thus increasing the potential bacterial load for fish at or near the surface. These two attributes of A+ *A. salmonicida* become relevant in the event that more than two mutations are involved — albeit a remote possibility — and the vaccine strain in question reverts to a wild-type virulence.

Another important aspect of virulence that is commonly overlooked in the construction of live vaccines is the role of toxins in both immunity and the undesirable side effects after exposure to a live vaccine. Recent reports, by other authors, on the toxins of *A. salmonicida* have revealed that the major toxic factor is a combination of protease, glycerophospholipid:cholesterol acyltransferase (GCAT), and LPS. More

specifically, it was shown that the addition of LPS to GCAT stabilized and enhanced the toxicity of this enzyme. Thus the exclusion, or alteration, of LPS in live vaccine strains should aid in the reduction of the toxic effects of the extracellular components of *A. salmonicida*, while still retaining the proteins produced by the live strain that aid in the stimulation of a protective immune response.

For these reasons, and for the fact that A<sup>-</sup> and LPS<sup>-</sup> cells are still effective vaccines, we propose that the inclusion of one or both of these mutations into a live vaccine strain for the control of furunculosis, while not essential, are important steps in the construction of effective live vaccines without potential or adverse biological and environmental impact.

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## RECENT AND UPCOMING BULLETIN ISSUES

**June 1995 — Proceedings of AquaTech 95**

**December 1995 — Invited presentations from Aquaculture Canada 95**

**March 1996 — Contributed papers from Aquaculture Canada 95**

**June 1996 — Use of All-female and Triploid Salmonids**

# Mailbox —

## RESPONSE TO A RECENT ARTICLE

I read with great interest the paper by D. L. Hugh-Jones on his work with the flat oyster, *Ostrea edulis*, in Ireland (AAC Bulletin 94-4). Mr. Hugh-Jones' experiences provide a number of object lessons for aspiring shellfish growers in the Maritimes. The most important is contained in the first sentence "*Oyster culture in Europe is an unfolding chapter of disease disasters*". And the cause is clear — an historic lack of adequate policy and effective control mechanisms on introductions and transfers.

The next statement, "*It probably started with an illegal importation of Pacific oysters, *Crassostrea gigas*, into Marennes, France, in 1967 ...*", concerns me. It signifies that the deliberate obfuscation of the true facts, which occurred at the time, still survives.

In November 1967, I received a letter from M. René Cordavault, an oyster grower in Chateau d'Oléron. He requested help in investigating losses and poor performance in oysters grown in the Bassin Marennes Oleron, an area where Pacific oyster seed from Japan had been planted the year before. This was the beginning of "Gill Disease" and the Hugh-Jones "Chapter Of Diseases". In the next year or so I corresponded with M. Cordavault, received and processed oyster material, including a visit to Ellerslie by a French worker for data comparison, and communicated with colleagues in the United Kingdom who were working on the same problem. All referred routinely to the 1966 introduction.

In the early 1970s, the French Government's posture was that the first introductions of Pacific oysters to the north coast of France were made after 1968 to rehabilitate an industry destroyed by disease. Statements to this effect occurred in correspondence and personal conversation at international meetings, and even found their way into the literature. When pressed on the matter there was occasionally a reluctant admission that there might have been an earlier private illegal introduction.

This was despite clear contemporary records in the scientific literature. In an early paper dealing with "Gill Disease", presented to the International Council for the Exploration of the Sea (I.C.E.S.) in 1968, L. Marteil, a biologist at the Trinité sur Mer laboratory of the I.S.T.P.M., reported "En mars 1966, quelques centaines de kilogrammes de jeunes huîtres *C. gigas* ont été importées du Japon dans la région de Marennes ..". Prof. Philippe Daste of the University of Poitiers in a report on an official visit to Japan early in 1969, partly to assess the incidence of the disease there, refers to the oyster shipments to France beginning in 1966, and the subsequent ban partly due to the I.S.T.P.M.'s association of the disease outbreak with the introduction. Pieter Korringa, in his book on farming *Crassostrea*, cites discussions with local growers in Marennes-Oleron including the introductions of *C. gigas* in 1966-7 and 1968-9 and their resistance to both the disease epizootics.

In order to clarify my records, in 1970 I asked the Canadian Department of Industry, Trade and Commerce for any information on imports of seed from Japan they could provide. Via embassy staff in Paris they obtained the following from official records:

*In February 1966, 900 kg of seed was imported, followed by an unspecified quantity in April 1967. Further imports were prohibited as of 15 November 1967. This prohibition was lifted in February 1969 and very substantial quantities of seed were imported in 1969 and 1970, together with the well documented large imports from the West Coast of North America.*

The facts are clear. The denials and deception are deplorable. We can assess and counter the potentially disastrous effects of transfers only in an atmosphere of openness and honesty. Any such transfers pose very serious threats which should not be obscured by deliberately inaccurate

rate reporting, no matter what the justification.

There has only been one approved transfer of stock of a native marine molluscan species into Atlantic Canada — oyster seed from New England brought into Prince Edward Island from 1910-14. This resulted in a series of major oyster mortalities with serious economic consequences over the next sixty years and some problems which still limit industry development. This was one of the earliest known such epidemics anywhere in the world and its memory has served as a spur to appropriate caution to the present time. But I fear that this is fading, that industry pressures and bureaucratic naivety are combining to lower the standards of scrutiny of such proposals and the very principles on which we have operated.

A major component of the pressure to consider stock transfers comes from a search for quick and easy answers to aquaculture development. There are no such magic bullets or free lunches. Success in aquaculture will come only from an adequate apprenticeship to gain knowledge and experience from which to develop competent husbandry. Stock transfers into Atlantic Canada, necessarily south to north and therefore to a more demanding environment, not only pose the threat of disease and nuisance organisms but also perhaps the greater threat of genetic damage to stocks at the extreme of their geographic range or adapted to a specific and rare environment. The potential for damage is not restricted to the species itself but can have much broader ecological implications. Such decisions and even their consideration must be made in a context of wide industry and even general public knowledge and input.

*Sincerely,*

Roy Drinnan  
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# Aquaculture Canada 96

## 13<sup>th</sup> Annual Meeting of the Aquaculture Association of Canada

Ottawa, Ontario  
2-5 June 1996

### Partial list of Special Sessions

Sea urchin culture and enhancement  
Government-industry relations  
Therapeutants  
Bottlenecks in juvenile production  
Regulatory issues  
Human resource issues  
Federal Aquaculture Strategy Review

Call for contributed papers will be mailed to AAC members early in 1996. If you are not a member of the Association but would like to receive mailings on this meeting, please contact AAC —

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# AQUACULTURE NEWS

## **Proceedings — Workshop on Federal Import Regulations**

The proceedings of the World Aquaculture Society **Workshop on Federal Import Regulations of Mexico, Canada, and the United States Relating to Aquatic Animal Health**, held 4 February 1995, are available as a mini-database for use on IBM-compatible computers. The database is on a 3<sup>1/2</sup>-inch diskette and consists of a simple menu-driven program that serves WordPerfect (DOS) versions of each paper presented at the workshop and allows the user to easily navigate the database. Also contained on the diskette are ASCII files of the papers for use with other word-processing programs. These papers are also available for viewing and downloading as Acrobat PDF files on the World Aquaculture Society web site on the AquaNIC server (point your web browser to <http://thorplus.lib.purdue.edu/AquaNIC/home.html> and follow the WAS link to the World Aquaculture Society materials). You will need the Adobe Acrobat Reader program to use the files. The Reader can be downloaded free of charge from many sites on the web, including Adobe's site (<http://www.adobe.com/>). If you would like to purchase a diskette version, send a cheque for US\$10 made out to Fish Disease Technology Training 254200, to: S.K. Johnson, Department of Wildlife and Fisheries Sciences, Texas A&M University, College Station, TX 77843-2258 U.S.A.

## **Ocean-Spar Co-Founder Wins Award**

Chief Engineer and Co-Founder of Ocean Spar Technologies, Gary Loverich received the 1995 Jerry Jurkovich Award for Innovation and Leadership in Gear Design. The award was presented on Saturday, 30 September at Fish Expo in Seattle. The award is presented every two years by the National Marine Fisheries Service (NMFS) in the United States. Jerry Jurkovich was a fisheries biologist who had the

unique ability to communicate scientific research to working fishermen. The award recognizes contributions to the promotion of mutual understanding and exchange of knowledge between fisheries researchers and fishing communities.

Gary Loverich worked with Jerry Jurkovich at NMFS in the Exploratory Fishing and Gear Research Department in the early 1970s. It was Jurkovich who encouraged Gary Loverich and Tom Croker to start NET Systems which grew into a multi-million dollar trawl gear company. "It is our way of saying "thank-you" for the outstanding contributions that Gary Loverich made to the trawl fishing industry", commented Gary Stauffer, Director of Resource Assessment & Conservation Engineering) and NMFS (the award is generally given to NMFS employees but an exception was made this year).

## **NRC Institute for Marine Dynamics Exploring Cage Design**

The Institute for Marine Dynamics (IMD) is collaborating with a consortium of British Columbia salmon farming companies—Pacific Aqua Salmon Farming Partners—and other government agencies to develop engineering and technological expertise in aquaculture cage design. The suitability of existing and new cage designs for deployment in locations with greater wave and current flow activity is being assessed. Better cage designs and mooring practices will prevent losses due to cage failure and will also address environmental concerns about farmed stock escaping to open waters. Better cage design to prevent intrusion by predators such as seals is also being studied. In seeking solutions to these challenges, IMD project manager Dr. Mehernosh Irani is employing field studies, numerical calculations, and physical modelling in the Institute's experimental test facilities. Wave and current loading on existing and conceptual cage designs is being evaluated, and effective design and deployment practices are being developed.

# AAC

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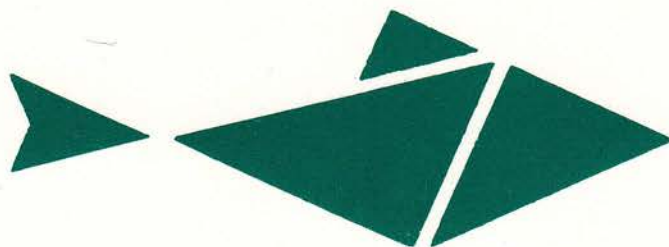
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