

UNIVERSITY  
of GUELPH



CHANGING LIVES  
IMPROVING LIFE

## ALMA AQUACULTURE RESEARCH STATION

University of Guelph, Office of Research

### RESEARCH HIGHLIGHTS 2008 – 2009

Since 1993, more than 140 research projects have been conducted at the Alma Aquaculture Research Station. The primary areas of research interest include:

*Breeding and genetics, culture methodology, engineering & system design, fish behaviour, fish health, fish welfare, growth physiology, nutrition, product development, reproduction and management, reproductive physiology, telemetry studies and waste management.*

A summary of some of the current research follows:

#### **ARS 132 – Genetic studies of growth, maturation timing, spawn timing, and family relatedness in salmonid fishes.**

This research addresses aspects of the influence of different genes on various phenotypic traits in salmonid fishes. These traits include growth, spawn timing, maturation timing, and stress/disease resistance. An understanding of the underlying genetic influence on these traits is of both basic and applied significance. The basic knowledge gained will increase our knowledge of where the genes influencing these traits are located in the salmonid genome. In addition, the fundamental knowledge gained from this research may also enhance the aquaculture of these species via the ability to select fish with desirable genes for the traits being studied. Aquaculture will grow in increasing importance in the future and may help to alleviate pressures on wild capture fisheries, many of which, are already greatly endangered. To facilitate these experiments fish from commercial broodstocks are selected and submitted to my lab for genetic analysis. These fish are directly derived from private or government researchers with whom we are conducting collaborative research. In addition we maintain our own source of experimental rainbow trout and Arctic charr at the Alma Aquaculture Research Station (AARRS) of the Ontario Ministry of Agriculture, Food and Rural Affairs. These fish are reared to maturity and periodically handled to measure growth and reproductive state. Atlantic salmon and Arctic charr from the east coast of Canada (used in the .aquaculture industry) are sampled by our collaborative partners. These fish are used to measure such indices as productive state and growth. Small gill tissue biopsies are taken from selected individuals that may be used in different experiments. DNA is extracted from these small tissue samples to determine genetic variation in DNA marker association studies.

#### CONTACT INFORMATION:

**Aquaculture Coordinator:**  
Professor Richard Moccia  
519-826-3800  
rmoccia@uoguelph.ca

**Station Manager:**  
Michael Burke  
519-669-5411  
aars2@hsfx.ca

#### WEBSITES:

**AARS**  
[http://www.uoguelph.ca/research/omafra/housing\\_animals.shtml](http://www.uoguelph.ca/research/omafra/housing_animals.shtml)

**AQUACULTURE CENTRE**  
[www.aps.uoguelph.ca/aquacentre](http://www.aps.uoguelph.ca/aquacentre)

*Continued from page 1*

The phenotypic manifestation of complex life-history traits such as the timing of first maturation in an animals life, the time within a reproductive season when a female's eggs are ovulated, and the developmental trajectories in an animals ontogeny is a complex interaction between the expression of specific genes controlling the onset of these specific traits, and modulation of these events through environmental interactions. Underlying the expression of all these important life-history developmental events is a requisite for the anabolic acquisition of energy resources to fuel subsequent developmental transitions. Growth rates per se, are known to be intricately coupled to the onset of early maturation rates in fishes, and this research furthers our understanding of this process in a group of model research species, the salmonid fishes.

Salmonids are important, not only commercially as a major food resource in the aquaculture industry (i.e. Atlantic salmon production accounts for greater than 95% of the revenue generated in the Canadian aquaculture industry), but also serve as primary fish experimental models in physiology and genomics research. Salmonid fishes are derived from an ancestor that underwent a whole genome duplication event and thus also serve as one of the few known vertebrate autopolyploid animal models. Furthermore, given that it is fairly well established that ray-finned fishes have undergone one additional whole genome duplication compared to extant tetrapod vertebrate species, the research helps to further our knowledge of how duplicated sets of genes (up to 16 copies for some gene families within salmonids) may be arranged within the genome compared to tetrapod vertebrate species (possessing only up to 4 gene copies/gene family).

We are studying the arrangement (chromosomal location and assignment to synteny blocks) and gene expression profiles of specific co-functioning gene families that prior research has indicated to be of importance in regulating the transitions of important life-history events in three model species of salmonid fishes (i.e. Atlantic salmon, *Salmo salar* rainbow trout, *Oncorhynchus mykiss*; and Arctic charr, *Salvelinus alpinus*). The species are important for two reasons. Firstly, they constitute the three major aquaculture species of salmonid fishes reared in Canada. Secondly, they represent an excellent model species group to study the process and consequences of genome duplication events in vertebrate species. The three species represent a continuum of chromosomal arrangements characterized by largely an acrocentric (single chromosome arms) based karyotype in the Arctic charr, to a highly derived karyotype in the Atlantic salmon, characterized by multiple chromosome arm fusions. The significance of this variation facilitates the study of how gene arrangements in other model teleost species (eg. zebrafish; medaka; sticklebacks; pufferfishes), are arranged (conserved, or re-arranged) in the salmonids. The identification of conserved synteny blocks can be of potential benefit to our understanding of gene regulatory networks and transcriptomics, given that regulatory sites within synteny blocks will be retained as haplotype blocks following meiosis. The manifestation of different allelic phases or genetic variants of the various genes contributing to a given phenotypic trait can only be studied in detail by examination of the whole animal phenotype. Hence, there is a requisite need to rear live fish that have been produced from controlled crosses

Previous research from our lab has implicated chromosomal regions bearing the circadian timing genes (i.e. multiple Clock genes, BMAL1, multiple Period genes, ROR complex, and RevErb complex) as being important in influencing the life- history traits mentioned. In some instances, we have directly cloned and mapped genes within this complex to known haplotype blocks that differentially associate with progeny expressing alternate life-history phenotypes (e.g. early vs. late maturing; early vs. late spawning). In other instances we are using comparative genomic approaches with more completely characterized genomic species such as zebrafish and medaka, to infer the expected location of key candidate genes and we are now currently cloning and mapping these genes in our model species. Our second group of target genes involve those regulating myogenic development and to a lesser extent lipid metabolism, and as such, growth differentials within fishes (i.e. mTOR; AKT; myogenic factor (Myf and MRF family genes); IGF1, IGF2, GH1, GH2; Grf/PACAP; SIRT gene family; ID regulatory family; etc). Concordant with these studies is the investigation of many of the genes important in regulating the initial sex determination, and the final sexual differentiation and sexual maturation pathways (i.e. aromatase (Cyp19); 11 $\beta$ -hydroxylase; amh; Nr5A gene family; GDF9; BMP15; DAX, DMRT-related; etc.).

**ARS 136 –Role of temperature and organic degradation on the persistence of C and N stable isotopes in aquaculture waste effluent.**

One of the common concerns in aquaculture practices deals with the dispersion of waste effluents and feces. Studies to assess, quantify and even predict these characteristics have been developed, but only a few of them utilize analyses of stable isotopes as a tool. Stable isotopes are widely distributed among the inorganic and organic compounds of our planet. The isotopes of carbon (C), nitrogen (N), sulfur (S), hydrogen (H) and oxygen (O) are the most studied, and the ones that show a better applicability for tracking waste materials in the environment. The ratio at which these stable isotopes present themselves in nature ( $\delta = \delta$ ) is unique, and are often characteristic of the source of materials containing these isotopes. When a source is considered isotopically distinct, and the isotope's  $\delta$  does not change, or changes in a predictable way, that particular SI ratio is designated as a “signature”. The stable isotopes ratios  $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$  and  $\delta^{34}\text{S}$  have been widely used as effective tracers for allochthonous sources of the elements N, C and S in aquatic ecosystems, but their utility to track aquaculture effluents is unknown. Isotope signaturing in aquaculture effluents may provide the tools for tracking aquaculture wastes in natural waters. The present study will try to identify unique signatures in wastes derived from aquaculture practices through the study of ordinary feed ingredients, feces and water.

There are several benefits of the present study: One will be the determination of the isotopic profiles of the most widely used ingredients in fish feed formulations. If we can find a unique signature that could be used as a tracking tool, this may be useful information in tracking the source and dispersal of aquaculture loadings and therefore develop techniques and strategies to minimize such loadings and their inherent impact. Hence, the benefits will be directed at establishing better management policies for the aquaculture industry and providing crucial information for regulatory agencies to develop realistic and appropriate waste monitoring guidelines to the industry. These practices will help in maintaining or even improving the health of natural ecosystems that act as reservoirs of aquaculture wastes. As well, with the proper interpretation, the isotopic analyses of the ingredients and the feces will also provide nutritional insight into the fractionation and retention of the ingredients used during the feeding trials.



**Fish feed – naturally enriched with  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  isotopes**

## **ARS 138 – Evaluating the effects of stressors on ovarian function, embryo development and growth of juvenile rainbow trout.**

Stress response patterns have been studied in juvenile and adult salmonid fishes however, few such studies have focused on the effects of stressors on embryo development energy partitioning and growth in embryos. Stressors (including environmental factors) are likely to divert energy resources of the embryos from normal growth and development and thus adversely affect growth and possibly other aspects of the animal's physiology; they might also exert epigenetic actions that result in altered adult phenotypes.

Some stressors, such as those associated with intensive aquaculture, particularly with regard to routine maintenance protocols, transfer and animals between tanks and between farms, hypoxia etc result in elevated stress hormone levels in the oocytes and therefore pose a potential risk to embryo development, particularly at key “windows of development” when growth-related and immune system-related genes are expressed. Other stressors, such as ubiquitous environmental chemicals also pose potential hazards. Aquatic animals are generally impacted more than terrestrial animals because the chemical factors commonly find their way into aquatic ecosystems. The study will focus on two major groups (based on their mode of action on biological systems), namely xenoestrogens and chlorinated hydrocarbon compounds. In juvenile and adult salmonid fish, xenoestrogens stimulate the inappropriate synthesis of the yolk phospholipoprotein, vitellogenin; chlorinated hydrocarbon compounds act as hepatic aryl hydrocarbon receptor (AhR)- initiating chemicals that stimulate the production of hepatic mixed function oxidase enzymes. In both situations, energy that would normally be invested in growth is redirected.

This study was conducted to determine the effects of elevating egg cortisol, xenoestrogen and AhR-activating chemicals on the development of the embryos produced from these eggs and the growth of juvenile stages.

The study was to be undertaken over a 2-year period. In year 1 (commencing September 2008), a pool of eggs were taken from 10-15 rainbow trout during the routine annual spawning collection at Alma. Approximately 20% of the eggs were retained in ovarian fluid for 3 hours before being fertilized, water hardened and transferred to Heath incubator trays (Control); two groups of eggs were incubated in ovarian fluid that was enriched with cortisol (stress hormone) at one of two concentrations (1 and 10 mg ml<sup>-1</sup>), and two groups of eggs were incubated in ovarian fluid that was enriched with an AhR-inducer (1 and 10 mg ml<sup>-1</sup>). In year 2 (commencing September 2009) a smaller experiment will be carried out using three treatment groups, the Control, and two levels of xenoestrogen exposure (1 and 10 mg ml<sup>-1</sup>).

For both studies (2008 and 2009) identical parameters and responses were to be measured:

- Growth performance of embryos and early to mid-stage Juveniles
- Time to reach key developmental stages of embryos
- Percent hatching and percent survival to the juvenile stage
- Expression profiles of growth-related genes (such as GH, IGF and their receptors)
- Expression profiles of immune system-related genes
- Stress response profiles (based on total body cortisol measurements) at two developmental stages.
- Determination of the ability of the embryos to metabolize and clear maternal steroid hormones (in vitro study).

## **ARS 140 – Evaluating the substitution of high quality vegetable oils for fish oil in rainbow trout feed.**

Dietary fats supply a major part of the energy of rainbow trout. Phospholipids and steroid components of body organs also rely on dietary lipids for synthesis of supply. Certain of the fatty acids are essential for health, growth and normal appearance of the fish. The best sources of essential fatty acids for rainbow trout diets are fish oils. However, the incorporation of fish oils into the diets has two major drawbacks. First, fish oil is expensive and because availability is expected to decline while demand continues to increase, feed prices are expected to increase, contributing to higher costs of rainbow trout production. Second, the aquaculture industry has come under scrutiny due to studies indicating that farm-raised salmon have significantly higher levels of dioxins, polychlorinated biphenyls (PCBs), chlorinated pesticides and heavy metals, versus their wild counterparts. Dioxins and PCBs were commonly used worldwide during several decades for agricultural and industrial purposes. They are not biodegradable, and bioaccumulate and biomagnify in the food chain. At high levels, organochlorines have direct toxic effects and are carcinogenic. Many are hormonally active and are thereby considered to be endocrine disrupters. Given that many PCBs are liposoluble, efforts should be directed at fish oil replacement in order to effectively solve contamination issues using nutritional approaches.

The search for alternative ingredients to reduce and/or replace fish oil continues to be a high priority for nutritionists and feed manufacturers. The proposed research project seeks to develop rainbow trout diets that reduces the requirement for fish oil by the partial replacement of fish oil with canola oil. This project examines the effect of partial replacement of fish oil by various levels of canola oil on fish growth. Replicate tanks of trout will be fed to excess on a control diet (no canola oil ) or diets that are otherwise identical but with increasing levels of fish oil replaced by canola oil.

The proposed trial will provide the aquaculture industry with credible solutions to the issue of contamination levels in farmed salmonids. An additional advantage to fish oil replacement is the significant benefit to be realized by the aquaculture industry given the relatively low costs and enhanced sustainability of feeding vegetable oil versus fish oil. Furthermore, the replacement of fish oil by vegetable oil will increase the efficiency and sustainability of the industry as critical shortfalls of marine oil are predicted in the short term.



**Herring oil and canola oil  
used in fish feed**



**Table 1. Research Projects Conducted at the Alma Aquaculture Research Station 2008-2009:**

<b>AARS #</b>	<b>Researcher(s)</b>	<b>Research Title</b>	<b>Fish Species</b>	<b>Start</b>	<b>Finish</b>
ARS119	Dr. Roy Danzmann & Dr. M. Ferguson Integrative Biology, University of Guelph	Evolutionary genetics of Arctic charr.		Sept/08	
ARS120	Dr. R. Smith, Dr. C. Mothersill, Dr. C. Seymour & R.D. Moccia Dept. Biology, McMaster University and Dept. Animal & Poultry Sciences, University of Guelph	Investigating the radiation bystander effect in fish.	<b>Rainbow Trout</b>	Sept/08	
ARS120b	Dr. R. Smith, Dr. C. Mothersill, Dr. C. Seymour and R.D. Moccia Dept. Biology, McMaster University and Dept. Animal and Poultry Sciences, University of Guelph	Investigating the radiation bystander effect in fish. Second generation	<b>Rainbow Trout</b>	Oct/08	Oct/09
ARS124	Dr. John Leatherland/Neel Aluru Dept. Biomedical Sciences, University of Guelph and Dept. of Biology, University of Waterloo	Effect of estrogenic compounds on gene expression in early embryogenesis using microarray analysis. ARS124b - Xenoestrogens. ARS124c - Dose response to bisphenol A	<b>Rainbow Trout</b>	Sept/08	June/09
ARS127	Dr. John Leatherland/Mao Li Dept. Biomedical Sciences, University of Guelph	Evaluation of stress response in rainbow trout embryos at different developmental stages.	<b>Rainbow Trout</b>	Oct/08	Sept/09
ARS129	AARS Office of Research, University of Guelph	Growth of McKay spring spawning rainbow trout ( <i>Oncorhynchus mykiss</i> ).	<b>Rainbow Trout</b>	Nov/08	Mar/09
ARS132	Dr. Roy Danzmann Integrative Biology, University of Guelph	Genetic studies of growth, maturation timing, spawn timing, and family relatedness in salmonid fishes: Broodstock development of Spring Valley rainbow trout.	<b>Rainbow Trout</b>	Sept/08	
ARS132b	Dr. Roy Danzmann Integrative Biology, University of Guelph	Genetic studies of growth, maturation timing, spawn timing, and family relatedness in salmonid fishes: Studies on compensatory growth dynamics in salmonid fishes.	<b>Rainbow Trout</b>	Sept/08	

<b>AARS #</b>	<b>Researcher(s)</b>	<b>Research Title</b>	<b>Fish Species</b>	<b>Start</b>	<b>Finish</b>
ARS133	Dr. John Leatherland/Mao Li Dept. Biomedical Sciences, University of Guelph	Evaluation of stress response in rainbow trout embryos: effect of two different incubation temperatures.	<b>Rainbow Trout</b>	Oct/08	Oct/09
ARS134	Dr. John Leatherland/Mao Li Dept. Biomedical Sciences, University of Guelph	Evaluation of stress response in rainbow trout embryos: maternal effects.	<b>Rainbow Trout</b>	Sept/08	June/09
ARS135	Dr. John Leatherland/Mao Li Dept. Biomedical Sciences, University of Guelph	Evaluation of stress response in Arctic charr embryos at different developmental stages.	<b>Arctic Charr</b>	Oct /08	Sept /09
ARS136	Richard Moccia/Kris Osuchowski Dept. Animal Poultry Science, University of Guelph	Role of temperature & organic degradation on the persistence of C and N stable isotopes in aquaculture waste effluent.	<b>Rainbow Trout</b>	Nov/08	Mar/09
ARS137	Dr. John Leatherland/Mao Li Dept. Biomedical Sciences, University of Guelph	Evaluation of stress response in rainbow trout embryos: test of RU486 inhibitory effect on cortisol.	<b>Rainbow Trout</b>	Mar /09	May/09
ARS138	Dr. John Leatherland/Mao Li Dept. Biomedical Sciences, University of Guelph	Evaluation of stress response in rainbow trout embryos: effect of cortisol implants in pre-ovulatory females.	<b>Rainbow Trout</b>	June /09	
ARS137	Dr. John Leatherland/Mao Li Dept. Biomedical Sciences, University of Guelph	Evaluation of stress response in rainbow trout embryos: test of RU486 inhibitory effect on cortisol.	<b>Rainbow Trout</b>	Mar /09	May /09
ARS138	Dr. John Leatherland/Mao Li Dept. Biomedical Sciences, University of Guelph	Evaluation of stress response in rainbow trout embryos: effect of cortisol implants in pre-ovulatory females.	<b>Rainbow Trout</b>	June /09	

<b>AARS #</b>	<b>Researcher(s)</b>	<b>Research Title</b>	<b>Fish Species</b>	<b>Start</b>	<b>Finish</b>
ARS140	Richard Moccia/Martin Feeds Mills Dept. Animal Poultry Science, University of Guelph	Evaluating the substitution of high quality vegetable oils for fish oil in rainbow trout feed.	<b>Rainbow Trout</b>	July/09	
ARS141	Dr. John Leatherland/Mao Li/Jackie Dept. Biomedical Sciences, University of Guelph	Mode of action of cortisol effects, GR/ER interactions.	<b>Rainbow Trout</b>	Oct /09	
ARS142	Dr. M. Vijayan/Oana Birceanu Dept. of Biology, University of Waterloo	Exposure of unfertilized rainbow trout eggs to BPA to mimic maternal transfer: determination of the amount of BPA accumulation in eggs after three hour exposure.	<b>Rainbow Trout</b>	Nov /09	Dec/09