Project Title: The Immunological Development of Larval Marine Fish

Targeted Research Area Code (e.g. TRA-10-01): TRA-21-2 **Project Duration** (months)**: 24**

Total Funding Requested from NRAC: $188,535

States with Participants in Project (circle / list):

CT DE (ME) MD MA NH NJ (NY) PA RI VT WV Wash, DC / Other: __

Project Coordinator

Ian Bricknell, PhD; School of Marine Science & Aquaculture Research Institute**,** University of Maine, 5735 Hitchner Hall, Orono, ME 04469
Business Phone/Fax: (207) 581-2767/4430 e-mail: Ian.bricknell@umit.maine.edu

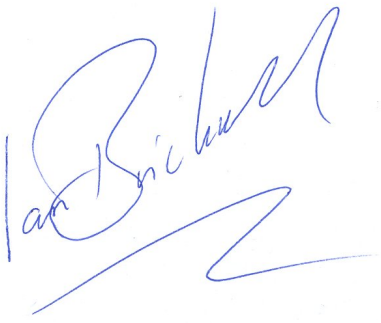
Principal Investigators

Rod Getchell, PhD Department of Microbiology and Immunology, Cornell University, College of Veterinary Medicine, Ithaca, New York 14853 rgg4@cornell.edu

Michael Pietrak PhD; USDA National Cold Water Marine Aquaculture Center:
Orono, Maine 04469 michael.pietrak@usda.gov

Chris Bartlett; Marine Extension Associate, Marine Technology Center, City of Eastport, 16 Deep Cove Road, Eastport, ME 04631 Tel 207.853.2518 cbartlett@maine.edu

Tim Bowden PhD; School of Foods and Agriculture & Aquaculture Research Institute**,** University of Maine, 5735 Hitchner Hall, Orono, ME 04469 Timoth.Bowden@maine.edu

Project Coordinator's Signature:  **Date: 14 July 2020**

Why:- Marine fish are becoming much more important to the US aquaculture industry. Often marine fish produce neotenous larvae that spend a long time as a yolk-sac animal. In this delicate state there are often mass mortalities that are attributed to disease. This can cause a bottleneck in production or the development of a species for commercial purposes. One of the most promising species currently being developed for large-scale culture is the lumpsucker (*Cyclopterus lumpus* L.). This species is not for human consumption, instead it is being used as a cleaner fish to remove sea lice, *Lepeophtheirus salmonis*, (Kroyer 1837) and *Caligus elongatus,* Nordmann, 1832) on Atlantic salmon and King trout (marine Rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792), in Maine and New Hampshire. A related species, the Pacific Spiny lumpsucker, *Eumicrotremus orbis*, Günther, 1861, has the potential to be an effective cleaner fish on salmon farms in the Pacific. However, both of these species suffer from unexpected losses of larval fish during those critical larval stages.

This preproposal sets out a project to investigate how the immune system of lumpsuckers develops during the critical egg-larvae-metamorphosis stage of development and establish if the maternal immunological investment into the eggs can be enhanced to protect the larvae as well as establishing the developmental pathways of the larval fish's immune system. This information will allow the use of immunostimulants on the larvae to enhance larval survival and establish when vaccines can be effectively delivered to larval lumpsuckers without adversely influencing the developing immune system of these fish.

What:- Most fish larvae hatch at an early stage of embryological development, usually the embryo has just undergone neural tube formation and gastrulation. The embryo sits on a large reserve of yolk, which will sustain it throughout the rest of its development until first feeding occurs. There are many major developmental steps a viable embryo has to overcome during this period. The development of all the major organs has yet to occur. Not least in this process is the immune system. It has been known for many years that the yolk of fish eggs contains many components of the mother's immune system. Immunoglobulins, complement, α-2 Macroglobulin and lysozyme will have been transferred to the eggs in the yolk during development within the ovary. The newly fertilized embryo will not show any gene activity for these important molecules to protect it from infection until the appearance and maturation of organs such as the thymus. This is the period when the larval fish is most vulnerable to infection as the only protection it has from infection is the maternal investment.

There is experimental evidence that female fish will transfer antibodies from its immune system to the larvae against pathogens it has recently encountered. Here it is proposed that we investigate the antibody investment in lumpsucker eggs by the mother. The ultimate outcome here is can the profile of egg immunoglobulin be influenced by vaccination of the mother to protect the developing larvae in lumpsuckers? This will be our first objective (**Objective 1**).

In the period after the eggs have been laid and fertilized the neotonous larvae will begin to develop its organs and physiology including the immune system. As these processes happen a molecular cascade will occur as the relevant genes start to be expressed that control tissue and organ development. This is particularly true of the immune system genes like IgM, lysozyme, RAG1/2, Interleukin-1, pentraxin, complement component C3, will be expressed sequentially as the larvae mature. These genes will inform us as to the status of the immune system and the defensive processes it is capable of mounting as the larvae develops and matures. Our second objective will be to determine the number of degree days after hatching that each component of the immune system begins to function due to the upregulation of key immune genes (**Objective 2**).

The uptick in immune genes will be associated with the development of key immunological organs such as the thymus and pronephros, the two key organs associated with B and T cell production in fish. One important factor is to establish where the immune genes are being expressed as this will help us target the delivery system of immunostimulants and vaccines for the developing larvae. To do this we will identify these key tissues by the use of *In situ* Hybridization (ISH) and Immuno-Histochemistry (IHC) to identify cell that are producing components for the immune system and where the genes are being expressed and where the proteins the genes encode for are being utilized in the embryo. So our third objective will be to identify the immunocompetent organs in the larval fish and the distribution of immunologically active proteins within the embryo (**Objective 3**)
Finally, for this work to be truly useful to the larval fish culturist we would investigate the effect of immunostimulants and vaccines delivered by immersion to these fish. The logic behind this research is that although the fish may not be sufficiently developed to respond to a vaccine initially. The components of the innate immune system usually develop first and can be upregulated by the use of immunostimulants (**Objective 4**). Using the novel method outlined above we would look at the effect of using immunostimulants and vaccines on the larvae and from which developmental point these can be used to help protect the larval fish from infection.
Hopefully we can answer the following questions:

1. Can vaccinated broodstock protect their offspring by the transfer of antibodies?
2. The optimal time to use immunostimulants to protect the larvae from pathogens
3. The optimal time to use vaccines to protect the larvae from pathogens

Who:- Dr Ian Bricknell will be responsible for running assays to investigate the functional immunology of the larval fish such as ELISA's, the distribution of immuno molecules in the larval fish and the development of reagents such as polyclonal antibodies against lumpsuckers antibodies, lysozyme etc. Dr Rod Getchell will be responsible for the *in-situ* hybridization and immunohistochemistry components of the project to identify and localize the tissues and organs expressing immune genes and immunologically active proteins. Dr Pietrak's group will be responsible for the culture and exposure of the lumpsucker larvae to immunostimulants and vaccines. Chris Bartlet will work as our extension agent and organize a workshop with the other Co-PIs at Aquaculture America 2022 (San Diego) and contribute to an open access review on larval vaccine husbandry in Aquaculture Reports. Dr Bowden will lead the molecular research investigating the ontogeny of genes in the larval fish for the key immunological components under investigation and analyze the level of expression of these genes under various scenarios using qPCR.
How:- At the start of year 1 a developmental sequence will be produced by NCWMAC and eggs and larvae preserved in 90% ethanol, RNA-later, formal saline and frozen for subsequent investigation. The material will be preserved at time intervals of 20-degree days to capture the changes in the functional and molecular immune response. This will then be distributed to the various laboratories. For the functional immunology analysis, the material will be thawed and the proteins extracted following PAGE electrophoresis and FPLC. This will identify the patterns of total proteins produced and when they appear in the time sequence. Specific proteins will be extracted from gels and further purified by electroelution, and used to create polyclonal and monoclonal antibody reagents by a commercial company. These reagents will be used to quantify the amount of target protein present in the egg and larvae tissue using quantitative ELISA assays, and used in western blots to identify the maternal contribution to the egg.
To localize where these proteins are produced the formalin fixed and ethanol fixed material will be cut into 5um sections on a microtome and the antibodies produced used to stain the section by

immunohistochemistry to identify the cells that are secreting the target proteins. The cells expressing the genes producing these proteins will be identified by *in-situ* hybridization. Where tagged molecular probes bind to the genes and light up the genes expressing cells.
To investigate the response of the larvae to immunostimulant and vaccines in the second spawning season in year two batches of larvae will be exposed to know immunostimulants such as β-1-3/β1-6 glucans and vaccines containing targeted antigens e.g. *Vibrio anguillarum*, a known pathogen of lumpsuckers, formulated for larval fish. Again, samples will be taken at 20-degree day intervals and preserved as described above. The same methods will be used to monitor changes in the immune response. At least one of the female broodstock will have been vaccinated with a targeted antigen and her immune response to it determined by ELSIA. Her offspring will also be analyzed to see if these targeted antibodies produced by the female and transferred to the eggs and subsequent larvae and if they have an immune function.
In-situ hybridization and gene expression will be used to investigate changes in distribution and expression of immune component genes. Primers will be developed for both *in-situ* hybridization and gene expression studies using conserved sequence homology of a range of immune markers such as IgM, lysozyme, RAG1/2, Interleukin-1, pentraxin, complement component C3, and cell markers CD4, CD8, TCRa/b.
To assist in transferring this technology to the industry it is proposed that a special session is run at Aquaculture America 2022 on vaccinating larval fish. It will use the PI of this grant as keynote speakers and also open the session up to other contributors both oral and poster presentations. It is proposed that a small e-book on larval vaccination is written by the PI's and distributed FoC to the industry via the Maine Seagrant and NRAC website. Any improved practices for vaccinating or administering immunostimulants will be incorporated into the lumpfish hatchery guidelines currently being produced by Dr Pietrak and Dr Fairchild under an existing NRAC project. A comprehensive paper on this research will be submitted for peer review in an open access journal at the end of the project

When:-

	Year 1				Year 2			
	Quarter 1	Quarter 2	Quarter 3	Quarter 4	Quarter 1	Quarter 2	Quarter 3	Quarter 4
Objective 1								
Objective 2								
Objective 3								
Objective 4								
Workshop								
Final Report								

BUDGET & JUSTIFICATION

SALARY AND WAGES:

A. PI/PD: I Bricknell has a 9-month faculty appointment at the University of Maine, School of Marine Sciences, and Aquaculture Research Institute. Dr. Bricknell is requesting funds for approximately 5 days of summer salary in each year ($2,500 yr1 and $2,500 yr 2) for his oversight and report writing for this project. ($5,000)

Dr Bowden has a 9-month faculty appointment at the University of Maine, School of Marine Sciences, and Aquaculture Research Institute. Dr. Bowden is requesting funds for approximately 5 days of summer salary in each year ($2,500 yr1 and $2,500 yr 2) for his experimental oversight and report writing for this project. ($5,000)

Dr Getchell is a research Assistant Professor at the Department of Microbiology and Immunology, Cornell University, College of Veterinary Medicine and is requesting $3,500 summer salary per year for his experimental oversight and report writing for this project. ($7,000) **Total $17,000**

C. Undergraduate Student Research Assistant: The undergraduate research assistant is a 12-month 25% (10 hours per week) appointment. The appointee will work closely with the PIs and will be responsible for aquarium maintenance and assisting the PI's in experiments. The undergraduate will be paid $12/hr for approximately 333 hours of time on the project **(total $4,000**).

Graduate Research assistants: The graduate research assistant is a 12-month 50% (20 hours per week) appointment. The appointee will work closely with the PIs and will be responsible for pathology INC and ISH staining and assisting the PI's in experiments. The undergraduate will be paid $21/hr for approximately 666 hours of time on the project (**total** $**13,986**).

Research Technician A full time research technician is required to support the rearing of fish, larval experiments assist with and optimize the function immune assays, qPCR assays and ISH and IHC staining. ($36,000 Yrs. 1 and 2 respectively, giving a total of $**72,000**) **Total Salaries $99,986**)

FRINGE BENIFITS:

Fringe benefits for the project are 8% for faculty summer salary $640. These rates are based on the University of Maine's current federal rate agreement. For the full time research technician indirect costs are $19,620 in year 1 and $19,188 in year 2 respectively. **Total $39,448**

MATERIAL/SUPPLIES:

Expendable supplies for q PCR and In-situ hybridization, $15,084: Fish and fish feed $7,061, heparin, syringes, needles, scalpels, microfuge and centrifuge tubes, pipette tips, pipets, freezer sample storage boxes *etc*. 6,600, Tank rental 5,365. **Total $34,110**

Travel (Regional): Travel to allow field work ($1215) research visits between the three sites and travel to project update meetings quarterly (two will be in person and two will be virtual and alternately hosted at UMaine and Cornell). Funds requested are for mileage 4,548 miles @ .44 cents per mile $1009), lodging ($150/night for 4 people for 4 nights per trip: $2400), travel, and meals $46 *per diem* for 16 days ($368). **Total $5,991**

Out of State: One interstate trip is planned to allow travel for two people to attend and present the research findings at NACE 2022. Funds requested are for mileage 982 miles @ .44 cents per mile $432), lodging ($150/night for 4 nights: $1,200), travel, and meals $46 *per diem* for 4 days ($368). **Total $2,000**

TOTAL AMOUNT OF THIS REQUEST: $188,535

Ian Robert Bricknell, Libra Professor of Aquaculture Biology
Business Address: Aquaculture Institute, School of Marine Science, U of ME, 5735 Hitchner Hall, Orono, ME 04469tel (207) 356 4119/581 4380; ORCID ID 0000-0001-6042-7143; e-mail: ian.bricknell@umit.maine.edu
Educational History: *1986-1989* University of Lancaster PhD Supervised by Prof. W.T.W. Potts awarded August 1990.
1983-1986 University of Reading B.Sc. (First Class Honors) Pure Zoology & Geology
Employment History: *September 2007 to date* Libra Professor of Aquaculture Biology School of Marine Sciences
September 2007-Nov 2013 Director of the Aquaculture Institute, School of Marine Science, University of Maine,
2000-Aug 2007 Fisheries Research Services (FRS) Marine Laboratory, Aberdeen (the FRS Marine Laboratory is an executive agency of the Scottish Executive), Principal Scientist, Aquaculture & Aquatic Animal Health.
1992-2000 Fisheries Research Services (FRS) Marine Laboratory, Aberdeen (the FRS Marine Laboratory is an agency of the Scottish Executive), Senior Scientist, Aquaculture & Aquatic Animal Health.
ICES Responsibilities :2005 Chair ICES Mariculture Committee (Term of Office 1 January 2006-31 December 2010) ICES Science Advisory Committee Member 2007-2010, Member of the ICES Marine Fish Culture Working group 1999-2003
Membership of Professional and Academic Bodies. Fellow of the Royal Entomological Society (*by invitation*), Associate of the Institute of Medical Laboratory Sciences, Member of the European Association of Fish Pathologists, Member of the Paleontology Association.
Membership of other organizations. Scientific Committee Sea Lice 2021, Faeroe's Islands September 2021, Scientific Committee The 14th ICOC, Kruger National Park, South Africa June 2021, AHJWG committee 1999-to date
Publications: Currently 133 peer reviewed papers & 3 Books (selected references follow)

Magnadottir B, Bragason BT, **Bricknell IR**, Bowden T, Nicholas AP, Hristova M, et al. Peptidylarginine deiminase and deiminated proteins are detected throughout early halibut ontogeny - Complement components C3 and C4 are post-translationally deiminated in halibut (*Hippoglossus hippoglossus* L.). Developmental and Comparative Immunology. 2019;92:1-19. doi: 10.1016/j.dci.2018.10.016.

Bowden TJ, **Bricknell IR**, Preziosi BM. Comparative pathogenicity of *Vibrio* spp., *Photobacterium damselae ssp damselae* and five isolates of *Aeromonas salmonicida ssp achromogenes* in juvenile Atlantic halibut (*Hippoglossus hippoglossus*). Journal of Fish Diseases. 2018;41(1):79-86.

Bowden TJ, **Bricknell IR**. Management of finfish and shellfish larval health in aquaculture hatcheries. In: Allan G, Burnell G, editors. Advances in Aquaculture Hatchery Technology. Woodhead Publishing in Food Science Technology and Nutrition 2013. p. 223-45.

McCarthy UM, Bron JE, Brown L, Pourahmad F, **Bricknell IR**, Thompson KD, et al. Survival and replication of *Piscirickettsia salmonis* in rainbow trout head kidney macrophages. Fish Shellfish Immunol. 2008;25(5):477-84. doi: 10.1016/j.fsi.2008.07.005. PubMed PMID: WOS:000261564400003.

Bricknell I, Dalmo RA. The use of immunostimulants in fish larval aquaculture. Fish Shellfish Immunol. 2006;19(5):457-72. doi: 10.1016/j.fsi.2005.03.008. PubMed PMID: WOS:000229684200006.

RESUME:

Rodman G. Getchell, Assistant Research Professor
Dept. of Microbiology and Immunology, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, Tel.: (607)-253-3393, E-Mail: rgg4@cornell.edu
ORCID ID: https://orcid.org/0000-0003-4063-4668

a. Professional Preparation

University of New Hampshire, Microbiology B.A. 1979; Oregon State University, Microbiology M.S. 1983; Cornell University, Veterinary Medicine PhD 2002.

b. Appointments

2016-pres. Assistant Research Professor, Aquatic Animal Health Program, Dept. of Microbiology and Immunology, College of Veterinary Medicine, Cornell University.
2014-2016 Research Scientist, Dept. of Microbiology and Immunology, College of Veterinary Medicine, Cornell University.
2013-2016 Northeast Regional Coordinator for the USDA NRSP-7 Program
2010-pres. Associate Director of AQUAVET®
2010-2013 Senior Research Associate, Dept. of Microbiology and Immunology, College of Veterinary Medicine, Cornell University.
2002-2010 Research Associate, Dept. of Microbiology and Immunology, College of Veterinary Medicine, Cornell University.
1994–2002 Research Support Specialist III, Dept. of Microbiology and Immunology, College of Veterinary Medicine, Cornell University.
1990-1993 Extension Associate II, Fish Pathology Laboratory, Dept. of Avian and Aquatic Animal Medicine, College of Veterinary Medicine, Cornell University.
1985-1990 Marine Resource Scientist I (Pathology), Fisheries Research Laboratory, Maine Dept. of Marine Resources.

c. Selected Publications (total: 75 refereed pubs)

(https://scholar.google.com/citations?user=7v9FiaAAAAAJ&hl=en)

1. Getchell RG, EJ First, SM Bogdanowicz, JA Andrés, Schulman AT, Kramer J, Eckerlin GE, Farrell JM, Marquis H. 2019. Investigation of round goby viral haemorrhagic septicaemia outbreak in New York. Journal of Fish Diseases 42:1023-1033. https://doi.org/10.1111/jfd.13003
2. Getchell, R.G., Cornwell, E.R., Bogdanowicz, S., Andrés,J. Batts, W.N., Kurath, G., Breyta, R., Choi, J.G., Farrell, J.M., and Bowser, P.R. 2017. Complete sequences of 4 viral hemorrhagic septicemia virus IVb isolates and their virulence in northern pike fry. Dis Aquat Org 126:211-227. https://doi.org/10.3354/dao03171
3. Getchell, R.G., E.E. Hatch, A.O. Johnson, E.R. Cornwell, G.A. Wooster, T. Erkinharju, and P.R. Bowser. 2015. Goldfish Carassius auratus susceptibility to viral hemorrhagic septicemia virus (VHSV) genotype IVb depends on route of exposure. Diseases of Aquatic Organisms 115:25-36. http://dx.doi.org/10.3354/dao02872
4. Warg, JV, T. Clement, E.R. Cornwell, A. Cruz, R.G. Getchell, C. Giray, A.E. Goodwin, G.H. Groocock, M. Faisal, R. Kim, G.E. Merry, N.B.D. Phelps, I. Standish, Y. Zhang, and K. Toohey-Kurth. 2014. Detection and surveillence of viral hemorrhagic septicemia virus using real-time RT-PCR. II. Diagnostic evaluation of two protocols. Diseases of Aquatic Organisms 111:15-22. https://doi.org/10.3354/dao02758

Dr. Michael Pietrak Ph.D.
USDA ARS National Cold Water Marine Aquaculture Center
25 Salmon Farm Rd, Franklin, ME 04634
Tel: (207) 812-0605, e-mail: michael.pietrak@usda.gov
ORCID ID: https://orcid.org/0000-0002-9301-342X

Education

May 2013 PhD in Marine Biology, University of Maine
Dec 2002 Masters of Science in Marine Biology, University of Maine
May 1997 B.A. in Biology, Ithaca College 1997

Professional Employment

2020 – Pres. Biologist USDA-ARS National Cold Water Marine Aquaculture Center
2019 - 2020 Biological Science Aid USDA-ARS NCWMAC
2015 - 2019 Research Geneticists (Animals) (Research Associate) USDA-ARS NCWMAC
2013 - 2015 Research Scientist Aquaculture Research Institute, University of Maine
2008 - 2013 Graduate Research Assistant, University of Maine

Selected Publications

Peterson, B., Burr, G., Pietrak, M., & Proestou, D. 2020. Genetic Improvement of North American Atlantic Salmon and the Eastern Oyster *Crassostrea virginica* at the U.S. Department of Agriculture-Agriculture Research Service National Cold Water Marine Aquaculture Center. North American Journal of Aquaculture. 82:321-330. doi:10.1002/naaq.10144

Gao, G., Pietrak, M., Burr, G., Rexroad, C., Peterson, B., & Palti, Y. 2020. A New Single Nucleotide Polymorphism Database for North American Atlantic Salmon Generated Through Whole Genome Resequencing. Frontiers in Genetics, 11. doi:10.3389/fgene.2020.00085

Pietrak, M. & T. G. Rosser. 2019. Morphologic and molecular characterization of Gyrodactylus cyclopteri Scyborskaja, 1948 from *Cyclopterus lumpus* L., 1758. Parasitology Research. doi:10.1007/s00436-019-06542-0.

Pietrak M, Backman S. 2018. Treatment of lumpfish (*Cyclopterus lumpus* L.) infected with *Gyrodactylus cyclopteri* (Scyborskaya 1948). J Fish Dis. 41:721–723. https://doi.org/10.1111/jfd.12781

Getchis, T. (ed). 2014. Northeastern U.S. Aquaculture Management Guide A manual for the identification and management of aquaculture production hazards. USDA Northeast Regional Aquaculture Center. https://agresearch.umd.edu/

Molloy, S.D., M.R. Pietrak, I. Bricknell, and D.A. Bouchard. 2013. Experimental Transmission of Infectious Pancreatic Necrosis Virus from the Blue Mussel, Mytilus edulis, to Cohabitating Atlantic Salmon (Salmo salar) Smolts. Applied and Environmental Microbiology. 79: 5882-5890.

Pietrak, M., Molloy, S.D., Bouchard, D., Singer, J.T., and Bricknell, I. 2012. Potential Role of *Mytilus edulis* as a vector of *Vibrio anguillarum* on an Integrated Multi-Trophic Aquaculture Farm. Aquaculture. 326-329: 36-39

Christopher A. Bartlett
Maine Sea Grant/University of Maine Cooperative Extension
16 Deep Cove Road, Eastport, Maine 04631,207 853-2518 cbartlett@maine.edu

EDUCATION

Bachelor of Science, Microbiology**,** 1990, University of Maine

EXPERIENCE

Marine Extension Associate**,** February, 1995 - Present
Maine Sea Grant/ University of Maine Cooperative Extension, Eastport, Maine
Finfish Aquaculture Specialist, September, 1992 - February, 1995
Maine Aquaculture Innovation Center, Eastport, Maine
Fish Culturist, June, 1990 - September, 1992
Northern Southeast Regional Aquaculture Association, Sitka Alaska

PROFESSIONAL SERVICE AND AFFILIATIONS

USDA/APHIS ISA Technical Board, 2004-Present
Downeast Institute for Marine Research and Education Board of Directors, 1999-Present
Maine Marine Technology Center Advisory Committee, 2007-Present
State of Maine Scallop Advisory Council, 2004-2007
State of Maine Fish Health Technical Committee, 1999-2003

RECENT OUTREACH

Publications:
Athearn, K, Bartlett, C.: Saltwater fishing in Cobscook Bay, Angler Profile and economic impact. Maine Sea Grant Marine Research in Focus, November 2008, Vol. 6

Presentations:

A Collaborative Effort to Examine New Strategies for Managing Closed Bottom Habitats for Sea Scallops; Maine Fishermen's Forum, March 1, 2008, Rockport, Maine
Conferences and Workshops:

Aquaculture Research and Economic Development- Foresight Planning
February 21-22, 2008, Portland, Maine, and July 16, 2008, Orono, Maine
Planning committee, staff, and facilitator

15th Annual Northeast Farmed Fish Health Management Workshop,
March 27, 2007, Calais, Maine
Conference organizer and planning committee chair

TIMOTHY J BOWDEN

School of Food & Agriculture, Department of Animal & Veterinary Sciences, University of Maine, 5735 Hitchner Hall, Room 237, Orono, ME 04469-5735.

Tel: (207) 581 2772. Fax: (207) 581 2729 Email: timothy.bowden@maine.edu ORCID: https://orcid.org/0000-0002-1185-1906

EDUCATION

B.Sc (Hons) Biological Sciences, University of Lancaster, Lancaster, UK. 1988

M.Phil., Biotechnology, Napier University, Edinburgh, UK. 1993

Ph.D., Microbiology, University of Aberdeen, Aberdeen, UK. 1998

EMPLOYMENT HISTORY AND APPOINTMENTS

2018- Associate Professor, University of Maine.

2011-2018 Assistant Professor, University of Maine.

2007-2011 Consultant Project Manager, Waverley Science, UK.

2006-2007 Marie Curie More Advanced Research Fellow, University of Valencia, Spain

2000-2004 Senior Scientist, Fisheries Research Services, UK

PROFESSIONAL ACTIVITIES

Executive Editor - Aquaculture Reports

Scientific committee – 2nd International Symposium for Fish and Shellfish Immunology 2019

Scientific committee – 19th International Conference on Diseases of Fish and Shellfish 2019

PROFESSIONAL ASSOCIATIONS

International Society of Fish and Shellfish Immunology

American Fisheries Society

European Association of Fish Pathologists

PUBLICATIONS

1. Magnadóttir B, Bragason, B, Bricknell IR, Bowden TJ, Nicholas AP, Hristova M, Guðmundsdóttir S, Dodds AW & Lange S. 2019. Peptidylarginine deiminase and deiminated proteins are detected throughout early halibut ontogeny – Complement components C3 and C4 are post-translationally deiminated in halibut (*Hippoglossus hippoglossus* L.). Developmental and Comparative Immunology. Mar;92:1-19. doi: https://doi.org/10.1016/j.dci.2018.10.016 .
2. Bowden TJ, Preziosi BM & Bricknell IR. 2017. Comparative susceptibility of juvenile Atlantic halibut (*Hippoglossus hippoglossus*) to different isolates of *Vibrio* sp. and *Aeromonas salmonicida* subsp. *achromogenes*. Journal of Fish Diseases, 41(1):79-86. DOI: 10.1111/jfd.12679
3. Bowden TJ. 2017. The humoral immune systems of the American lobster (*Homarus americanus*) and the European lobster (*Homarus gammarus*). Fisheries Research, 186 (part 1): 367-371. DOI: 10.1016/j.fishres.2016.07.023
4. Bowden TJ, Bricknell IR. Management of finfish and shellfish larval health in aquaculture hatcheries. In: Allan G, Burnell G, editors. Advances in Aquaculture Hatchery Technology. Woodhead Publishing in Food Science Technology and Nutrition 2013. p. 223-45.
5. Makrinos DL & Bowden TJ. 2016. Growth characteristics of the intracellular pathogen, *Piscirickettsia salmonis*, in tissue culture and cell-free media. Journal of Fish Diseases, 40(8): 1115-1127. DOI: 10.1111/jfd.12578

Logic Model

Logic Model of: The Immunological Development of Larval Marine Fish

Situation Statement: The needs for developing an optimum strategy for the use of cleaner fish in cold water aquaculture in the USA
Outcome Summary: Produce A cleaner fish strategy that optimizes the development and cleaning efficiency of cleaner fish on fish farms

Inputs	Outputs: Activities	Outputs: Participation	Outcomes – Impact: Short Term	Outcomes – Impact: Medium Term	Outcomes – Impact: Long Term
What we invest: 4 Research Scientists with wide range of knowledge and expertise in fish culture, fish parasitology, fish immunology and pathobiology 1 Technician Undergraduate student technicians graduate student technicians research Associates Laboratory and Aquarium Facilities Time Equipment Experimental fish lumpsuckers (*Cyclopterus lumpus*) Expertise and knowledge	**What we do:** Understand the development and immunology of larval lumpsuckers Investigate factors that favor increased survival of larval fish Determine the efficacy vaccines and immunostimulants in larval fish and how maternal investment int eh eggs can be optimized, Reporting and data analysis Transfer technology fish health Industry Extension workshops Scientific meeting presentations.	**Who we reach:** Scientific researchers Outreach Marine fish industry Research Associates Graduate and undergraduate students State, national and international collaborators Scientific community General public Fish farmers, veterinarians and animal health companies	**What the short-term results are:** Will vaccinating broodstock lumpsuckers improve larval survival Variables that impact or benefit larval lumpsucker immunological development Knowledge developmental processes key to developing an otogenoic series for larval fish Graduate Student research & education experience Fish farmers gain knowledge and understanding of larval fish vaccines Communication between researchers marine fish hatcheries and Vaccine industry a increased	**What the medium-term results are:** Optimum Conditions and treatments for the prevention of disease in larval lumpsuckers Production costs for fish farming is decreased Animal welfare is improved Scientific publications The working relationship between UMaine and the fish health industry is enhanced and increased Fact sheet and Web site presence of research Educate students	**That the ultimate results are:** Mitigation of the impact of Pathogenic disease for lumpsuckers production Significant expansion of the marine fish aquaculture industry in the US Improved food security Reduced reliance of chemical control for sea lice as there is a reliable aquacultured source of lumpsuckers as cleaner fish. Increased sustainability and competitiveness of US agriculture [aquaculture]

Assumptions
The Lumpsuckers have similar conserved biological process in their larval development that are found in other fish species. This included the development of the thymus, maternal transfer of immunological

External Factors
Environmental conditions of use, time, temperature and health status of the lumpsucker larvae.
That the environmental conditions encountered in the experimental region have an optimum deployment window for immune modulation of

defenses into the eggs and conserved gene sequences for key immunological genes	broodstock and larval fish. Education of veterinarians, farmers and best management practices to optimizing the production environment

Evaluation - How will you measure and report your outcomes?

This project will investigate the potential of manipulating the immune development of larval lumpsuckers to improve survival. Improving fish health welfare and production levels by reducing epizootics of disease in the hatchery. Disease outbreaks are one of the major limiting factors in fish aquaculture growth both nationally and internationally and larval mortality to infectious diseases is considered to be a significant bottleneck in marine fish larval production. .Experiments will determine the performance of the larval immune system under hatchery conditions and external factors such as broodstock vaccination of the appropriate use of immunostimulants and immunomodulators to improve larval survival and increase cleaner fish production; It will investigate larval performance and any adverse or beneficial effects these treatment have on the immune system and larval survival. Data will be collected, analyzed, interpreted and shared for each objective as outlined in the milestone. Regular meetings will occur between investigators and collaborators to share results and assess objectives completed. There will be a continual feedback loop with objectives completed and information gained. Outcomes will be reported via extension workshops, publications in peer reviewed journals, a fact sheet, NRAC required reports, The ARI web site and via scientific conferences and trade shows and social media.