Immunological Mechanisms of Intensively Reared Warmwater and Coolwater Finfish

Termination Report

Project Work Period November 1, 2003–November 1, 2007

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Participants

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Reason for Termination Funding ended

PROJECT OBJECTIVES

1. Develop a suite of reagents and assays that can be used to quantify the specific humoral and cellular immune responses of warmwater and coolwater finfish that could be used as indicators of healthy finfish immune systems and assess the efficacy of novel vaccines and methods of immunization.
2. Determine the immune response kinetics of hybrid striped bass and rainbow trout to *S. iniae* and *A. hydrophila*.
3. Determine the effects of hatchery practices on immune functions of hybrid striped bass and rainbow trout.
4. Assess the immune response and cytokine gene expression of hybrid striped bass and rainbow trout to *S. iniae* following delivery by a novel method for mass immunization.
5. Transfer the tools and research findings from this project to industry.

PRINCIPAL ACCOMPLISHMENTS

Objective 1. Develop a suite of reagents and assays that can be used to quantify the specific humoral and cellular immune responses of warmwater and coolwater finfish that could be used as indicators of healthy finfish immune systems and assess the efficacy of novel vaccines and methods of immunization.

This project has enabled the successful development of both humoral and cellular immune assays for assessing immune function of two economically important finfish species—hybrid striped bass (HSB) and rainbow trout (RBT). The serum immunoglobulin assays (enzyme-linked immunosorbent assay or ELISA) developed for this project allowed us to study the serum immunoglobulin response following exposure to live Gram-negative and Gram-positive bacterial pathogens. Furthermore, we developed three macrophage-based cellular assays (migration, phagocytosis, chemiluminescence) in HSB and RBT and all have utility for studying how bacterial pathogens, various vaccine preparations, and methods of immunization can affect cellular immune function.

To augment the humoral and cellular assay development efforts, quantitative PCR (qPCR) assays to assess expression of RBT cytokines were developed previously at the Western Fisheries Research Center and used to identify which HSB inflammatory response genes were modulated after experimental exposure to *S. iniae*. A qPCR assay for HSB hepcidin gene was chosen initially since this gene had been sequenced and published previously and was used to design authentic hepcidin primers. Using these reagents, a greater than 1000-fold increase in hepcidin gene expression was seen in the infected fish. This assay will allow biologists to study the impact of aquaculture on healthy finfish immune systems.

***Objective 2. Determine the immune response kinetics of hybrid striped bass and rainbow trout to* S. iniae *and* A. hydrophila.**

Standardized challenge models were developed for experimental infection of HSB and RBT with *S. iniae* and *A. hydrophila*. A standard injection challenge model was developed for *S. iniae* infection in HSB. The LD50 of *S. iniae* for HSB was estimated to range from 2.95 x 105 to 3.93 x 105 (mean 3.44 x 105) CFU in a 0.1 ml injection volume. A standard challenge model for *A. hydrophila* in rainbow trout and HSB was developed. The LD50 for *A. hydrophila* in HSB was estimated to range from 5.53 x 106 to 4.15 x 106 (mean 4.84 x 106) CFU in a 0.1 ml injection volume. The LD50 for A. hydrophila in rainbow trout has been estimated at 3.37 x 107 CFU.

The development of the RBT/*A. hydrophila* challenge model has led to research designed to develop an injectable adjuvanted vaccine against *A. hydrophila*. Our first experiments demonstrated that RBT could be passively immunized against *A. hydrophila* and that survivors post infection where virtually completely resistant to re-infection. These results led to injection vaccination experiments that were quite encouraging because relative percent survival of RBT post challenge ranged from 69-100 depending on the challenge dose. It appears that this type of vaccine may be feasible and that an immersion delivered vaccine may also be attainable.

Objective 3. Determine the effects of hatchery practices on immune functions of hybrid striped bass and rainbow trout.

An experiment was conducted to study the effect of loading density on the humoral immunoglobulin response in HSB and RBT following injection vaccination with bovine serum albumin. Serum immunoglobulin levels of both RBT and HSB were measured after maintaining fish at low, medium, and high densities up to 10 weeks post vaccination. Both HSB and RBT held at low density displayed a slight but positive increase of serum immunoglobulin over the course of the six-week experiment. When held at normal density, serum immunoglobulin levels increased over the duration of the trial, but in the high-density group, HSB serum immunoglobulin levels increased slightly from 1 to 3 weeks post vaccination but almost doubled this level by 6 weeks post immunization. A similar trend was observed in RBT; the highest levels of serum antibody were detected in the high-density group at 6, 8, and 10 weeks post-immunization. These results seem counter-intuitive in that one might hypothesize that loading density would be immunosuppressive.

Objective 4. Assess the immune response and cytokine gene expression of hybrid striped bass and rainbow trout to* S. iniae *following delivery by a novel method for mass immunization.

Northwest Marine Technologies (NMT), the world leader in the design and manufacture of equipment for the automated mass coded wire tagging of salmonids, developed proprietary technology for mass vaccination of salmonids without the need for anesthesia. The WRAC Immunology group assisted in the first proof of principle studies for the safety and efficacy of this technology for salmonids under laboratory conditions. We demonstrated that the NMT AutoFish SV vaccination platform successfully delivered the vaccine and thus provided effective protection of coho salmon against a *V. anguillarum* challenge. This effort is expected to lead to the availability of a novel mass vaccine delivery technology that will significantly improve the health and disease resistance of fish reared at, or released from, federal, state, tribal, and private sector facilities. This will provide a significant new benefit to fish stocks in aquaculture, resource enhancement, and threatened and endangered captive broodstock programs.

Objective 5. Transfer the tools and research findings from this project to industry.

To date two extension publications, "Measurement of the Innate Cellular Immune Responses of Hybrid Striped Bass and Rainbow Trout" and "Measurement of rainbow trout and hybrid striped bass antibody using an enzyme-linked immunosorbent assay (ELISA)," have been written, submitted, and reviewed by our extension specialist. Based on feedback from outside sources, these findings are suitable for publication and are currently in the final editorial stages prior to printing. A third extension publication, "Testing of novel mass delivery methods for fish vaccines,"

has been written and is in final stages of preparation. A fourth product, "Assessment of the feasibility of developing a vaccine against Aeromonas hydrophila in rainbow trout," is in the planning stage.

IMPACTS

Objective 1. The HSB serum immunoglobulin ELISA developed for this project is being tested to determine whether it could serve as a predictor of infection or previous exposure to an infectious agent(s). Also HSB serum immunoglobulin levels may correlate with a dose-dependent response to vaccination and serve as a predictor of vaccine efficacy in clinical trials.

Objective 2. The development of an efficacious vaccine to reduce *A. hydrophila* mortality associated with commercially reared rainbow trout will have a direct impact on survival and cost of production for this cultured fish species.

Objective 3. Our results suggest that the elevated stocking densities typically encountered during intensive aquaculture practices do not seem to negatively impact humoral immunoglobulin production. This finding needs to be repeated to confirm our initial findings. It appears that domestication may select for animals that can survive and respond under similar conditions.

Objective 4. Mass vaccination technology is desperately needed to ensure reliable, safe, and effective delivery of vaccines (and other biologics) to finfish. Our efforts with NMT to prove their technology were highly successful. At this time however, it is felt that the economics of introducing mass injection vaccination technology appear to greatly exceed the economics that aquaculture is willing to pay to have relatively low-priced animals injection vaccinated.

Objective 5. The fish immunology-related extension products currently submitted for publication will assist fish health researchers, biologists, and aquaculturists with management decisions regarding the effect of intensive culture conditions on fish immune function.

RECOMMENDED FOLLOW-UP ACTIVITIES

Investigate in greater detail the role that hatchery practices (for example, stocking density) play in modulation of fish immune function. Given the tools that have been developed here, further insights into how healthy HSB and RBT immune systems function in response to intensive culture practices could have significant practical implications for animal husbandry and animal welfare issues in the western US.

There is a desperate need for affordable mass vaccination technology in US aquaculture. The importance of vaccination in aquatic animal health has been realized and is generally necessary to successfully rear economically important fish species. There is now a need for ways of economically delivering immunologically relevant (i.e., protective) antigens to finfish. This achievement should be considered the cornerstone to the future economic viability of US aquaculture.

PUBLICATIONS, MANUSCRIPTS, AND PAPERS PRESENTED

Publications in print

None

Manuscripts

Outreach products

1. Measurement of the innate cellular immune responses of hybrid striped bass and rainbow trout. Authors: Alcorn S, Ostland V, LaPatra S, Friedman C, and Winton J. Final manuscript submitted and in production.
2. Measurement of rainbow trout and hybrid striped bass antibody using an enzyme-linked immunosorbent assay (ELISA). Authors: Ostland V, Alcorn S, LaPatra S, Friedman C, and Winton J. Final manuscript submitted and in production.

3. Testing of novel mass delivery methods for fish vaccines. Authors: Winton J, Alcorn S, Ostland V, LaPatra S, and Friedman C. Final version in preparation.
4. Assessment of the feasibility of developing a vaccine against *Aeromonas hydrophila* in rainbow trout. Authors: LaPatra S, Alcorn S, Ostland V, Friedman C, and Winton J. Final version in preparation.

Peer-reviewed manuscripts

1. Development of assays to measure the cell-mediated innate immune response of hybrid striped bass and rainbow trout. Alcorn et al. *Journal of Fish Diseases*. Manuscript in preparation.
2. Measurement of hybrid striped bass antibody using a novel enzyme-linked immunosorbent assay. Ostland et al. *Fish and Shellfish Immunology.* Manuscript in preparation.
3. The adaptive humoral response of hybrid striped bass and rainbow trout is density dependent. LaPatra et al. Manuscript in preparation.

Papers presented

None

SUPPORT

YEAR	WRAC-USDA FUNDS	OTHER SUPPORT					TOTAL SUPPORT
		University	Industry	Other Federal	Other	Total	
2003	100,000	7,000[a]	25,530[b]	25,000[c]		57,530	$157,530
2004	100,000	7,000[a]	25,530[b]	25,000[c]	1,000[d]	58,530	$158,530
2005	100,000	7,000[a]	25,530[b]	25,000[c]		57,530	$157,530
2006	100,000	7,000[a]	25,530[b]	25,000[c]	2,000[d]	59,530	$159,539
Total	400,000	28,000	102,120	100,000	3,000	233,120	$633,120

a in-kind salary for Friedman (0.1 FTE)
b in-kind support from Kent SeaTech, Clear Springs Foods
c in-kind salary, ancillary laboratory equipment, facilities, reagents for Winton
d in-kind support from Northwest Marine Technologies
e in-kind contribution (reagents, salary) from Aquatic Diagnostics Limited, Stirling Scotland