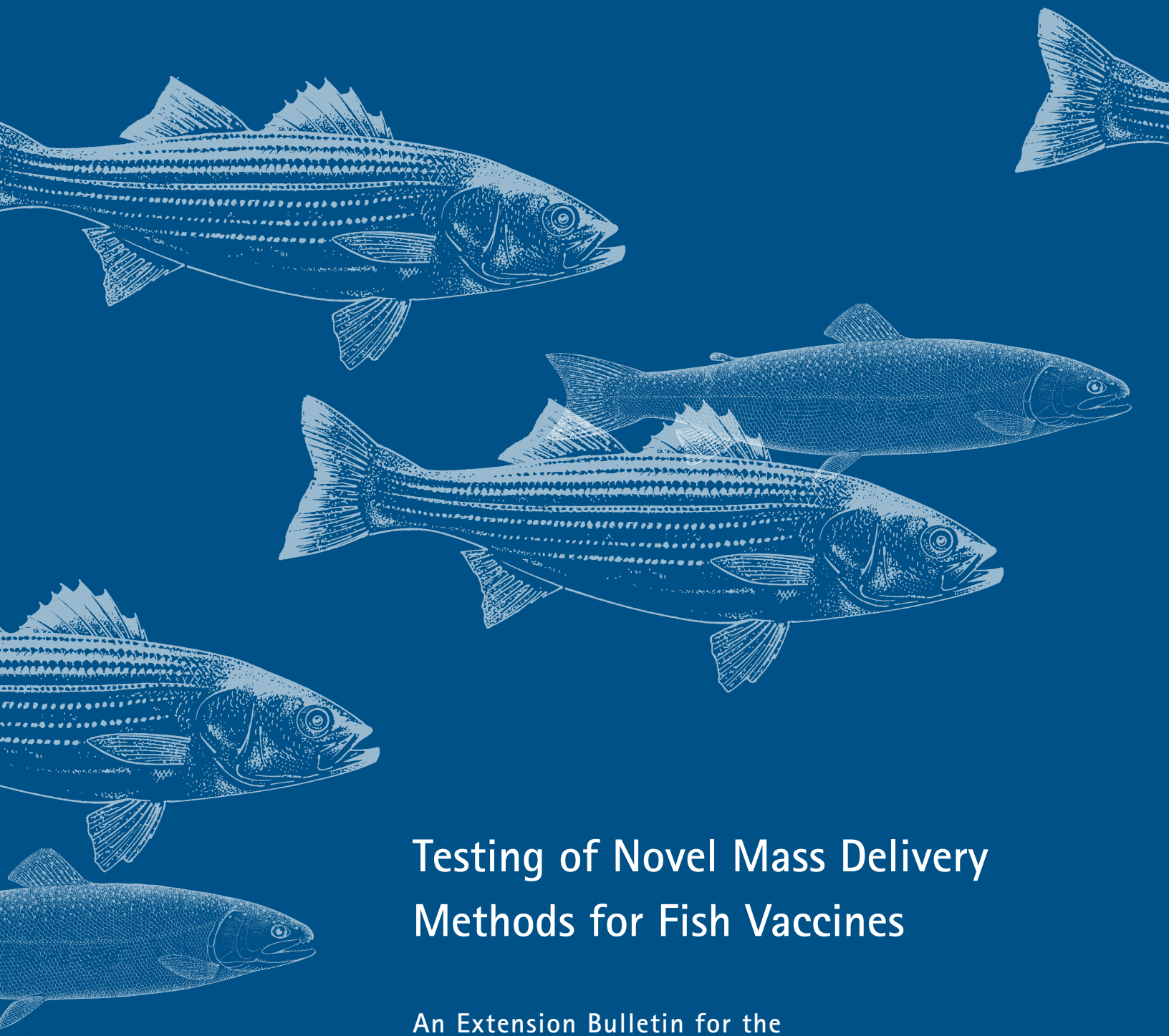

Testing of Novel Mass Delivery Methods for Fish Vaccines

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J. Winton, S. LaPatra, V. Ostland,
C. Friedman, and S. Harbell

Introduction

For more than 50 years, researchers have tested a variety of killed, attenuated, and subunit vaccines for control of fish diseases. The earliest fish vaccines killed preparations of whole bacteria, viruses, or parasites. Today, several bacterins have become commercially successful with more expected as improved delivery systems and better adjuvants are developed. Live, attenuated vaccines have been created by serial passage of a pathogen in laboratory cultures as well as by using naturally occurring mutants or cross-reacting strains. These generally offer excellent protection and are cost-effective, but concerns about residual virulence or their effects on other aquatic species can make them difficult candidates for licensing.

While most live vaccines and some bacterins can be delivered by immersion, many of these first generation fish vaccines have only demonstrated efficacy when injected by hand, either intraperitoneally (IP) or intramuscularly (IM). This method of vaccinating fish is relatively expensive, time-consuming, and not commercially viable for large aquaculture operations or for animals of low individual value. Although the throughput for hand immunization can be increased by use of automated syringes in a production line, new approaches are needed to further increase the potential application and delivery of fish vaccines.

In recent years, the tools of molecular biology have been applied to the construction of a variety of recombinant, engineered, or subunit vaccines for fish. Preparations representing each of these second-generation approaches have been tested in laboratory or field trials with various results and such vaccines promise to be safe and relatively inexpensive if they are able to provide protection when delivered by a low-cost approach.

A significant advantage of genetically engineered vaccines is the ability to construct multivalent preparations that can protect fish against several pathogens or different strains of the same pathogen. While many of these novel vaccine strategies have been effective at stimulating specific immunity in the laboratory when injected by IP or IM methods, more work is needed to develop better delivery systems and to overcome potential regulatory concerns. One objective of the WRAC Fish Immunology Project was to test novel delivery systems for fish vaccines.

Courtesy of Kent SeaTech

Hand immunization of hybrid striped bass with an autogenous vaccine. Throughput can be increased by use of automatic syringes, but the process still requires significant labor.

WRAC Immunology Project

Researchers working on the WRAC Immunology Project tested two proprietary methods for mass vaccination of finfish developed by Northwest Marine Technology (NMT), the world leader in the design and manufacture of equipment for the automated mass-coded wire tagging of salmonids. The first approach used a novel technology developed under an Advanced Technology Program grant from the US Department of Commerce that allowed NMT to complete the initial development of automated equipment for the mass IP injection of salmonids. The AutoFish SV vaccination system not only has the capability of IP vaccinating individual fish without the use of an anesthetic, but can also size separate and enumerate the fish in each size class simultaneously.

As part of a collaborative effort, WRAC researchers conducted controlled laboratory experiments to assess the level of protection provided to coho salmon (*Oncorhynchus kisutch*) that were mass vaccinated against *Vibrio anguillarum* at a hatchery using this equipment. The commercially

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Photos: Cover and at left: Courtesy of Northwest Marine Technology

available vibrio bacterin delivered using the AutoFish SV vaccination system provided strong protection against a laboratory challenge with high doses of live bacteria, thus demonstrating the first proof-of-principle for the safety and efficacy of this new equipment.

To explore the application of the AutoFish SV vaccination system for other finfish species, NMT biologists visited Kent SeaTech for a preliminary testing of the volitional entry device for the system, using hybrid striped bass fingerlings. They discovered that hybrid striped bass preferred not to enter the automated handling system in the same manner as salmonids, but would enter the device (and therefore be orientated properly for vaccination) when altered water flow rates and differing patterns of light/shade were tested.

Biologists with NMT also conducted collaborative studies with Clear Springs Foods. The first study consisted of an evaluation of the volitional entry device with rainbow trout juveniles (Clear Springs strain). The fish preformed extremely well during these laboratory studies and plans were made for a field evaluation using the mass IP injection capability of the AutoFish SV vaccination system.

A field study was initiated with rainbow trout at Clear Springs Foods in May 2007. Five separate vaccination "lines" were calibrated for four different sizes of fish, including 85–92 mm, 92–101 mm, 101–112 mm, and 112–124 mm. The fifth line was calibrated at 40–89 mm and would be considered the "bottom" population of this group. Testing was initiated once all the lines had been calibrated and NMT biologists were comfortable that fish in each size range were being injected in the proper area and that the vaccine was entering the peritoneal cavity without inducing any injury from the automated injection. The initial throughput was approximately 5,000 fish per hour and the equipment appeared to be performing well. However, as the testing progressed, fish became more resistant to entering the volitional entry device and throughput decreased.

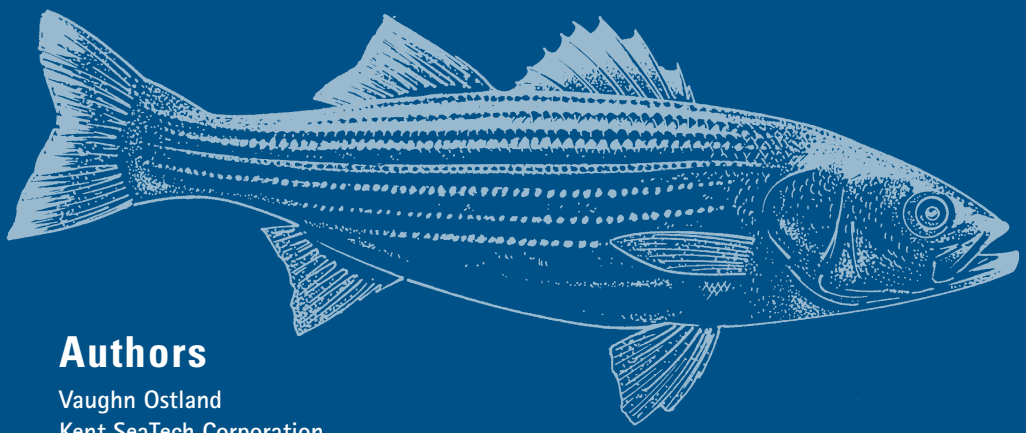
We speculated that the longer the fish were held off feed during the testing period, the more they cued on the biologists as a potential source of feed since they had been hand fed previously. Overall 18,883 fish with a mean length of 94 mm were injected using this equipment. There were 7,740 fish that entered the device but were not injected because they were below the size range that the units were calibrated to inject. This collaborative work has confirmed that the AutoFish SV vaccination system would enable fish culturists to rapidly deliver vaccines to hybrid striped bass as well as salmonids, thus expanding the application of this mass vaccination system to other segments of the aquaculture industry in the western US. At present, NMT has put this project on hold pending further developments in the technology or a move toward the more widespread injection vaccination of Pacific Salmon.

During the third year of the WRAC Immunology Project, we began testing a novel and proprietary method for automated delivery of DNA vaccines developed at NMT with support from the Washington Technology Center. Preliminary results using a DNA vaccine against infectious hematopoietic necrosis (IHN) that was originally developed by the WRAC-funded IHN research project showed that the location for injection of the DNA vaccine, more than the dose, controlled the level of protection conferred by the vaccine against a lethal virus challenge. A quantitative gene expression assay was used to measure levels of induction of the trout Mx-1 gene, an interferon-responsive gene that serves as a marker for activation of the innate immune system. An important finding from this work was that the level of induction of the Mx-1 gene in groups of trout administered the DNA vaccine by various methods correlated well with the level of protection seen in the groups following laboratory challenge

This finding confirmed the utility of the quantitative gene expression assays developed by the WRAC Immunology Project to serve as a rapid and effective proxy for the state of the immunity conferred by different types of vaccine preparations or by different delivery methods. The findings from this effort are expected to further the development of new vaccine delivery technologies that will significantly improve the health and disease resistance of fish reared by federal, state, tribal, and private sector facilities.

Photo courtesy of Northwest Marine Technology.

Northwest Marine Technology's AutoFish SV vaccination system.



Authors

Vaughn Ostland
Kent SeaTech Corporation
11125 Flintkote Ave, Suite J, San Diego, CA 92121

Stewart Alcorn
School of Aquatic and Fisheries Sciences
University of Washington, Seattle, WA 98195
(current address: Washington Department of Fish and Wildlife,
600 Capitol Way North, Olympia, WA 98501-1091)

Scott LaPatra
3 Clear Springs Foods, Inc.
PO Box 712, Buhl, ID 83316

Steve Harbell
Washington State University Cooperative Extension
PO Box 88, South Bend, WA 98586

Carolyn Friedman
School of Aquatic and Fisheries Sciences
University of Washington, Seattle, WA 98195

James Winton
Western Fisheries Research Center
6505 NE 65th Street, Seattle, WA 98115

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