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ALMA AQUACULTURE RESEARCH STATION
University of Guelph, Office of Research

RESEARCH HIGHLIGHTS 2007 – 2008

Project ARS 120 - Investigating the Radiation Bystander Effect in Fish.

This collaborative project with McMaster University explores the unique aspects of radiation induced damage in aquatic animals. In radiation biology, the accepted principle is that the effect of radiation is directly related to the dose which is received; i.e. an increase in the dose results in a directly proportional increase in the effect. However, at very low radiation doses, the effects begin to deviate from what would be predicted. This deviation is thought to be a result of the bystander effect. The bystander effect has been demonstrated in cultured cells and occurs when cells which have not been irradiated but are in proximity of a radiated cell begin to exhibit some or all of the effects of direct irradiation. However, the bystander effect has not been adequately investigated in whole animals. Since the cause of the bystander effect is thought to be a chemical signal, the effect may have particular importance in the aquatic environment since chemical transmission is easier through water than through air. As a result, fish may be a particularly important animal group in which to investigate this phenomena. Therefore the aim of this investigation is to use very low (i.e. non-lethal) levels of X-radiation to investigate the bystander effect in rainbow trout. The importance of this research relates to environmental protection when legislating the use of nuclear power, specifically exposure to low levels of radiation present in cooling water discharge or, more seriously, accidental radiation leakage from nuclear facilities.

Given the unique and demanding requirements for fish stocks, appropriate rearing tanks and stringent water quality assurances coupled with an ability to conduct trans-generational fish research, the OMAFRA facilities at Alma are the only place that this kind of project can be done.

Rainbow trout eggs being X-rayed

The project's objective's are fivefold:

- investigate the bystander effect in whole animals i.e. address the question of whether a directly irradiated fish can initiate a response in a neighbouring, non-irradiated fish.
- investigate and characterize the effects of direct irradiation and this secondary bystander effect.
- characterize the bystander effect in terms of X-ray dose required for induction, duration and attenuation.
- initiate a stock of rainbow trout which have been irradiated at the earliest life history stages and then maintained for long term effect and trans-generational studies.
- determine at what early life history stage is the bystander affect most evident.

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

AQUACULTURE CENTRE
www.aps.uoguelph.ca/~aquacentre

Project ARS 125 - Evaluation of Alternative Energy Levels and Fat Sources in Rainbow Trout Feeds.

This nutritional research project was conducted by the Alma Aquaculture Research Station on behalf of the Martin Mills Inc. The cost of feed is the single most significant factor in the economics of rainbow trout production. Fish oil sources in trout diets are expensive and because availability is expected to decline while demand continues to increase, feed prices are expected to increase, contributing to higher costs of fish production. Fats and lipids are essential nutrients for fish. They are an important energy source, allow absorption of fat-soluble nutrients, play a role in membrane structure and are components of hormones. There are several alternative sources of fats suitable for commercial trout diets. These sources can be used to manufacture a variety of diets, containing different protein:energy ratios, that may result in different growth responses. The purpose of this project is to determine if these substitutes are suitable in rainbow trout diet formulation such that they maintain nutrient quality in a cost efficient manner. The anticipated benefit will be the ability to evaluate the effects of formulation on growth efficiency and overall profitability for both the trout producer and on carcass yield for the processor.

Traditionally research into salmonid diets have been conducted on young (< 50 g) fish due to limitations in physical resources (ie. rearing tanks are small and large volumes of high quality influent water are unavailable). However, given the market target weight of one kilogram fish, 75 to 80% of a grower's feed costs occur after the fish reach 250 g. Therefore, the feed manufacturer desires to test these experimental diets on fish from 250 g to an endpoint of one kilogram, a size range which is most meaningful to the aquacultural community. In order to test diets using large fish at commercial densities in a statistically relevant study, numerous large tanks (1,500 litres) requiring large volumes of high quality influent water is necessary. Alma's physical resources and expertise conducting this kind of applied research has allowed it to successfully complete this and similar nutritional trials.

Project ARS 127: Evaluation of Stress Response in Rainbow Trout Embryos at Different Developmental Stages.

Many of the currently-applied aquaculture husbandry practices, particularly in intensive culture situations, such as netting, handling, transfer of animals between tanks, transportation of animals between sites, testing for the stage of ovarian maturation, etc. are stressful on the animal. This not only impacts the welfare of the animals, but has potential economic consequences of reduced growth, reduced feed efficiency, and potentially elevated susceptibility to infections. The stress responses of juvenile and adult salmonid fishes have been well studied and the recovery profiles are well established, so that aquaculture protocols can be adjusted accordingly, and stressors kept to a minimum. On the contrary, similar profiles have not been developed for early developmental stages; the animals are too small to bleed and thus stress markers, such as changes in plasma cortisol, epinephrine, and glucose cannot be measured. Recent studies have found that whole body cortisol levels can be used to monitor stress in small fish, such as zebrafish.

Obtaining tissue samples from rainbow trout

This study uses similar techniques to determine the stress-response profiles in embryonic stages of a salmonid fish, rainbow trout, which is a key species used by the aquaculture industry of Ontario. The information gained will facilitate the development of appropriate management protocols in intensive aquaculture, particularly with regard to routine maintenance protocols, transfer of animals between tanks and between farms, etc.

An extension of the study is to determine if stress imposed on the adult female during the ovarian growth phase (which elevates maternal plasma cortisol levels and thus elevates egg cortisol levels) has an impact on the stress response of the embryos reared from these eggs. In the study, the maternal stress response will be simulated by immersing newly stripped eggs in cortisol enriched ovarian fluid to increase oocyte cortisol content. In addition, the study examines if environmental contaminants (endocrine mimics, in this case xenoestrogens) that are transferred to the eggs from the maternal circulation also affect the stress response in the embryos; freshly stripped oocytes will be immersed in xenoestrogen-enriched ovarian fluid to simulate the maternal transfer.

(continued)

In addition to the stress response, the study examines the possible effects of cortisol and xenoestrogen on the embryo's ability to metabolize steroid hormones from its tissues. Previous studies have shown early embryos to be able to biotransform steroid hormones that enter the oocyte from the maternal circulation during ovarian growth; all the major classes of ovarian steroids can be metabolized. One possible route for the action of xenobiotics is the impairment of this ability to biotransform and clear these maternal steroids. An in vitro test will be applied to examine the rate of clearance of pregnenolone (P5) from body tissues of embryos in each of the treatment groups (only the embryo stages will be examined).treated ovarian fluid.

Outcomes of the study:

- The study will provide information needed to ensure that handling practices applied to embryos will have the least impact on the animals during key developmental periods.
- The study will also provide, for the first time, concrete information about the stress sensitivity of different embryo stages.
- The study will provide information about the effects of maternal stress episodes and maternally transferred xenobiotics on the stress susceptibility of the offspring.
- The study will provide information about the possible impact of stress and xenoestrogens on embryo steroid clearance capacity.

For Additional Information:

Professor Richard Moccia
Aquaculture Coordinator
Office of Research
University of Guelph, Guelph,
Ontario
Canada N1G 4Y2
Tel. 519-826-3800
Tel. 519-824-4120 ext 52616
Email: rmoccia@uoguelph.ca

Mr. Michael Burke
Station Manager
Alma Aquaculture Research Station
6957 Eighth Line West, R.R. #1, Elora,
Ontario
Canada N0B 1S0
Tel. 519-669-5411
Fax. 519-669-5412
Email: aars2@hsfx.ca

Table 1. Research Projects Conducted at the Alma Aquaculture Research Station: 2007-2008.

Project Number	Researcher(s)	Research Title Fish Species	No. Fish at Start	Location Tanks	A.U.P. #	Duration
ARS-75	Dr. Laura McKay/Dr. Ian McMillan Dept. Animal & Poultry Science Ontario Agriculture College	Strategies for the genetic improvement of salmonids for aquaculture. **Rainbow Trout**	own stock (RT00) own stock (RT01) own stock (RT02) own stock (RT03) own stock (RT03)	I-Lab incubator x 1 A-Lab 0.7 m x 24 1.0 m x 16	99R042 03R030	02/00 ↓ 05/07
ARS-101 **ACTIVE**	Dr. Roy Danzmann/ Dr. M. Ferguson Dept. Zoology College of Biological Sciences	Genetic studies of growth, maturation, spawn timing, stress resistance ad family relatedness in salmonid fishes. **Rainbow Trout**		I-Lab incubator x 2 A-Lab 0.7 m x 19	00R044 04R024 08R069	10/03 ↓
ARS-102	Dr. Laura McKay/Dr. Ian McMillan Dept. Animal & Poultry Science Ontario Agriculture College	Improvement of rainbow trout strains using quantitative genetics. b - production of RT06 stock for commercial distribution **Rainbow Trout**	5,700 eggs 12 ♂♂ (RT99) own stock (RT03) own stock (RT06)	I-Lab incubator x 1 A-Lab 0.7 m x 24 1.0 m x 16	03R030	10/03 ↓ 05/07
ARS-119 **ACTIVE**	Dr. Roy Danzmann/ Dr. M. Ferguson Integrative Biology College of Biological Sciences	Evolutionary genetics of Arctic charr. **Arctic Charr**	7,057	I-Lab incubator x 1 H-Lab trough x 5	04R024 08R069	02/06 ↓
ARS-120 **ACTIVE**	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. **Rainbow Trout**	19,600 eggs resulting in 8,673 fry	B-Lab 2.0m x 20	McMaster 06-12-65 07R079	11/06 ↓
ARS-121	Civil and Environmental Engineering	Surface water quality modelling considering riperian cover	discharge water	WWTC and pond discharge	NA	06/06 ↓ 03/07

Project Number	Researcher(s)	Research Title Fish Species	No. Fish at Start	Location Tanks	A.U.P. #	Duration
ARS-122	Richard Moccia/Jen Campbell Dept. Animal Poultry Science Ontario Agriculture College	Comparing two commercial diets for tilapia growout. **Nile Tilapia**	1,198	QR1 12 tanks	04G004	10/06 ↓ 11/07
ARS-123	AARS Office of Research	Evaluation of different commercial starter feeds for rainbow trout (*Oncorhynchus mykiss*). **Rainbow Trout**	5,100	C-Lab 0.7 m x 12 1.0 m x 12	04G004	12/06 ↓ 06/07
ARS-124 **ACTIVE**	Dr. John Leatherland/Neel Aluru Dept. Biomedical Sciences Ontario Veterinary College, U of Guelph Dept. of Biology University of Waterloo	Effect of estrogenic compounds on gene expression in early embryogenesis using microarray analysis: Xenoestrogens. **Rainbow Trout**	1,700 fertilized eggs resulting in the use of 821 fry 5,500 fertilized eggs resulting in the use of xxx fry	I-Lab incubator x 2 H-Lab trough x 3 C-Lab 0.7 x 3	05R141 08R076 & 04R091	10/06 ↓
ARS-125	Richard Moccia/ Martin Feeds Mills Dept. Animal Poultry Science Ontario Agriculture College	Evaluation of alternative energy levels and fat sources in rainbow trout feeds. **Rainbow Trout**	1,650	B-Lab 2.0 m x 21	07R092	05/07 ↓ 10/07
ARS-126	Dr. G. Van Der Kraak/ Dr. J. Sherry Integrative Biology, CBS, Uof Guelph and Environment Canada (CCIW)	Use of fish feeding experiments to study the transmission of estrogenic effects through the food chain to rainbow trout. **Rainbow Trout**	200	A-Lab 2.0 m x 1	07R028	08/07 ↓ 02/08
ARS-127 **ACTIVE**	Dr. John Leatherland/Mao Li Dept. Biomedical Sciences Ontario Veterinary College	Evaluation of stress response in rainbow trout embryos at different developmental stages. **Rainbow Trout**	16,500 fertilized eggs resulting in the use of xxxx fry	A-Lab 0.7 m x 9 1.0 m x 15	07R108	09/07 ↓

Project Number	Researcher(s)	Research Title Fish Species	No. Fish at Start	Location Tanks	A.U.P. #	Duration
ARS-128	Dr. Roy Danzmann/ Dr. M. Ferguson Integrative Biology College of Biological Sciences	Evolutionary genetics of rainbow trout: crosses using domestic rainbow trout and Idaho rainbow trout. **Rainbow Trout**	3,200 fry	I-Lab incubator x 2	04R024 08R069	10/07 ↓ 07/08
ARS-129 **ACTIVE**	AARS Office of Research	Growth of McKay spring spawning rainbow trout (*Oncorhynchus mykiss*). **Rainbow Trout**	3,600	Hatchery 0.7 m x 12 C-Lab 1.0 m x 12	04G004	04/08 ↓
ARS-130	Dr. Roy Danzmann/ Evan Timusk Integrative Biology College of Biological Sciences	QTL for time to hatch in Arctic charr. **Arctic Charr**	15,000 fertilized eggs	I-Lab incubator x 1	04R024	11/07 ↓ 02/08
ARS-131 **ACTIVE**	Dr. John Leatherland/Neel Aluru Dept. Biomedical Sciences Ontario Veterinary College, U of Guelph Dept. of Biology University of Waterloo	Effect of stress on reproduction in rainbow trout. **Rainbow Trout**	30	B-Lab 2.0 m x 2	08R076	07/08 ↓
ARS-132 **ACTIVE**	Dr. Roy Danzmann Integrative Biology College of Biological Sciences	Broodstock development of Spring Valley rainbow trout. **Rainbow Trout**	11,500 fertilized eggs from Lyndon Fish Farms resulting in the use of xxxx fry	I-Lab incubator x 1	08R069	09/08 ↓
ARS-133 **ACTIVE**	Dr. John Leatherland/Mao Li Dept. Biomedical Sciences Ontario Veterinary College	Evaluation of stress response in rainbow trout embryos: effect of two different incubation temperatures. **Rainbow Trout**	18,000 fertilized eggs	I-Lab incubator x 1	07R108	09/08 ↓
ARS-134 **ACTIVE**	Dr. John Leatherland/Mao Li Dept. Biomedical Sciences Ontario Veterinary College	Evaluation of stress response in rainbow trout embryos: maternal effects. **Rainbow Trout**	18,000 fertilized eggs	I-Lab incubator x 1	07R108	09/08 ↓