2015 ANNUAL REPORT & MEETING

Lake Superior State University's Aquatic Research Laboratory & Michigan Department of Natural Resources

LAKE SUPERIOR STATE UNIVERSITY

DEPARTMENT OF NATURAL RESOURCES · DNR · MICHIGAN

Cloverland ELECTRIC COOPERATIVE

Lighthouse.Net

TABLE OF CONTENTS

2015 ANNUAL ARL-MDNR MEETING 3

Agenda 3

Optional Evening Activities 3

INVITED PARTICIPANTS 4

ACKNOWLEDGMENTS 7

HATCHERY OPERATIONS 7

Stocking & Disease Testing 7

Sub-adult Rearing and Use for Education and Research 9

Broodstock Collection, Disease Testing, Gamete Collection, and Egg Treatments 10

Concentration of Thiamine in Eggs of Chinook and Coho Salmon 15

RESEARCH ACTIVITIES 16

Grants & Contracts 16

Peer-reviewed Publications 17

Presentations 24

SENIOR THESIS ABSTRACTS 25

APPENDICES 32

2015 ANNUAL ARL-MDNR MEETING

12:00 PM – 4:30 PM
June 2, 2015
Anchor Room, Cisler Center, LSSU

Agenda

12:00-1:00	Welcome, introductions, & lunch (provided)
1:00-1:30	MDNR update – Aaron Switzer
1:30-1:40	Hatchery operations – Roger Greil ATS planned stocking 2015 ATS rearing 2014-2015 ATS brood stock netting report 2014
1:40-1:50	Fish Disease Lab update
1:50-3:00	Research activities – LSSU faculty & students
3:00-3:10	Break
3:10-3:20	CFRE update
3:20-4:20	Discussion of future research directions
4:20-4:30	Other business
4:30	Adjourn

Optional Evening Activities

6:00	Dinner at The Antlers (at own expense)
9:00-10:00	Open house at ARL
10:00	Atlantic Salmon release!

INVITED PARTICIPANTS

Edward Baker
Marquette Research Station Mgr
Fisheries Division, MDNR
Marquette, MI 49855
bakere1@michigan.gov
(906) 249-1611

David Borgeson
Northern Lake Huron Unit Supervisor
Fisheries Division, MDNR
Gaylord, MI 49735
borgesond@michigan.gov
(989) 732-3541 Ext. 5070

Dave Caroffino
Fisheries Biologist
Fisheries Division,MDNR
Charlevoix, MI 49720
caroffinod@michigan.gov
(231) 547-2914

Dave Clapp
Charlevoix Research Station Manager
Fisheries Division, MDNR
Charlevoix, MI 49720
clappd@michigan.gov
(231) 547-2914

Randy Claramunt
Research Biologist
Fisheries Division, MDNR
Charlevoix, MI 49720
claramuntr@michigan.gov
(231) 547-2914

Jim Dexter
Fisheries Division Chief, MDNR
Lansing, MI 48909
dexterj1@michigan.gov
(517) 373-3375

Ed Eisch
Fish Production Manger
Fisheries Division, MDNR
eische@michigan.gov
(231) 347-4689

Randy Espinoza
Thompson Hatchery Manager
Fisheries Division, MDNR
espinozar@michigan.gov
(906) 341-5587

Barbara Evans
Professor, Biology
Lake Superior State University
Sault Ste. Marie, MI 49783
bevans@lssu.edu
(906) 635-2164

Dave Fielder
Fisheries Research Biologist, MDNR
Alpena Great Lakes Fisheries Station
Alpena, MI 49707
fielderd@michigan.gov
(989) 356-3232

Roger Greil
Lab Manager of the ARL
Lake Superior State University
Sault Ste. Marie, MI 49783
rgreil@lssu.edu
(906) 635-1949

Brandon Gerig
PhD Student
Department of Biological Sciences
University of Notre Dame
bgerig@lssu.edu

Sheri Glowinski
Asst. Professor, Biology
Lake Superior State University
Sault Ste. Marie, MI 49783
sglowinski@lssu.edu
(906) 635-2147

Neal Godby
Fisheries Biologist
Northern Lake Huron Management Unit
Fisheries Division, MDNR
Gaylord, MI 49735
godbyn@michigan.gov
(989) 732-3541, ext. 5071

Roger Greil
Manager, ARL
Lake Superior State University
650 W. Easterday Ave.
Sault Ste. Marie, MI 49783
rgreil@lssu.edu
(906)635-1949

Todd Grischke
Lake Huron Basin Coordinator
MDNR Fisheries Division
PO Box 30446
Lansing, MI 48909
grischket@michigan.gov
(517) 373-6762

Kevin Kapuscinski
Asst. Prof. & Co-Director of ARL
Lake Superior State University
Sault Ste. Marie, MI 49783
kkapuscinski@lssu.edu
(906) 635-2093

Barb Keller
Dean
Lake Superior State University
650 W. Easterday Ave.
Sault Ste. Marie, MI 49783
bkeller@lssu.edu
(906) 635-2267

Megan Kelly
Asst. Professor, Environmental Science
Lake Superior State University
Sault Ste. Marie, MI 49783
mkelly6@lssu.edu
(906) 635-2894

Nancy Kirkpatrick
Dept. Chair and Professor of Biology
Lake Superior State University
650 W. Easterday Ave.
Sault Ste. Marie, MI 49783
nkirkpatrick@lssu.edu
(906) 635-2894

Cory K. Kovacs
Fisheries Biologist
Eastern Lake Superior Management
Newberry Operations Service Center
5100 State Highway M-123
Newberry, MI 49868
kovacsc@michigan.gov
(906)-293-5131

Jun Li
Assistant Professor of Biology
Lake Superior State University
650 W. Easterday Ave.
Sault Ste. Marie, MI 49783
jli@lssu.edu
(906) 635-2281

Ashley Moerke
Professor & Co-Director of ARL
Lake Superior State University
650 W. Easterday Ave.
Sault Ste. Marie, MI 49783
amoerke@lssu.edu
(906) 635-2153

John Roese
Professor & Interim Co-Director of ARL
Lake Superior State University
Sault Ste. Marie, MI 49783
jroese@lssu.edu
(906) 635-2648

Philip J. Schneeberger
Lake Superior Basin Coordinator,
MDNR
Marquette Fisheries Res. Station 484
Marquette, MI 49855
schneebergerp@michigan.gov
(906) 249-1611

Steven Scott
Fisheries Biologist
Fisheries Division, MDNR
Newberry, MI 49868
scotts4@michigan.gov
(906) 293-5131, ext. 4070

Aaron Switzer
NLP Area Hatchery Manager
Beulah, MI 49617
switzera1@michigan.gov
(231) 325-4611

Jan P. VanAmberg
UP Hatchery Area Manager
Marquette and Thompson SFH
Manistique, MI 49854
vanambej@michigan.gov
(906) 341-5587 ext 202

Gary Whelan
Research Program Manager
MI DNR Fisheries Division
Lansing, MI 48909
whelang@michigan.gov
(517) 373-6948

Martha Wolgamood
Wolf Lake State Fish Hatchery Mgr
Mattawan, MI 49071
wolgamoodm@michigan.gov
(269) 668-2696, ext 25

Derek Wright
Assistant Professor of Chemistry
Lake Superior State University
650 W. Easterday Ave.
Sault Ste. Marie, MI 49783
dwright1@lssu.edu
(906) 635-2628

Greg Zimmerman
Professor of Biology
Lake Superior State University
650 W. Easterday Ave.
Sault Ste. Marie, MI 49783
Gzimmerman@lssu.edu
(906) 635-2470

Troy Zorn
Research Biology Specialist
MDNR Fisheries Division
Marquette, MI 49855
Zornt@michigan.gov
(906) 249-1611

ACKNOWLEDGMENTS

We are extremely grateful to Cloverland Electric Cooperative for providing space within their facility and electricity, which are essential to our hatchery operations. We are also appreciative of Lighthouse.net for graciously providing broadband internet service for broadcasting our fishcam (http://www.lssu.edu/arl/fishcam.php). The Michigan Department of Natural Resources supplies the feed necessary to sustain our hatchery operation, funds all disease testing, and provides expertise and additional supplies as needed. We also acknowledge John Roese for serving as interim co-director and overseeing newsletter and website publications, as well as the numerous student employees and volunteers that contribute to the success of the Aquatic Research Laboratory.

HATCHERY OPERATIONS

Stocking & Disease Testing

A total of 40,908 age-1 and 9,816 age-0 Atlantic Salmon (*Salmo salar*) that were reared at Lake Superior State University's Aquatic Research Laboratory (ARL) were stocked into Michigan waters in 2014 (Table 1). Age-1 fish (lot P-ATS-LL-W-12-SM-LS-LS) received a left pelvic fin clip, averaged 181 mm in total length, and were stocked into the St. Marys River on 12 June 2014. Survival of these age-1 fish was about 80% from the eyed-egg stage until the time of stocking, similar to the survival rate observed for the previous five year-classes (Appendix 1). Age-0 fish (lot P-ATS-LL-W-13-SM-LS-LS) did not receive a fin clip, averaged 136 mm in total length, and were transported and stocked into Torch Lake (Antrim County) by Michigan Department of Natural Resources (MDNR) personnel on 27 October 2014. Sixty age-1 fish and 60 age-0 fish were tested for the presence of *Aeromonas salmonicida*, Bacterial Kidney Disease (BKD), Infectious Hematopoietic Necrosis Virus, Infectious Pancreatic Necrosis Virus, Viral Hemorrhagic Septicemia Virus, and *Yersinia ruckeri* by Michigan State University (MSU) personnel prior to stocking; age-0 fish were also tested for *Myxobolus cerebralis*. All fish tested negative for pathogens (Appendices 2 & 3).

Table 1. Number and mean total length of age-1 Atlantic Salmon stocked during 1987-2014. Stocking typically occurred between mid-May and mid-June of each year.

Year	# stocked	Mean total length
1987	19,000	189
1988	12,751	196
1989	19,966	170
1990	31,702	131
1991	8,367	127
1992	8,048	179
1993	47,716	191
1994	20,350	174
1995	29,060	185
1996	33,756	183
1997	43,373	150
1998	41,721	142
1999	49,818	181
2000	46,220	179
2001	35,909	172
2002	29,313	154
2003	54,743	180
2004	24,811	211*
2005	29,665	201*
2006	38,032	186
2007	20,437	178
2008	29,373	186
2009	28,400	185
2010	26,301	184
2011	31,100	200
2012	35,230	189
2013	35,000	196
2014	40,908	181

*Fish were held until August because they were treated for Bacterial Kidney Disease and furunculosis

Sub-adult Rearing and Use for Education and Research

A total of 42,992 age-0 Atlantic Salmon were moved into fry raceways in early March of 2014 at the time of early feeding and reared in heated water until June. About 40,800 fry survived through early June of 2014, at which time they were transferred into large raceways and were reared in ambient river water. On average, these fish grew about 125 mm in total length during March-November 2014 (Table 2). A total of 29,907 age-1 Atlantic Salmon were fin clipped in May 2015 and will and be stocked into the St. Marys River on 2 June 2015. During May 2014-May 2015, a total of 1,051 Atlantic Salmon of various life stages were used and sacrificed for education and research activities (Table 3).

Table 2. Biweekly rearing data of age-0 Atlantic Salmon reared in heated water in 2014 (lot: P-ATS-LL-W-13-SM-LS-LS). The number of fish initially moved into fry raceways was 42,992.

Mid date of biweekly summary	Ending # of fish	Mean temp (°C)	Mean length (mm)	Mean biomass (kg)	TUGR (mm/C)	FCR	Biweekly mortality (%)	Avg. density (kg/m^3)	Flow (L/min)
24-Mar	42,692	9.54	29.24	10.24	0.019	1.23	0.70	5.23	65
7-Apr	41,935	8.46	30.71	11.83	0.004	9.30	1.77	6.03	98
21-Apr	41,234	9.06	32.16	13.40	0.020	1.87	1.67	6.84	98
5-May	40,906	9.84	35.55	18.05	0.031	1.21	0.80	9.21	131
19-May	40,835	10.56	40.63	27.23	0.040	1.24	0.17	13.89	224
2-Jun	40,773	10.19	47.18	43.37	0.051	1.07	0.15	22.13	224
16-Jun	40,760	8.74	54.95	69.87	0.068	0.70	0.03	4.81	484
30-Jun	40,667	9.44	63.72	111.01	0.070	0.74	0.23	3.82	1,452
14-Jul	40,569	13.55	73.24	171.53	0.052	0.85	0.24	5.91	1,452
28-Jul	40,440	14.89	83.28	256.07	0.049	1.07	0.32	8.82	1,936
11-Aug	40,322	17.47	93.61	368.54	0.043	1.28	0.29	12.69	1,936
25-Aug	40,204	15.29	104.21	514.61	0.050	1.14	0.29	11.15	3,076
8-Sep	40,137	15.59	114.41	688.77	0.044	1.40	0.17	14.93	3,076
22-Sep	40,115	13.84	123.95	884.68	0.049	1.18	0.05	19.17	3,076
6-Oct	40,100	11.60	133.07	1,105.03	0.054	1.14	0.04	23.95	3,076
20-Oct	30,267	9.98	141.29	1,158.81	0.055	0.92	24.52	25.12	3,076
3-Nov	30,199	7.82	148.39	1,172.92	0.059	0.85	0.22	37.09	2,108
17-Nov	30,188	4.09	154.12	1,319.42	0.087	0.75	0.04	41.73	2,108

*High densities were because fish were combined in raceways to accommodate brood stock collection

Table 3. Summary data of 1,051 Atlantic Salmon used and sacrificed for education and research activities during May 2014-April 2015.

Date	Number	Size category	Use
28-May-14	21	Yearlings	Quality Assessment Dissection
29-May-14	5	Yearlings	Quality Assessment Dissection
25-Aug-14	60	Fall fingerlings	MSU-health inspection
2-Sep-14	6	Fall fingerlings	Dr. Li-class
9-Sep-14	25	Fall fingerlings	Dr. Li-class and Luke Bradburn-thesis
11-Sep-14	5	Fall fingerlings	Dr. Li-class
16-Sep-14	5	Fall fingerlings	Dr. Li-class
30-Sep-14	5	Fall fingerlings	Dr. Li-class
5-Feb-15	250	Sac fry	Dr. Evans-class
9-Feb-15	30	Sac fry	Dr. Evans-class
23-Feb-15	60	Yearlings	MSU-health inspection
19-Mar-15	12	Yearlings	JKL-school dissections
26-Mar-15	15	Yearlings	Dr. Kapuscinski-class
9-Apr-15	150	Swim-up fry	Dr. Li-feeding study
13-Apr-15	2	Yearlings	Jeff Panich-conservation officer school
8-May-15	400	Fry	Dr. Li-feeding study
20-May-15	120	Yearlings	Dr. Li-feeding study

Broodstock Collection, Disease Testing, Gamete Collection, and Egg Treatments

Personnel from the ARL and MDNR collected returning adult Atlantic Salmon for broodstock on 30-31 October 2014. Fish were captured from the St. Marys River at the Cloverland Hydroelectric Plant using a gill net that covered the opening of the first inactive turbine tailrace on the east side of the closest active turbine. The gill net used was 15.2 m (50 ft) long, 3.4 m (10 ft) high, with a 10.2 cm (4 in) stretch mesh. The net was continuously observed until a fish became entangled in the net, at which time the net was lifted and the fish was immediately removed. Each fish was identified to species, measured for length and weight, and examined to determine sex, maturity (ripe or unripe), presence of fin clips, tags, and Sea Lamprey (*Petromyzon marinus*) scars. After examination, Atlantic Salmon were retained for subsequent gamete collection in one of two raceways based on sex.

The net was fished from 8:25-13:15 on 30 October (4.83 hr) and from 13:20-14:48 on 31 October (0.97 hr) for a total of 5.8 hr. A total of 225 Atlantic Salmon were collected; 182 on 30 October and 43 on 31 October. The catch rate of Atlantic Salmon was about 39 fish per hr, the highest on record (Table 4). The 113 male fish sampled averaged 602 mm in total length and 2.15 kg in weight, whereas the 112 females sampled averaged 612 mm in total length and 2.55 kg in weight. Brood fish were age 2-5, with 71% of all fish captured being age-2 (Table 5). The average Fulton's condition factor *K* for all fish was 1.02 in 2014, and there was no discernable trend since record-keeping began in 1990 (Figure 1). Data on individual Atlantic Salmon used as

broodstock is presented in Appendix 4. Fourteen of the 225 fish (6.2%) were possibly reared by the MDNR based on the presence of eroded fins and double fin clips (none possessed an adipose fin clip). About 21% of all Atlantic Salmon had at least one Sea Lamprey scar, the second lowest percentage observed since 1990 (Figure 2). Type B, stage IV scars were the most common among fish that had scars (about 42%), and about 72% of all scars were type B, whereas about 28% were type A (Table 6). No species other than Atlantic Salmon were captured during broodstock netting in 2014.

Table 4. Summary data from gill-netting of Atlantic Salmon broodstock from 1990-1994 and 1998-2014.

Year	# of fish	Mean hr/d	# of d	Net size (m^2)	Mean # fish per hr	Mean # fish/d
1990	46	-	23	47	-	2
1991	65	6.5	23	47	0.43	2.83
1992	19	6.7	28	58	0.1	0.68
1993	11	2.5	18	56	0.24	0.61
1994	18	2.6	23	65	0.31	0.78
1998	87	2.6	17	47	1.98	5.12
1999	49	3	26	56	0.63	1.88
2000	105	2.8	18	47	2	5.83
2001	116	2.5	13	47	3.61	8.92
2002	104	2.7	13	56	2.94	8
2003	158	2.8	9	56	6.36	17.56
2004	196	3.1	14	56	4.5	14
2005	210	4.11	6	56	8.52	35
2006	111	2.71	6	56	6.83	18.5
2007	276	2.62	6	56	17.52	46
2008	172	2.79	4	47	15.4	43
2009	140	4.5	3	47	10.37	47
2010	212	4.78	3	47	14.78	70.67
2011	240	4.23	4	47	14.19	42.4
2012	313	1.99	6	47	26.21	52.17
2013	378	3.46	4	47	27.35	94.49
2014	225	2.9	2	47	38.79	112.5

Table 5. Age of Atlantic Salmon captured in fall 2014 broodstock collection efforts.

Age	Type of clip	# of males	# of females	Total # of fish
1	Left pelvic	0	0	0
2	Left pectoral	96	63	159
3	Right pelvic	8	23	31
4	Right pectoral	3	10	13
5	Left pelvic	2	6	8
	None/regenerated	4	10	14
	Grand total	113	112	225

Table 6. Classification of Sea Lamprey scars observed on Atlantic Salmon captured in 2014 broodstock collection efforts. Percentages are based on fish that had scars.

Sex	Scar type	Scar stage	# scars	Percent
Male	A	I	1	1.9
	A	II	0	0.0
	A	III	2	3.8
	A	IV	3	5.7
	Subtotal		6	11.3
	B	I	1	1.9
	B	II	2	3.8
	B	III	1	1.9
	B	IV	4	7.5
	Subtotal		8	15.1
Female	A	I	2	3.8
	A	II	2	3.8
	A	III	2	3.8
	A	IV	3	5.7
	Subtotal		9	17.0
	B	I	3	5.7
	B	II	2	3.8
	B	III	7	13.2
	B	IV	18	34.0
	Subtotal		30	56.6
	Grand total of A		15	28.3
	Grand total of B		38	71.7

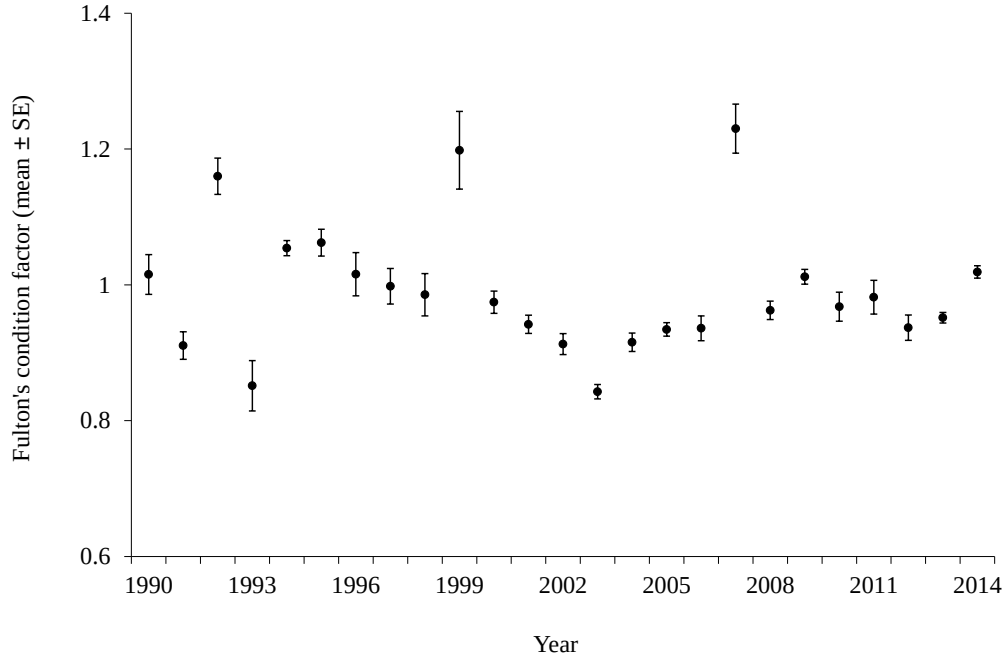


Figure 1. Annual mean (±SE) Fulton's condition factor *K* for Atlantic Salmon netted during 1990-2014.

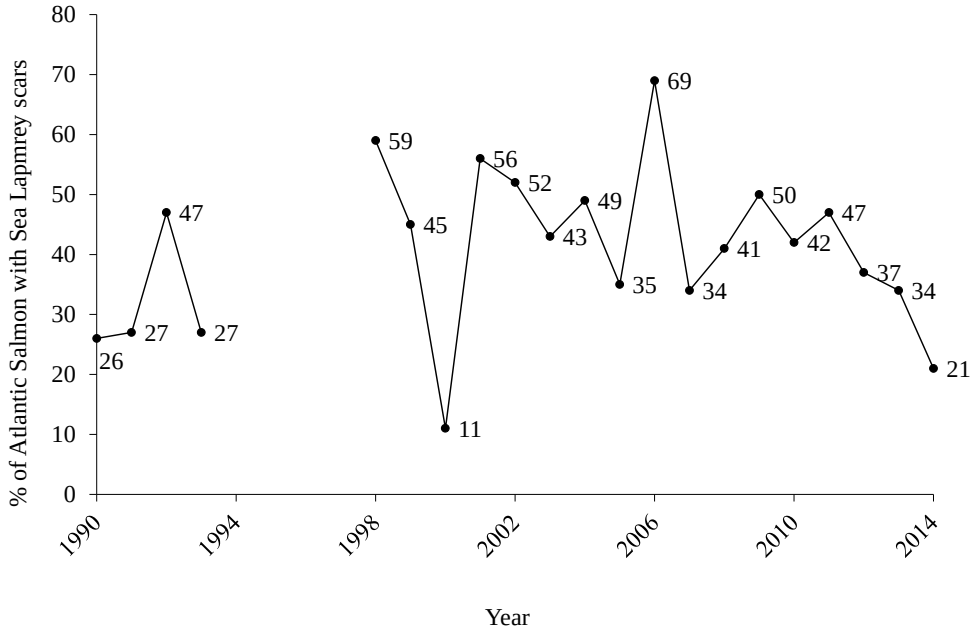


Figure 2. Percent of Atlantic Salmon broodstock that had at least one lamprey scar during 1990-1993 and 1998-2014.

Fish were held in raceways for at least one week prior to gamete collection, which occurred on 5, 10, 13, and 17 November 2014. A 1 female: 1 male crossing scheme and the dry method were used during artificial spawning of 104 pairs of Atlantic Salmon. Fertilized eggs from each cross were isolated in buckets until testing was completed for BKD by LSSU's Fish Disease Laboratory (usually within 24 hr). After gametes from all 208 Atlantic Salmon brood fish tested negative for BKD (Appendix 5), fertilized eggs were mixed together according to the date of collection and placed into egg trays. Personnel from MSU arrived on 11 November 2014 and completed a broodstock health inspection for the presence of *Aeromonas salmonicida*, BKD, Infectious Hematopoietic Necrosis Virus, Infectious Pancreatic Necrosis Virus, *Myxobolus cerebralis*, Viral Hemorrhagic Septicemia Virus, and *Yersinia ruckeri* on 60 adult fish that had been previously tested for BKD by LSSU. One fish tested positive for *Aeromonas salmonicida*; all other tests were negative (Appendix 6). In addition, MSU personnel tested the gametes from these 60 broodstock fish for BKD, Infectious Hematopoietic Necrosis Virus, Infectious Pancreatic Necrosis Virus, *Oncorhynchus masu* Virus, and Viral Hemorrhagic Septicemia Virus; all fish tested negative (Appendix 6).

One female Atlantic Salmon captured during broodstock netting was previously captured on 27 August 2014 by the Ontario Ministry of Natural Resources and implanted with an ultrasonic transmitter in the lower abdomen and a yellow Floy tag behind the dorsal fin. Eggs were taken from this fish and it was released back into the St. Marys River. In addition, two male and two female Atlantic Salmon captured during broodstock netting were taken by personnel from the Platte River State Fish Hatchery for display at their visitor's center.

A total of 372,872 Atlantic Salmon eggs were collected in 2014 (Table 7). All fertilized eggs were treated according to MDNR protocols, which included saline baths, erythromycin treatment, and iodine disinfectant. Dosages for each treatment are described in Appendix 8. On 30 December 2014, 322,000 eyed eggs were transported by MDNR personnel to the Platte River State Fish Hatchery.

After hatching, sac fry at the ARL were given a static bath of 2,000 ppm thiamine for ≥ 4 hr. We used one tote per egg tray of sac fry for the static bath. For each tote, 12 L of filtered river water was added and initial pH was measured. Next, 24 g of thiamine was added and mixed, and then pH was measured again. Initial pH readings ranged from 7.2-7.7, whereas pH ranged from 3.1-4.4 after thiamine was added. Baking soda was added to the thiamine bath (ranging from 37-58 g) to get the pH back up to initial levels, and then air stones were placed in the totes. Finally, sac fry from a single egg tray (about 2,500 fry per tray) were added to each tote, a lid was placed over them, and the fry were checked every hr. Thiamine treatments for eggs collected in 2014 occurred on 11, 12, 16, 17 and 20 January 2015. Two egg trays were treated twice due to the pale appearance of the sac-fry after the first treatment. The duration of static baths averaged 6.8 hr, and the sac fry appeared healthy after the treatments.

Table 7. Summary data of egg collection efforts in 2014.

Date	# of pairs	Mean # of eggs/fish	Total # eggs
6-Nov-14	23	3,773	86,774
10-Nov-14	26	3,229	83,965
13-Nov-14	36	3,344	120,386
17-Nov-14	19	4,302	81,747
Grand total	104	3,662	372,872

Concentration of Thiamine in Eggs of Chinook and Coho Salmon

Concentrations of thiamine in eggs of 30 Chinook Salmon (*Oncorhynchus tshawytscha*) and 30 Coho Salmon (*Oncorhynchus kisutch*) collected by the MDNR in 2013 were quantified at the LSSU Fish Disease Lab by Dr. Jun Li. The mean concentration of total thiamine in eggs was 4.33 nMol / g of egg (SE = 0.43, range = 1.58-13.81) for Chinook Salmon and 6.01 nMol / g of egg (SE = 0.34, range = 3.48-10.47) for Coho Salmon (Table 8). All eggs tested contained concentrations of thiamine above 1 nMol / g of egg and therefore were not considered to be at risk for Early Mortality Syndrome.

Table 8. Summary data from tests of concentrations of thiamine in eggs from 30 Chinook Salmon and 30 Coho Salmon collected by the MDNR in 2013.

Species		Total phosphorylated thiamine per gram egg (nMol / g)	Total nonphosphorylated thiamine per gram egg (nMol / g)	Total thiamine per gram egg (nMol / g)
Chinook Salmon	Mean	3.18	1.15	4.33
	SE	0.32	0.14	0.43
	Minimum	0.79	0.51	1.58
	Maximum	10.24	3.57	13.81
Coho Salmon	Mean	2.83	3.18	6.01
	SE	0.14	0.23	0.34
	Minimum	1.36	2.12	3.48
	Maximum	4.35	6.27	10.47

RESEARCH ACTIVITIES

Grants & Contracts

Conservation of native fish communities in tributaries to the Great Lakes: Predicting the impacts of contaminants delivered by spawning Pacific salmon. Great Lakes Fishery Trust (2013-2015; Chaloner , D., Lamberti, G., **Moerke, A.**, Janetski, D., and R. Rediske)

Use of Fin Rays for Estimating Age of Muskellunge. Hugh C. Becker Foundation (2015-2016; **Crane, D.P.**, Isermann, D.A., Simonson, T.D., Kampa, J.M, and **K.L. Kapuscinski**)

Engaging active STEM Education through aquaponic. Michigan STEM Partnership (2014-2015; **B. Evans, S. Glowinksi**, P. Weber, **J. Li, R. Greil**, and R. Wilhems)

Re-emergence of epizootic epitheliotropic disease virus: potential effects and development of improved diagnostics & control measures, Great Lakes Fishery Trust (2014-2016; Faisal, M., and **J. Li**)

Development and management of St. Lawrence River fisheries. New York State Department of Environmental Conservation (2013-2016; Farrell, J.M., Whipps, C.M., and **K.L. Kapuscinski**)

Continuing work toward removal of BUIs in the St. Marys River AOC. Great Lakes Commission (2014-2015; **Glowinski, S.**, and **A. Moerke**)

Quantifying relationships between fish assemblages and nearshore habitat characteristics of the Niagara River. Niagara River Greenway Ecological Fund (2013-2016; **Kapuscinski, K.L.**, and **D.P. Crane**)

Thiamine analysis of Coho and Chinook Salmon eggs. Michigan Department of Natural Resources (2014**; J. Li**)

Bacterial Kidney Disease analysis of Atlantic Salmon. Michigan Department of Natural Resources (2014**; J. Li**)

Effect of dietary beta-glucan derived from algae on growth performance, disease resistance and immune response in Atlantic salmon. Algal Scientific Company (2014-2015; **Li, J.**, and **B. Evans**)

Furthering capacity to maintain high quality coastal wetlands in northern Michigan. US Environmental Protection Agency (2014-2015; Lishawa, S., Tuchman, N., Treering, D., **Zimmerman**, **G.,** and others)

Ecological responses to restoration of flow to the Little Rapids area. Great Lakes Commission and National Oceanic and Atmospheric Administration (2014-2016; **A. Moerke**).

Cooperative agreement for Sea Lamprey monitoring. US Fish and Wildlife Service (2015-2017; **Moerke, A.**, and **R. Greil**)

State of Michigan's Eurasian Watermilfoil biocontrol pilot study. Michigan Department of Environmental Quality (2014-2016; **Moerke, A.** and **G. Zimmerman)**

Monitoring fish movement and fish condition in tributaries of Whitefish Bay. Bureau of Indian Affairs-Great Lakes Restoration Initiative Funds (2015; Ripple, P., Zomer, F., **Moerke, A.**, **Kapuscinski, K.**, and **J. Li**)

Delineation of natural boundaries of muskellunge in the Great Lakes and the effects of supplementation on genetic integrity of remnant stocks. Great Lakes Fishery Commission (2013-2014; Sloss, B.L., Hanchin, P., Farrell, J.M., **Kapuscinski, K.L.**, Miller, L., Scribner, K., and C. Wilson)

GLIC Implementing Great Lakes coastal wetland monitoring. US Environmental Protection Agency, Great Lakes Restoration Initiative (2011-2015; Uzarski, D., Brady, V., Cooper, M., Albert, D., Ciborowski, J., Danz, N., Gathman, J., Grabas, G., Hokansen, A., Howe, B., Lamberti, G., **Moerke, A.**, Ruetz, C., Steinman, A., Tozer, D., and D. Wilcox)

Peer-reviewed Publications

Crane, D.P., Farrell, J.M., and **K.L. Kapuscinski**. 2014. Predicting muskellunge spawning habitat using a Maxent-based approach to model selection. Journal of Great Lakes Research 40:325-335. DOI: 10.1016/j.jglr.2014.02.016

Conserving and restoring muskellunge (*Esox masquinongy*) spawning habitat are essential for maintaining self-sustaining populations. A Maxent model was developed based on presence and background data to investigate the relationship between the occurrence of spawning muskellunge and habitat features in the upper Niagara River. Muskellunge spawning points (n = 15) were determined by direct observation of spawning pairs. Model inputs were based on micro-habitat features collected at each spawning point and a sample of 250 background habitat points. The full model was reduced to a four variable model to remove uninformative variables and reduce overfitting and redundancy. Model performance was evaluated based on the mean test gain of cross-validated models (n= 15). Model outputs identified aquatic macrophyte/algae coverage as the most important habitat feature at spawning locations. The relative probability of muskellunge spawning increased with the percent rank of total aquatic macrophyte/algae coverage, water velocity, and water depth and it was highest at points with muddy-sand to sand substrates. Mean test gain (0.68; SE = 0.52) of the cross-validated models indicated that the likelihood of an average muskellunge spawning point was nearly two times greater than an average background point. Results from this research advance our knowledge of muskellunge reproductive ecology, while providing scientists and managers with quantitative measures to guide habitat conservation and restoration.

Farrell, J.M., **Kapuscinski, K.L.**, and H.B. Underwood. 2014. Fine scale habitat use by age-1 stocked muskellunge and wild northern pike in an upper St. Lawrence River bay. Journal of Great Lakes Research 40:148-153. DOI: 10.1016/j.jglr.2014.01.014

Radio telemetry of stocked muskellunge (n=6) and wild northern pike (n=6) was used to track late summer and fall movements from a common release point in a known shared nursery bay to test the hypothesis that age-1 northern pike and stocked muskellunge segregate and have different habitat affinities. Water depth, temperature, substrate and aquatic vegetation variables were estimated for each muskellunge (n = 103) and northern pike (n=131) position and nested ANOVA comparisons by species indicated differences in habitat use. Muskellunge exhibited a greater displacement from the release point and used habitat in shallower water depths (mean=0.85m, SE= 0.10) than northern pike (mean=1.45 m, SE= 0.08). Both principal components analysis (PCA) and principal components ordination (PCO) were used to interpret underlying gradients relative to fish positions in two-dimensional space. Our analysis indicated that a separation of age-1 northern pike and muskellunge occurred 7 d post-release. This first principal component explained 48% of the variation in habitat use. Northern pike locations were associated with deeper habitats that generally had softer silt substrates and dense submersed vegetation. Muskellunge locations post-acclimation showed greater association with shallower habitats containing firmer sandy and clay substrates and emergent vegetation. The observed differences in habitat use suggest that fine-scale ecological separation occurred between these stocked muskellunge and wild northern pike, but small sample sizes and potential for individual variation limit extension of these conclusions. Further research is needed to determine if these patterns exist between larger samples of fishes over a greater range of habitats.

Guinan, M.E., **Kapuscinski, K.L.**, and M.A. Teece. 2015. Seasonal diet shifts and trophic position of an invasive cyprinid, the rudd *Scardinius erythrophthalmus*, in the upper Niagara River. Aquatic Invasions 10(2): 217-225. Available at: http://dx.doi.org/10.3391/ai.2015.10.2.10

Dietary shifts of invasive rudds *Scardinius erythrophthalmus* (Linnaeus, 1758) and food web structure of the upper Niagara River were examined. Stable isotope analysis (SIA) of liver tissue was used to test the hypothesis that rudds shifted their diets from piscivory during early spring months when macrophyte availability was low towards herbivory when macrophytes were abundant and warmer water temperatures facilitated digestion. Muscle tissue was used to evaluate the trophic position of rudds and other invasive species relative to native species. SIA revealed enriched $\delta^{15}N$ and depleted $\delta^{13}C$ in liver tissue of rudds during early spring months, suggesting a mostly piscivorous diet of pelagic origin when macrophyte availability was low, and depressed $\delta^{15}N$ and elevated $\delta^{13}C$ values during warmer summer months when littoral macrophytes were abundant. Analysis of muscle tissue from late summer indicated that rudds and other invasive fishes, including common carp *Cyprinus carpio* (Linnaeus, 1758) and goldfish *Carassius auratus* (Linnaeus, 1758), had similar trophic positions that may be attributed to their omnivorous feeding strategies. The ability of rudds to shift their diets from feeding on fishes of pelagic origin towards consuming littoral macrophytes is an adaptation that is likely to both facilitate invasion and create novel pathways of nutrient transfer among habitat types. Our results provide an increased understanding of the feeding ecology of the rudd and the role of this invasive species in the food web of the upper Niagara River.

Janetski, D., Chaloner, D., **Moerke**, **A.H.**, Levi, P., and G.A. Lamberti. 2014. Novel environmental conditions alter subsidy and engineering effects by introduced Pacific salmon. Canadian Journal of Fisheries and Aquatic Sciences 71(4):502-513. Available at: http://dx.doi.org/10.1139/cjfas-2013-0292

We demonstrate that the major ecological functions of Pacific salmon (*Oncorhynchus* spp.) can be altered or re-ordered in non-native habitats where environmental conditions differ from native ranges. We compared subsidy and engineering effects of spawning Pacific salmon in six streams within their introduced range (Laurentian Great Lakes) with responses reported in their native range (northern Pacific Rim). Streamwater nutrient responses (i.e., subsidy effects) in Great Lakes streams were generally weak compared with those reported in native streams, whereas disturbance (i.e., engineering effects) was often strong where salmon were abundant. We attribute the relatively weak nutrient response to high background nutrient concentrations and low salmon biomass. In contrast, in Great Lakes streams with high salmon biomass, sediment routing was intense and pervasive and, consequently, benthic biofilm and macroinvertebrate abundance often declined by over 90% during the salmon run. These strong disturbance effects were likely facilitated by the small sediments that typified the Great Lakes streams. Our study provides evidence that salmon effects are context-dependent at much broader spatial scales than has been reported previously.

Kapuscinski, K.L., Farrell, J.M., and M.A. Wilkinson. 2015. Abundance, biomass, and macrophyte consumption by rudd in Buffalo Harbor and the Niagara River, and potential herbivory by grass carp. Journal of Great Lakes Research. Available at: http://dx.doi.org/10.1016/j.jglr.2015.02.006

The invasive rudd and grass carp consume aquatic macrophytes, thereby altering important habitat, creating novel trophic pathways, and adversely affecting indigenous species. We estimated the abundance and biomass of rudd and their consumption of macrophytes in Buffalo Harbor (northeastern Lake Erie) and the upper Niagara River during 2012–2013. We also estimated consumption of macrophytes by hypothetical populations of grass carp equal in biomass to rudd populations. Using mark-recapture methods, we estimated that 2571 (95% CI: 2362–2821) rudd were present in Buffalo Harbor and 142,957 (95% CI: 135,127–151,751) were present in the upper Niagara River. Biomass of rudd was estimated at 1.89 mt (95% CI: 1.73–2.07) in Buffalo Harbor and 100.21 mt (95% CI: 94.72–106.38) in the upper Niagara River. Using observed water temperatures and published consumption rates, we estimated that (1) rudd could have consumed 98 mt of macrophytes in Buffalo Harbor and 5210 mt in the upper Niagara River, and (2) hypothetical populations of grass carp equal in biomass to rudd could consume96 mt of macrophytes in Buffalo Harbor and 4941 mt in the upper Niagara River. Rudd abundance and biomass are substantial in these two waters, and consumption of aquatic macrophytes by rudd may threaten existing aquatic habitat and restoration projects. Annual standardized surveys of fish and macrophyte assemblages would provide data necessary to monitor rudd populations, detect invasion and reproduction by grass carp, and assess habitat changes resulting from herbivory by these two invasive fishes.

Kapuscinski, K.L., Farrell, J.M., S.V. Stehman, G.L. Boyer, D.D. Fernando, M.A. Teece, and T.J. Tschaplinski. 2014. Selective herbivory by an invasive cyprinid, the rudd *Scardinius erythrophthalmus*. Freshwater Biology 59: 2315-2327. DOI: 10.1111/fwb.12433

Herbivory by non-native animals is a problem of growing concern globally, especially for ecosystems where there were few native herbivores or where they have been replaced by non-natives. The rudd (*Scardinius erythrophthalmus*) is an omnivorous cyprinid (native to Europe) now very widespread due to human introductions, yet it is unknown whether the invasive rudd feeds selectively among aquatic macrophyte species common in North America. We tested feeding selectivity by rudd using five species of aquatic macrophytes: *Ceratophyllum demersum*, *Elodea canadensis*, *Najas flexilis*, *Stuckenia pectinata* and *Vallisneria americana*. Fish were presented with known quantities of each macrophyte species in a randomized complete block design, and we measured the mean per cent mass remaining in each case. We also quantified differences in the chemical attributes of the five macrophyte species and determined whether feeding by rudd was related to dry matter content (DMC), per cent C by dry mass (%C), per cent N by dry mass (%N), and the concentrations of total soluble proteins, two organic acids (aconitic and oxalic acid), total soluble phenolic compounds (<1000 Da), nine soluble phenolic metabolites and total phenolic compounds. Rudd fed selectively, with consumption declining in the order *N. flexilis* > *E. canadensis* > *S. pectinata* > *V. americana* > *C. demersum*. Selection was positively related to %C and atomic C : N, but not DMC, %N or concentration of total soluble proteins, contrary to the expectation that rudd would select the most nutritious plants available. The concentration of aconitic acid was greatest in *N. flexilis*, a preferred macrophyte, contrary to the expectation that this compound provides resistance to herbivory. The concentration of oxalic acid, which negatively affects palatability, was highest in *C. demersum*, the least preferred macrophyte. Selection was also positively related to the concentration of total (and some specific) soluble phenolic compounds. The concentrations of caffeic acid, trans-caftaric acid and quercetin were positively related to macrophyte preference by rudd, whereas concentrations of cis-4-O- and trans-4-O-ferulic acid glucoside were negatively related. Selective feeding by rudd (which can be very numerous in North American fresh waters) could evidently alter macrophyte assemblages and hinder attempts to restore plant communities.

Kapuscinski, K.L., Farrell, J.M., Paterson, G., Wilkinson, M.A., Skinner, L.C., and A.J. Gudlewski. 2014. Low concentrations of contaminants in an invasive, omnivorous cyprinid, the rudd (*Scardinius erythrophthalmus*), in a Great Lakes Area of Concern. Bulletin of Environmental Contamination & Toxicology 93(5): 567-573. DOI: 10.1007/s00128-014-1325-3

The invasive, omnivorous rudd *Scardinius erythrophthalmus* is abundant in the upper Niagara River, a Great Lakes Area of Concern. Invasive species can alter trophic and contaminant pathways, but complex ontogenetic and seasonal diet shifts by rudds make it difficult to predict their chemical burdens relative to other fishes. We quantified concentrations of chemical residues in rudds and compared them to six fish species from various trophic levels. Rudds contained low concentrations of total dichlorodiphenyltrichloroethane (mean = 0.02 lg/g), Hg (mean = 0.03 lg/g), and polychlorinated biphenyls (mean = 0.06 lg/g); these concentrations were among the lowest for all species examined. Concentrations of aldrin, heptachlor, hexachlorobenzene, total hexachlorocyclohexanes, mirex, and total chlordanes were less than the

method detection limit for all rudds examined. If reducing rudd biomass is determined to be beneficial, resource managers could consider targeted harvest, given the low concentrations of contaminant in rudds and their susceptibility to capture.

Kelly, M.M., Fleishhacker, N.T., Rearick, D., Arnold, W.A., Schoenfuss, H.L., and P.J. Novak. 2014. Phytoestrogens in the environment, II: microbiological degradation of phytoestrogens and the response of fathead minnows to degradate exposure. Environmental Toxicology and Chemistry 33(3):560-566. Available at: http://dx.doi.org/10.1002/etc.2462

Phytoestrogens are endocrine active compounds derived from plants, including the isoflavones genistein and daidzein, and their methylated derivatives biochanin A and formononetin. These compounds have been detected at the µg/L level in the effluents of plant-processing industries and municipal treatment plants and at the ng/L level in surface waters worldwide. The present study assessed the persistence of genistein and daidzein in natural aquatic systems, specifically riverine samples. Initial concentration, temperature, sample location, and time of sample collection varied. Genistein and daidzein were found to be readily biodegradable at all tested concentrations, at both 10 °C and 20 °C, in samples collected during different seasons, and in samples from 3 different rivers. In addition, organismal responses in larval and sexually mature fathead minnows (*Pimephales promelas*) were quantified following exposure to microbiologically degraded phytoestrogens (genistein, daidzein, and formononetin). Products of the microbiological degradation of parent phytoestrogens did not affect larval survival, growth, or predator avoidance. Female adult fathead minnows exposed to these degradation products produced significantly fewer eggs than those exposed to a control, but no other morphological, physiological, or behavioral changes were observed with male or female minnows. The present research suggests that although phytoestrogens are not likely to persist in aquatic systems, they may pseudo-persist if discharges are continuous; in addition, caution should be exercised with respect to high-concentration effluents because of the potentially antiestrogenic effects of phytoestrogen degradates.

Li, M.F., Sun, L., and **J. Li**. 2015. *Edwardsiella tarda* evades serum killing by preventing complement activation via the alternative pathway. Fish and Shellfish Immunology 43(2):325-329

Edwardsiella tarda is a Gram-negative bacterium with a broad host range that includes a wide variety of farmed fish as well as humans. *E. tarda* has long been known to be able to survive in host serum, but the relevant mechanism is unclear. In this study, we investigated the fundamental question, i.e. whether*E. tarda* activated serum complement or not. We found that (i) when incubated with flounder serum,*E. tarda* exhibited a high survival rate (87.6%), which was slightly but significantly reduced in the presence of Mg^{2+}; (ii) *E. tarda*-incubated serum possessed strong hemolytic activity and bactericidal activity, (iii) compared to the serum incubated with a complement-sensitive laboratory *Escherichia coli* strain, *E. tarda*-incubated serum exhibited much less chemotactic activity, (iv) in contrast to the serum incubated with live*E. tarda*, the serum incubated with heat-inactivated *E. tarda* exhibited no apparent hemolytic capacity. Taken together, these results indicate for the first time that *E. tarda* circumvents serum attack by preventing, to a large extent, complement activation via the alternative pathway, and

that heat-labile surface structures likely play an essential role in the complement evasion of *E. tarda*.

Liu, X.D., Wen, Y., Hu, X.Q., Wang, W., Liang, X.F., **Li, J.**, Vakharia, V., and L. Lin. 2015. Breaking the host range: Mandarin fish was susceptible to a vesiculovirus derived from snakehead fish. Journal of General Virology 96:775-781

Members of the genus *Vesiculovirus*, which belongs to the family*Rhabdoviridae*, can cause great economic loss in fish culture. In the present report, a vesiculovirus [named snakehead fish vesiculovirus (SHVV)] was isolated from diseased hybrid snakehead fish. SHVV shared 94% nucleotide sequence identity at the genomic level with *Siniperca chuatsi* rhabdovirus (SCRV), which infects mandarin fish (*S. chuatsi*). We showed that SHVV was able to replicate and proliferate well in SSN-1 cells, which originate from striped snakehead fish (*Channa striatus*). Furthermore, mandarin fish was susceptible to SHVV by bath exposure, as well as by intraperitoneal injection. The infected fish showed typical clinical signs of rhabdovirus infection, including haemorrhage and oedema. Histopathological analysis revealed that extensive inflammation and necrosis were observed in the spleen, kidney, liver, heart and brain of the moribund mandarin fish. These results will shed new light on the epidemic of vesiculovirus infections among fish.

Rearick, D., Fleishhacker, N.T., **Kelly**, **M.M.**, Arnold, W.A., Novak, P.J., and H.L. Shoenfuss. 2014. Phytoestrogens in the environment, I: Occurrence and exposure effects on fathead minnows. Environmental Toxicology and Chemistry 33(3):553-559. Available at: http://dx.doi.org/10.1002/etc.2461

Naturally occurring phytoestrogens may mimic biogenic estrogens and modulate endocrine action in vertebrates. Little is known, however, about their temporal and spatial variability in the environment and the biological effects associated with exposures. The present study assessed the environmental presence of phytoestrogens in human-impacted and relatively pristine areas. The response in larval and sexually mature fathead minnows to environmentally relevant concentrations of 3 common phytoestrogens (genistein, daidzein, and formononetin), both singly and in mixture, was also quantified. Phytoestrogens were only present in the human-impacted surface waters. When detected, mean concentrations were low (±standard deviation) in an urban lake: 1.4 ± 0.5 ng/L, 1.6 ± 0.7 ng/L, and 1.1 ± 0.2 ng/L for genistein, daidzein, and formononetin, respectively, and in treated wastewater effluent: 1.6 ± 0.4 ng/L, 1.8 ± 1.3 ng/L, and 2.0 ng/L. Biochanin A was detected twice, whereas zearalenone and coumestrol were never detected. No clear temporal trends of aqueous phytoestrogen concentration were evident. Larval survival was significantly reduced in genistein, formononetin, and mixture treatments, whereas adult male fish only exhibited subtle changes to their anatomy, physiology, and behavior. Daidzein-exposed adult females produced greater quantities of eggs. The present study indicates that genistein, daidzein, and formononetin are likely attenuated rapidly and are unlikely to cause widespread ecological harm in the absence of other stressors.

Tucker, S., Moerke, A., Steinhart, **G.,** and **R. Greil**. 2014. Note: First record of natural reproduction by Atlantic Salmon (*Salmo salar*) in the St. Marys River, Michigan. Journal of

Great Lakes Research 40:1022-1026. Available at: http://dx.doi.org/10.1013/j.jglr.2014.08.009

Atlantic salmon *(Salmo salar)* are native to Lake Ontario; but their populations severely declined by the late 1800s due to human influences. During the early to mid-1900s, Atlantic salmon were stocked throughout the Great Lakes in effort to reestablish them into Lake Ontario and introduce the species into the upper Great Lakes. However, these efforts experienced minimal success. In 1987, Lake Superior State University and the Michigan Department of Natural Resources began stocking Atlantic salmon in the St. Marys River, Michigan, which has resulted in a successful, self-supporting hatchery operation and stable recreational Atlantic salmon fishery. Possibly due to a combination of competition with other salmonid species for spawning habitat, prey selection causing detrimental effects on early life stages and high rates of early mortality syndrome, Atlantic salmon appeared to be severely limited in their ability to naturally reproduce within the upper Great Lakes. In 2012, the first unequivocal documentation of naturally reproduced Atlantic salmon in the St. Marys River was recorded, downstream from the compensation works and parallel to the Soo Locks in Sault Ste. Marie, Michigan.

Wu, N., LaPatra, S.C., **Li**, **J.**, Sunyer, J.O., and Y.A. Zhang. 2014. Complement C5a acts as a molecular adjuvant in fish by enhancing antibody response to soluble antigen. Fish and Shellfish Immunology 40(2):616-623. Available at: http://dx.doi.org/10.1016/j.fsi.2014.08.013

C5a, the most potent anaphylatoxin generated during complement activation, has important pro-inflammatory actions and has also been shown to enhance antigen-specific antibody response in mammals, thereby acting as a molecular adjuvant. In rainbow trout, C5a has been shown to have a chemoattractant ability and its receptor has also been found on potential APCs. In this study, we tested the possible role of trout C5a as a molecular adjuvant. We demonstrated the presence of native C5a in trout serum using the antibody generated by recombinant trout C5a, and then we generated recombinant infectious hematopoietic necrosis virus glycoprotein (G), and a G-C5a fusion protein to test the adjuvant activity of trout C5a. Recombinant G-C5a displayed a potent chemoattractant activity in contrast to G alone, indicating that the C5a portion of the fusion protein was functional. Thereafter, G-C5a, partially emulsified in a small quantity of IFA, was injected into one group of trout, while the other group of trout was inoculated with the same dose of recombinant G. At four to sixteen weeks post-injection, the serum IgM antibody levels of the fish injected with recombinant G-C5a were obviously higher than those injected with G protein alone. Thus, these results suggest, for the first time, that C5a acts as molecular adjuvant in teleost fish by enhancing antibody response to a soluble antigen.

Zhao, X.H., Liu, L.C., Hegazy, A.M., Wang, H., Li, J., Zheng, F., Zhou, Y., Wang, W.M., **Li, J.**, Liu, X.L., and L. Lin. 2015. Mannose receptor mediated phagocytosis of bacteria in macrophages of blunt snout bream (*Megalobrama amblycephala*) in a Ca^{2+}-dependent manner. Fish and Shellfish Immunology 43(2):357-363

Mannose receptor (MR) is an important pattern-recognition receptor in macrophages and plays a critical role in immune responses. It is has been reported that mammalian macrophages are able to engulf a wide range of microorganisms mediated by Ca^{2+}-dependent MR binding to terminal

mannose residues which are frequently found on the pathogen surfaces. However, little is known about the MR-mediated phagocytosis in macrophages of fish. In this report, the distributions of MR in the macrophage and head kidney tissue from blunt snout bream were examined using MaMR specific antibody generated in our lab. Mannan and MaMR specific antibody inhibition experiments results collectively showed that MR was involved in the GFP-expressed *E. coli* engulfed in the macrophages, resulting in respiratory burst, nitric oxide production as well as inflammatory cytokines secretion, and the MaMR-mediated phagocytosis was Ca^{2+}-dependent. These results will shed a new light on the immune functions of teleost MRs.

Presentations

Gerig, B.S., Chaloner, D.T., Janetski, D.J., **Moerke, A.H.**, Rediske, R.R., and G.A. Lamberti. 2014. Tracing Salmon-Derived Persistent Organic Pollutants in Great Lakes Tributaries Using Congener Analyses. Joint Aquatic Sciences Meeting, Portland, OR

Pacific salmon (Oncorhynchus spp.) contribute large quantities of organic material to Great Lakes tributaries where they have been stocked since the 1960s. In the Great Lakes, salmon are semelparous and potamodromous, and may biotransport persistent organic pollutants (POP), such as polychlorinated biphenyls (PCBs). In our study, we compared the proportion of different PCBs congeners (i.e., unique PCB compounds) in spawning salmon and resident stream fish, both among lake basins and within streams, using analysis of similarity (ANOSIM) and non-metric multidimensional scaling (NMDS). We hypothesized that stream biota exposed to salmon spawners would have congener patterns similar to salmon, the presumed contaminant source. Using ANOSIM, we found significant differences in PCB congener pattern between fish species, location within stream, and lake basin. Based on NMDS, PCB congeners for salmon were strongly differentiated among lake basins. PCB congeners for resident fish were more variable but NMDS did discriminate between locations with and without salmon. Overall, our analysis may be useful in identifying salmon as a source of POP contaminants to stream ecosystems throughout the Great Lakes.

Moerke, A.H., M.D. Elya, B.S. Gerig, D.T. Chaloner, M.A. Brueseke, G.A. Lamberti. 2015. Potential for Contrasting Nutrient Subsidies To Great Lakes Tributaries By Native And Non-Native Migratory Fishes. Society for Freshwater Science Annual Meeting, Milwaukee, WI

Resource subsidies have been shown to be ecologically important to aquatic ecosystems. However, little evaluation has been conducted of the subsidies associated with abundant fish migrations that occur between the Great Lakes and their tributaries. We compared the dynamics of dissolved nutrients excreted and dissolved carbon secreted by live non-native Chinook Salmon (*Oncorhynchus tshawytscha*) and Atlantic Salmon (*Salmo salar*), and native White Sucker (*Catostomus commersonii*) spawners. During spawning runs, five adults of each species were placed into separate containers of stream water, and then filtered water samples were collected hourly over 8 hours. Samples were analyzed for dissolved phosphorus (SRP), nitrogen (NH_4^+), and carbon (DOC), and then standardized by wet mass. Mean hourly NH_4^+ excretion rates were similar for all species, whereas SRP excretion rates were 2-4x higher for Atlantic Salmon and DOC secretion rates were 3-10x higher for Chinook Salmon. Thus, historical and ongoing

changes in Great Lakes migratory fish run size and composition may alter nutrient loading to tributaries with broader implications for stream productivity and food web dynamics.

Riley, J.B., **Moerke**, **A.H.**, and S.D. Tiegs. 2014. Factors Influencing Fish and Invertebrate Communities along an Environmental Gradient in the St. Marys River Coastal Wetlands. Joint Aquatic Sciences Meeting, Portland, OR

Coastal wetlands of the Laurentian Great Lakes contain diverse populations of invertebrates, and serve as nursery and spawning habitats for ecologically and economically significant fish species. Our objective was to explore possible relationships between environmental variables (e.g., wave exposure, sediment depth, and water quality) and fish and invertebrate-community attributes. Fish and invertebrate communities were sampled along a gradient of wave exposure (as fetch) in nine coastal wetlands of the St. Marys River, Michigan (USA). Fish species richness values ranged from 3 to 21, while invertebrate genus richness ranged from 24 to 43. pH had a significant positive relationship to both invertebrate and fish richness (R^2= 0.45 and 0.60 respectively). Fetch played a role in invertebrate community distribution, with invertebrate diversity being negatively related to wave exposure (R^2=0.55). Fish diversity was positively related to organic-sediment depth and turbidity (R^2= 0.52 and 0.55 respectively). This research lends insights into the composition of fish and invertebrate communities in the St. Marys River and should be useful in effectively managing these valuable resources.

Wang, Y., Wang, X., Huang, J., and J. Li. 2015. Effect of *Quillaja Saponaria Saponin* (QSS) In Turbot (*Scophthalmus maximus*) Upon Bath Vaccination. 40th Eastern Fish Health Workshop, Charleston, SC

The adjuvant effect of QSS on protection of turbot fry was investigated with bath vaccination of formalin-killed *Vibrio anguillarum* O1 and various concentrations of saponin (5, 25, 45 and 65mg/L). Fish were challenged at day 7, 14 and 28 post-vaccination. Significantly high RPS [(59.1±13.6)%, (81.7±8.2)%, (77.8±9.6)%] were recorded in the fish received bacterin bath with QSS at 45mg/L which are comparable to the positive control group vaccinated by intraperitoneal injection(IP). Moreover, a remarkable higher serum antibody titer was also demonstrated after 28 days in the vaccinated fish with QSS (45mg/L) than those vaccinated fish without QSS (P<0.05), but lower than the IP immunized fish (P<0.05). Significant up-regulation of IgM gene expression has also been identified in the tissues of skin, gill, spleen and kidney from the immunized fish in comparison to the control fish. Taken together, the present study indicated that QSS could remarkably evoke systemic and mucosal immune responses in immunized fish. Therefore, QSS might be a promising adjuvant candidate for fish vaccination through bath administrating route.

SENIOR THESIS ABSTRACTS

Each student whose major is within the School of Biological Sciences or School of Physical Sciences at LSSU must complete a senior thesis project. Below are abstracts from 13 student projects related to the ARL and Fish Disease Lab that were completed during the 2014-2015 academic year.

Bactericidal Activity of Salmonid Serum

Ashley Alexander, Jun Li

The bacterial killing ability of the complement system in the serum of Atlantic salmon (*Salmo salar*), pink salmon (*Onchorynchus gorbuscha*), and lake trout (*Salvelinus namaycush*) was examined. The purpose was to determine whether there was a difference between the bactericidal activity of serum obtained from these three different fish species. The sera were diluted using phosphate buffered saline with calcium and magnesium ions (PBS/$Ca^{2+}Mg^{2+}$). Serial dilutions of a model bacteria (*Escherichia coli*) were incubated with the diluted sera and then inoculated onto agar plates. After two to three days, the colonies on these plates were counted and the survival rates of the *E. coli* in the different incubation conditions were calculated. A difference was observed between the survival rate of the *E. coli* incubated with the fish sera and the negative control (incubated with PBS/$Ca^{2+}Mg^{2+}$), as well as between the *E. coli* incubated with fish serum and the positive control (fetal bovine serum). However, there was no significant difference for the bactericidal activity of the serum from the three fish species.

Assessment of Angling and Recreational Use in Trout Brook Pond, MI

Robert Barta, Neal Godby

Michigan has more than 11,000 inland lakes ranging from large natural systems to small man-made impoundments. Small impoundments can create popular fisheries in areas that lack fishing opportunities in a lake setting. Unfortunately, the amount of recreational use these impoundments receive is unknown and remains an issue for management and funding implications. In this study Trout Brook Pond, a small secluded impoundment located in the Eastern Upper Peninsula of Michigan, was assessed for total angling and recreational usage. A random stratified creel survey aided by mechanical equipment (trail cameras) from April 26, 2014 until September 30, 2014 was implemented. When estimated overall angling pressure was low at a total of 15 angler trips consisting of 21 angling hours. Given the results, trail cameras proved a viable method of data collection for inland lake creel survey counts and have further implications in the fisheries field. This data collected along with past knowledge of the system will allow managers to assess the future of Trout Brook Pond by conducting a cost benefit analysis.

Status and trends of mercury contamination in Lake Whitefish *Coregonus clupeaformis* in Whitefish Bay, Lake Superior

Bill Bernier, Ashley Moerke

Lake Superior is home to the Lake Whitefish (*Coregonus clupeaformis*), which is one of the most important commercial and subsistence fisheries. PCB monitoring is performed to ensure the safe consumption of these fish. The objective of this study was to quantify the amount of mercury in local (i.e., eastern Lake Superior waters) Lake Whitefish and also evaluate temporal trends from 1992 to 2013. Fish were collected from Whitefish Bay using gill nets at 3 different

sites. Tissue samples were then extracted and analyzed for Hg concentration using EPA method 1631 rev. E., which oxidizes and runs the mercury through a series of reductions before being analyzed by a cold-vapor atomic fluorescence spectrometer. The average mercury concentration from the 2013 Lake Whitefish samples was 0.0548 ppm (SE + 0.0156). The concentrations indicated a slight increase in mercury concentrations from 1992 to 2013, but the difference was not significant.

Effects of Salmon Carcasses on Resident Trout Behavior

Ryan Cass, Ashley Moerke

Pacific Salmon (*Onchorhynchus spp.*) were introduced into the great lakes region beginning in 1967. Many of Michigan's trout streams now experience a semelparous salmon run each fall resulting in massive carcass inputs to the stream. Much of the research on these salmon occurs on the west coast where these fish are native and is lacking in the great lakes region where they are non-native. This study explores the effects that salmon carcasses may have on resident trout behavior. Behavior was observed in pools in Hunt Creek MI, Salmon carcasses and eggs were planted and observation was repeated. Trout were found to be less aggressive after the carcass additions. The number of feeding attempts per trout did not vary and many trout were observed eating eggs. Overall trout behavior was affected by the additions of carcasses and eggs.

Changes in terrestrial invertebrate subsidies and Rainbow Trout (*Oncorhynchus mykiss*) diets following a wildfire

Addie Dutton, Ashley Moerke

In the western US, wildfires have been shown to alter terrestrial-aquatic linkages by reducing terrestrial inputs that fuel stream food webs. Recently, a wildfire in northern Michigan burned forest landscapes across the Two Hearted River watershed. The objective of this study was to determine: 1) the effects of the fire on the vegetation in the riparian zone, 2) the effects of wildfire on terrestrial invertebrate subsidies to the river, and 3) if the diets of Rainbow Trout (*Oncorhynchus mykiss*) change in response to the changes in terrestrial subsidies present. Riparian vegetation (i.e. canopy cover and % living), terrestrial insect subsidies, and rainbow trout diets were compared over two years after the wildfire. Significantly higher amounts of canopy cover, terrestrial invertebrate subsidies, and proportion of terrestrial invertebrates present in Rainbow Trout diets were observed in the unburned reach of the Two Hearted River when compared to the burned reach. This suggests that wildfires influence aquatic-terrestrial linkages in the Great Lakes stream region in a similar manner as has been observed in the western US.

Return of Lake Whitefish (*Coregonus clupeaformis*) to Historic Lake Michigan Spawning Tributaries

Kyle Hafeman, Kevin Kapuscinski

Lake Whitefish (*Coregonus clupeaformis*) are one of the most commercially important species of fish found in the Great Lakes. Lake Whitefish once spawned in the many tributaries of the Green

Bay of Lake Michigan until the early 1900's. Recently it has been found that Lake Whitefish are returning to some of these historic spawning sites. While Lake Whitefish have been observed in some of these old spawning tributaries there are many that have not been studied. The objective of this study was to determine if Lake Whitefish are spawning in the Cedar, Ford and Escanaba Rivers. This study will also determine if there are any significant differences between the rivers that could help explain the return of Lake Whitefish. To determine if Lake Whitefish were present in the historic tributaries larval drift nets were set in each river to collect larval fish. Habitat and water quality parameters were also taken from each river to look for significant differences. Results showed Lake Whitefish were present in the Escanaba and Ford Rivers but were not present in Cedar River. Water quality parameters that varied significantly between the experimental rivers were alkalinity and hardness. While these parameters varied between the rivers, they still fell within the normal range for rivers in the Great Lakes region. The exact reason for the return to these tributaries is unknown but one current theory is that these whitefish are genetically different from the normal reef spawning Lake Whitefish population. These findings along with other current research could provide a definitive answer.

Changes in Fish Populations After Barrier Removal on Thompson Creek, MI

Donald M. Hiltz, Ashley Moerke

In 2010, a dam that prevented upstream movement by salmon was removed on Thompson Creek (Upper Peninsula of Michigan) which allowed salmon (*Oncorhynchus* spp.) to interact with the upstream Brook Trout (*Salvelinus fontinalis*) population. The objective of this study was to determine: 1) if adult spawning salmon were using the spawning habitat upstream of the barrier after removal, and 2) quantify changes in the resident Brook Trout population upstream of the barrier once spawning salmon could colonize after dam removal. Adult salmon spawner abundance and Brook Trout mean individual length, condition factor, and abundance were compared between the reaches upstream and downstream of the dam site for two years before and three years after dam removal. Adult salmon were found spawning in the reach upstream of the barrier site every year after barrier removal at similar numbers to downstream. Brook Trout were larger in the upstream reach before dam removal, however mean individual length was similar between reaches after dam removal. Brook Trout abundance was higher in the upstream reach before and after barrier removal, but Brook Trout condition did not differ between reaches. Our findings suggest that colonization of upstream habitat by salmon may not negatively impact the abundance of Brook Trout, but may impact individual growth throughout the stream. Longer-term surveys are necessary to assess impacts on recruitment and population level changes.

Transfer of Polychlorinated Biphenyls from Stream Ecosystems to Riparian Ecosystems by Semi-aquatic Mammals

Garret L. Price, Ashley Moerke

Polychlorinated Biphenyls (PCB) are persistent environmental contaminants which were produced for industrial applications in the 1920s, and were banned in 1979 due to widespread environmental contamination. PCBs are toxic to humans and wildlife and have been well documented to bioaccumulate in salmon. Resident fish in Great-Lakes-salmon-influenced

reaches of the AuSable, Manistee, and Muskegon Rivers have been shown to observe higher levels of PCBs, than those observed in fish upstream of the first dam, on each of the rivers, where no salmon run is present. Semi-aquatic piscivorous mammals, such as mink and otter, are at risk for consuming dangerous levels of PCBs due to the higher levels observed in both salmon, and resident fish in the Great-Lakes-salmon-influenced reaches of these rivers. The objective of the current study was to determine if mink observed higher concentrations of PCBs in the Great-Lakes-salmon-influenced reaches as opposed to the upper reaches where no salmon run was present in the AuSable, Manistee, and Muskegon Rivers. No mink could be collected from the Great-Lakes-salmon-influenced reaches of the study rivers. The mink collected from the upper reaches observed concentrations (measured by PCB ELISA test kit) orders of magnitude below the NOAEC levels reported by Giesey et al. 1994. No significant difference could be determined for PCB concentrations in mink between the study rivers however, the average concentrations observed were lowest in the AuSable and highest in the Muskegon which parallels the predictions made by Giesey et al. (1994). The sample mink in the current study showed that in the upper reaches of the three study rivers PCBs are not currently at dangerous levels for mink.

Do Case-building Caddisfly Larvae Select Substrate for Crypsis?

Tori Roznowski, Ashley Moerke

Case building by caddisfly larvae often is considered a predator defense mechanism. This strategy suggests that they would select case materials that promote crypsis and reduce their visibility to predators, but little researched has addressed this question. The objective of this study was to determine if caddisfly larvae do select case substrates for crypsis. To test this objective, eight aquaria were set up with a combination of background color and substrate condition. 50 *Pycnopsyche* sp. larvae were collected from a local stream, stripped of their cases, and two to five were placed randomly into an aquarium. Four of the aquaria had light backgrounds and four had dark backgrounds. Within each background treatment there were four substrate conditions: all light substrate, all dark substrate, evenly mixed light and dark substrate, and light and dark substrate separated. Case composition was compared to background color and substrate condition using a nested ANOVA, and Ivlev's Selectivity Index was calculated to determine color preference in each treatment. Neither background color nor substrate availability affected case substrate selection by *Pycnopsyche*. However, all larvae tested did significantly select for dark substrate, regardless of the background color and substrate condition. This increase in dark pigment may improve crypsis against objects in the environment.

Does Bluegill colony size influence potential nest predators?

Abby Schoonyan, Kevin Kapuscinski

Bluegill sunfish *Lepomis macrochirus* reproduce by laying externally-fertilized eggs in a nest that is build and guarded by the male. Most Bluegill spawn in colonies, but <5 % will spawn in solitary nests. The effect of nest location within a colony on nest predation has previously been studied, but the effects of colony size and the proximity of neighboring nests on nest predation has not. I studied nests from small and large colonies near South Bass Island, Lake Erie. Male Bluegills were caught off their nests and potential nest predators were filmed using an

underwater camera. I found that nests in large colonies were closer together than nests in small colonies. Then mean number of potential nest predators did not change with distance to the three nearest neighboring nests. The mean number of potential predators stayed consistent each minute after the male was removed from nests within large and small colonies; however, the mean number of predators appeared to be greater in large colonies. The mean number of potential predators was high in larger colonies because parental Bluegill from neighboring nests were observed chasing off predators that enter the nests and then consuming the eggs off of the nest where the guarding male was removed. Knowing how Bluegill interact within colonies of differing sizes allows for better understanding as to whether they would exert energy to guard or consume eggs off the nearest neighboring nests when given the opportunity.

Bioassessment of the St. Marys River Little Rapids Area (MI) Pre-Restoration

Nate Sleight, Ashley Moerke

The St. Marys River, a Great Lakes Area of Concern, suffers from historical losses of rapids habitat and degradation of fish and macroinvertebrate communities. In an attempt to reverse historical impairments, a proposed restoration of the Little Rapids area, located next to Sugar Island, is scheduled for the spring of 2016. Flow will be restored by replacing a causeway with a bridge spanning 600'. The restoration goals are to restore higher current velocities and increase fish habitat and macroinvertebrate diversity. The objective of this study was to collect baseline data on fish and macroinvertebrate communities. From May 2013 - July 2014 sampling of larval, juvenile, and adult fishes, as well as macroinvertebrates, was conducted in the Little Rapids and Main Rapids (2013 only). Sampling revealed a highly diverse assemblage of fish species, but 98% of species caught were fish common in lentic systems and there was a lack of a sport fish present. Natural reproduction of salmonids was observed, but it was five times less in the Little Rapids than what was observed in the Main Rapids. The macroinvertebrate community had low diversity and indicated poor to moderate water quality. Therefore, the restoration of flow in the Little Rapids area is expected to increase sport fish populations and macroinvertebrate diversity, contributing to the long-term goal of delisting the St. Marys River as an Area of Concern.

Genotyping Lake Sturgeon (*Acipenser fulvescens*) Populations from the St. Marys River and Garden River

Lilja Strang, Barbara Evans

Over the last 15 years, LSSU researchers have discovered and characterized a population of lake sturgeon residing in the north channel of the St. Mary's river; however, the spawning site had not been identified. In 2013, the Anishinabek Ontario Fisheries performed a larval drift study in the Garden River ON, and obtained 107 larval lake sturgeon. The objective of the current study is to determine if the Garden River is the spawning site of the north channel lake sturgeon population. DNA was extracted from the larval samples, and using PCR was amplified at five microsatellite loci (AfuG 68, AfuG 68b, AfuG 9, Spl 120, and AfuG 112). Allele peaks were compared to those of existing data for the local adults of the St. Marys River and also other populations throughout the Great Lakes. Data were analyzed using in the program STRUCTURE, which showed the most probable number of distinct populations to be two (K=2). The bar plot provided

by the program indicated the Garden River larva and the St. Marys River adults are one population, distinct from the remainder of the Great Lakes populations. These findings support what has been alluded to in previous research, that the Garden River is the spawning site for the St. Marys River lake sturgeon adults. Furthermore, the uniqueness of this population parallels the trends seen in walleye and yellow perch in the same geographic area, suggesting this site should be protected and that great care should be exercised when stocking.

Seasonal Survey of *Renibacterium salmoninarum* in Minnows (*Cyprinidae)* from EUP Michigan Bait Shops

Angelina Walker, Jun Li

Bacterial Kidney Disease, caused by *Renibacterium salmoninarum*, is well-established in the Great Lakes basin. It has been mostly known as one of the leading bacterial diseases affecting salmonid fish. With the use of minnows (*Cyprinidae spp*.) as bait throughout the Great Lakes area, they could be transmitting various pathogens that infect the Great Lakes fish. A total of 960 minnows were surveyed throughout the beginning and end of October and January, followed by an Enzyme Linked Immuno-Sorbent Assay (ELISA) diagnosis of BKD contamination in the minnows. After ELISA testing the beginning and end of each season from two separate sources, there was no BKD pathogen detectable in the minnows (*Cyprinidae spp.*) from either bait shops or seasons. There was no change in bacteria detected over the time of season in either bait shop. The amount of pathogens that were carried in the bait shop after being distributed did not make a difference on the amount of BKD active in the minnows. Bacterial Kidney Disease is still a huge problem in the Great Lakes and this research relieves the understanding that minnows are a harmless bait to use with no correlation to the amount of BKD in the Michigan lakes.

APPENDICES

Appendix 1. Historical summary of the Atlantic Salmon propagation program at Lake Superior State University's Aquatic Research Laboratory.

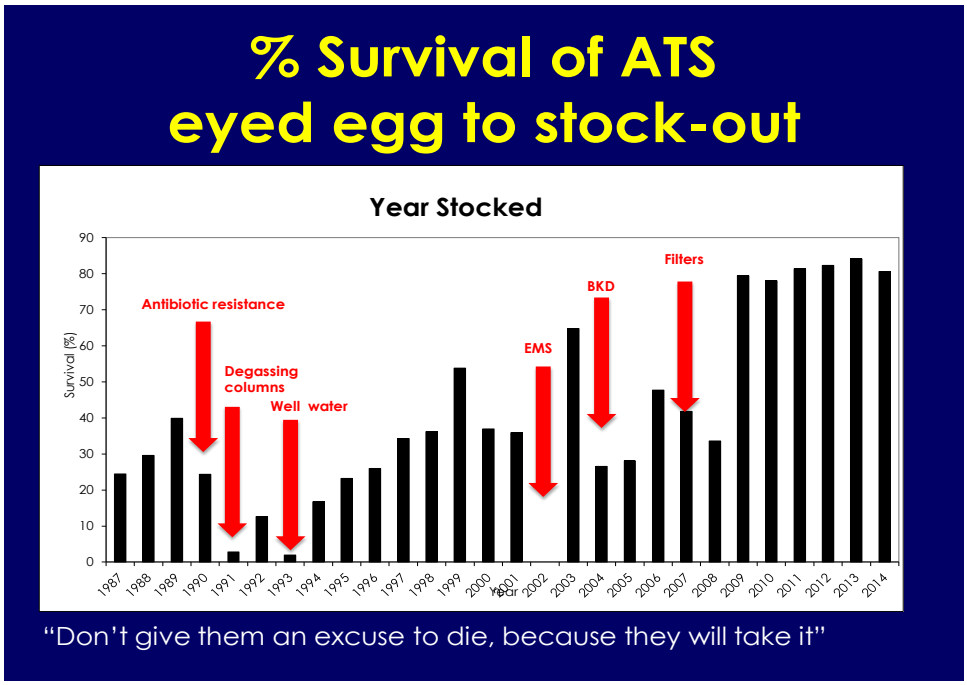
Lake Superior State University (LSSU), in conjunction with Cloverland Electric Cooperative and the Michigan Department of Natural Resources (MDNR) Fisheries Division, have been making great strides in introducing Atlantic salmon, one of the world's premier sportfish, into the St. Marys River. As of now, it is the only successful stocking program in the Upper Great Lakes with a fishable return in Northern Lake Huron and the St. Marys River.

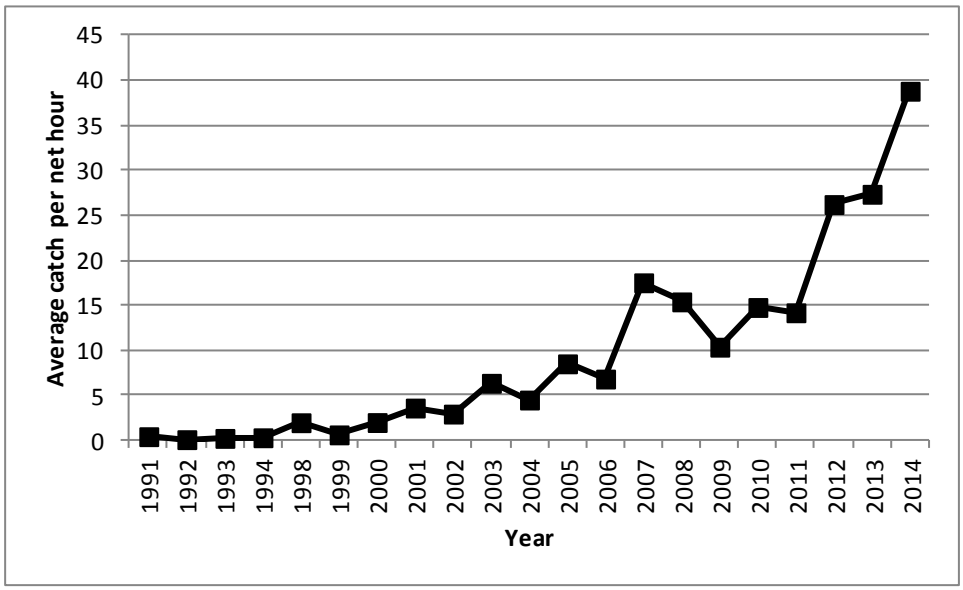
The LSSU Aquatic Research Laboratory (ARL) has been rearing and stocking Atlantic salmon (*Salmo salar*) into the St. Marys River in Sault Ste. Marie, (Chippewa County) Michigan since the mid-1980s. The first stocking was in 1987, with yearling smolts, and since then just over 767,000 Atlantic salmon have been stocked at an average of 30,700 per year, averaging 178 mm in length. Fall fingerlings were stocked for a few years in the mid-to-late 1990s but the returns were very poor.

The university's primary mission is not to stock fish, but rather to use the hatchery as a training tool for undergraduates. Students are trained in all aspects of hatchery work, from collecting returning adults from the St. Marys River for egg-take, to stocking fish 18 months later, and everything in-between. Students handle all aspects that are required in rearing (yearling) production fish.

In the early years, the ARL had some difficult times, losing over 90% of its eggs or fish because of a lack of knowledge or understanding of Atlantic salmon and how to rear them (see graph below). It took us about 10 years to begin to understand the fish and still today the ARL staff will not lay claim to knowing it all on them; Atlantics still give staff problems from time to time in the hatchery.

From 2004-2013, ARL fish were a lot healthier at stock-out and the returns rates showed it. During this time, we stocked an average of just under 29,000 spring yearlings annually with average lengths of just over 192 mm. Atlantic salmon stocked by LSSU have consistently provided a better catch-per-number-of-fish-stocked (return-to-creel) than any other salmonid stocked in Lake Huron (Johnson, 2012). After the alewife collapse in 2004 in Lake Huron, the return rates for Atlantic salmon averaged about 5.5 %, which is nearly 10 times the return-to-creel for steelhead during the same period (Johnson 2012). The same increase is shown from our brood stock collection over the year (we use a gill net in the discharge of the hatchery in the St. Marys River) as shown below. The fish are stocked in the first week of June if the river is at least 8 degrees Celsius; if not, the fish are held until it is 8 C.





Since 2003, the ARL has been self-supporting in its egg-take needs (and getting enough eggs for the MDNR, when needed) through harvesting eggs from returning fish in the St. Marys River. We feel the fish we collect each year are a land-locked strain from West Grand Lake in Maine, as this is the strain we worked with the most. We did work with Penobscots from the USGS Tunison Lab in New York and Sebago from Maine, but we had very poor survival at that time and we stocked very few fish.

How are we successful? This is a question we hear a lot, and here is what we think:

- Fish are reared in the same water in which they are stocked
- Fish are held until they are 1 ½ year-old smolts
- We now have a better understanding of the fish – our biggest reason for success.
- St. Marys River is a dynamic system that can accommodate a great variety of fish
- The biggest reason why is **"time"** to work with the fish and to get to understand them

- ARL has the luxury of being a university with a primary mission of training students and doing research and not a government agency that must respond quickly to anglers who may not have much patience and want quick results.

Literature Cited

Johnson, James E. 2012. Review of Attributes of Landlocked Atlantic Salmon in Relation to their Management in Lake Huron. Michigan Department of Natural Resources. Fisheries Brief Report 01.

Appendix 2. Results of fish health inspection of age-1 Atlantic Salmon from lot P-ATS-LL-W-12-SM-LS-LS conducted by Michigan State University.

FISH HEALTH INSPECTION REPORT--FISHERIES DIVISION
MICHIGAN DEPARTMENT OF NATURAL RESOURCES
Fish Health Inspection Report

AAHL Number: 140305-1-PI-LSSU

This report is not evidence of future disease status. To determine current status, contact Fish Health Official below.

Name and Location of Fish Source:	Owner/Manager:	Inspection Date(s): Spring 2014
Lake Superior State University Sault Ste. Marie, MI	Roger Greil	This: 3/5/14 Prior: 3/12/13 Classification: B

Type of Water Supply:
Stream
Origin of Fish Examined:
Hatchery
Type of Fish Examined:
Salmonid

Species[2]	Designation	AAHL #	Age[1]	Number in Lot	Obtained as Eggs (E) or Fish (F) From:	Pathogens[3] Inspected for and Results[4]						
						Aa	Fr	Rs[a]	VHS	IHN	IPN	WD
ATS-LL	P-ATS-LL-W-12-SM-LS-LS*	140305-1-PI-LSSU	10	31,000	E-St. Mary's River	60 (0)	60 (0)	60 (0)	60 (0)	60 (0)	60 (0)	60 (0)

Remarks/Recommendations:

Lot P-ATS-LL-W-12-SM-LS-LS can be stocked in Michigan's public waters.

[4] Laboratory assays were conducted in accordance with the guidelines of the Great Lakes Fishery Commission (GLFC), the American Fisheries Society (AFS), and the World Organization for Animal Health (OIE).

[a]: The presence of *Renibacterium salmoninarum* was tested with quantitative QELISA, which is more sensitive than the direct fluorescent antibody technique. H=high, M=medium, L=low antigen concentrations.

-: Test not required.

*: *Nucleospora salmonis* was not detected in the 12 pools of tissue tested by nPCR (5 fish per pool). *N. salmonis* is not currently a pathogen of concern.

Address/Phone of Contracted Fish Health Official:

Aquatic Animal Health Laboratory
College of Veterinary Medicine
Michigan State University
Food Safety & Toxicology Building
1129 Farm Lane, room 177K
East Lansing, MI 48824
PHONE: 517/884-2024
FAX: 517/432-2310

Signature of Contracted Fish Health Official:

Mohamed Faisal

Mohamed Faisal, D.V.M., PhD.

[1] For juv. hatchery fish give age in months; for feral and adult hatchery fish use symbols e=eggs or fry; f=fingerlings; y=yearlings; b=older fish.

[2] See list of pathogen and spp. abbreviations (pg 2).

cc: Gary Whelan

Appendix 2 continued

FISH HEALTH INSPECTION REPORT CONTINUED. REPORT NUMBER:

Species	Designation	AAHL #	Age	Number in Lot	Obtained as Eggs (E) or Fish (F) From:	Pathogens Inspected for and Results						
						As	Yr	Rs	VHS	IHN	IPN	WD

SUPPLEMENTAL INSPECTION INFORMATION

Date	Species	Lot #	Findings

Pathogen Abbreviations

IHN	Infectious Hematopoietic Necrosis Virus	IPN	Infectious Pancreatic Necrosis Virus
VHS	Viral Hemorrhagic Septicemia Virus	*As*	(BF) *Aeromonas salmonicida* (furunculosis)
Rs	(BK) *Renibacterium salmoninarum* (BKD)	WD	*Myxobolus cerebralis* (whirling disease)
Yr	*Yersinia ruckeri* (ERM)	X	Various (see remarks box)
Y	Various (see remarks box)	Z	Various (see remarks box)

Species Abbreviations

ATS	Atlantic Salmon	BKT	Brook Trout	BNT	Brown Trout	CHS	Chinook Salmon
COS	Coho Salmon	FCS	Fall Chinook Salmon	LAT	Lake Trout	Mixed	Mixed species
OSA	Other Salmonids	RBT	Rainbow Trout	SPL	Splake (Brook x Lake)	STN	Sturgeon
STT	Steelhead Trout						

This is to certify that the above-mentioned fish were collected and laboratory assays conducted in accordance with the guidelines of the Great Lakes Fishery Commission (GLFC), the American Fisheries Society (AFS), and the World Organization for Animal Health (OIE). Samples received in the laboratory were examined for disease signs and were subjected to laboratory testing for the fish pathogens as listed.

[1]For juv. hatchery fish give age in months; for feral and adult hatchery fish use symbols e=eggs or fry; f=fingerlings; y=yearlings; b=older fish.

[2]See list of pathogen and spp. abbreviations (pg 2).

cc: Gary Whelan

Appendix 3. Results of fish health inspection of age-0 Atlantic Salmon from lot P-ATS-LL-W-13-SM-LS-LS conducted by Michigan State University.

FISH HEALTH INSPECTION REPORT--FISHERIES DIVISION
MICHIGAN DEPARTMENT OF NATURAL RESOURCES
Fish Health Inspection Report

AAHL Number: 140826-1-PI-LSSU

This report is not evidence of future disease status. To determine current status, contact Fish Health Official below.

Name and Location of Fish Source:	Owner/Manager:	Inspection Date(s): Fall 2014
Lake Superior State University Sault Ste. Marie, MI	Roger Greil	This: 8/26/14 Prior: 9/4/13

Type of Water Supply:	Classification:
Stream	B
Origin of Fish Examined:	
Hatchery	
Type of Fish Examined:	
Salmonid	

Species[2]	Designation	AAHL #	Age[1]	Number in Lot	Obtained as Eggs (E) or Fish (F) From:	Pathogens[a] Inspected for and Results						
						Ac	Fc	Rs[b]	VHS	IHN	IPN	WD
ATS-LL	P-ATS-LL-13-SM-LS-LS	140826-1-PI-LSSU	5	42,000	E - St Marys River	60 (0)	60 (0)	60 (0)	60 (0)	60 (0)	60 (0)	NR[c]

Remarks/Recommendations:

Lot P-ATS-LL-W-13-SM-LS-LS can be stocked in Michigan's public waters.

[a] Laboratory assays were conducted in accordance with the guidelines of the Great Lakes Fishery Commission (GLFC), the American Fisheries Society (AFS), and the World Organization for Animal Health (OIE).

[b]: The presence of *Renibacterium salmoninarum* was tested with quantitative QELISA, which is more sensitive than the direct fluorescent antibody technique; H=high, M=medium, L=low antigen concentrations.

[c]: Test not required.

Address/Phone of Contracted Fish Health Official:

Aquatic Animal Health Laboratory
College of Veterinary Medicine
Michigan State University
1129 Farm Lane, room 177K
Food Safety & Toxicology Building
East Lansing, MI 48824
PHONE: 517/884-2024; FAX: 517/432-2310

Signature of Contracted Fish Health Official:

Mohamed Faisal

Mohamed Faisal, D.V.M., Ph.D.

[1]For juv. hatchery fish give age in months; for feral and adult hatchery fish use symbols e=eggs or fry; f=fingerlings; y=yearlings; b=older fish.

[2]See list of pathogen and spp. abbreviations (pg 2).

cc: Gary Whelan

Appendix 4. Data on individual Atlantic Salmon used for gamete collection in 2014. Note: 000-099 and 000A-003A are females, and 100-199 and 100A-103A are males.

Date eggs taken	Total length (cm)	ID #	Wt of 50 eggs (g)	Total Wt of eggs (g)	Total # of eggs
11/5/2014	55	000	9.7	149.2	763
11/5/2014	62	100			
11/5/2014	63	001	8.3	650.3	3914
11/5/2014	61	101			
11/5/2014	55	002	8.5	693.9	4069
11/5/2014	58	102			
11/5/2014	68	003	9.8	962.5	4895
11/5/2014	59	103			
11/5/2014	70	004	8.6	531.5	3086
11/5/2014	68	104			
11/5/2014	74	005	9.1	962.1	5313
11/5/2014	60	105			
11/5/2014	73	006	7.5	668.2	4474
11/5/2014	56	106			
11/5/2014	67	007	8.5	958.9	5416
11/5/2014	62	107			
11/5/2014	70	008	8.3	630.1	3790
11/5/2014	57	108			
11/5/2014	55	009	5.6	289.2	2587
11/5/2014	54	109			
11/5/2014	56	010	6.2	463	3758
11/5/2014	58	110			
11/5/2014	56	011	7.6	498	3291
11/5/2014	58	111			
11/5/2014	77	012	6.9	620.3	4489
11/5/2014	65.5	112			
11/5/2014	52.6	013	5.4	282.6	2635
11/5/2014	57	113			
11/5/2014	75	014	8.5	1130.7	6656
11/5/2014	66.5	114			
11/5/2014	55	015	6.4	500.4	3885
11/5/2014	61.3	115			
11/5/2014	56.2	016	6.2	252.4	2043
11/5/2014	61.5	116			

11/5/2014	64.5	017	8.1	797.3	4965
11/5/2014	60.5	117			
11/5/2014	55.4	018	5.4	352.7	3265
11/5/2014	59	118			
11/5/2014	53.7	019	4.8	341.6	3552
11/5/2014	64.4	119			
11/5/2014	49	020	5.3	366.7	3451
11/5/2014	61	120			
11/5/2014	57	021	6.0	461.4	3799
11/5/2014	56.9	121			
11/5/2014	52	022	5.6	301.3	2678
11/5/2014	78.8	122			
11/10/2014	65.3	023	9.0	670.9	3727
11/10/2014	62	123			
11/10/2014	59	024	6.8	429.1	3139
11/10/2014	74.5	124			
11/10/2014	58	025	6.3	365.2	2891
11/10/2014	51	125			
11/10/2014	66.5	026	8.2	391.4	2385
11/10/2014	52.5	126			
11/10/2014	70	027	6.7	388.6	2899
11/10/2014	57.5	127			
11/10/2014	70.5	028	8.3	122.9	737
11/10/2014	60.5	128			
11/10/2014	71	029	7.9	626.8	3942
11/10/2014	61.5	129			
11/10/2014	78	030	7.8	436.6	2806
11/10/2014	74	130			
11/10/2014	67.8	031	8.3	682.2	4148
11/10/2014	57	131			
11/10/2014	57.5	032	4.6	348.8	3772
11/10/2014	61	132			
11/10/2014	56	033	4.4	275.8	3113
11/10/2014	60	133			
11/10/2014	54.7	034	4.4	252.8	2889
11/10/2014	61.4	134			
11/10/2014	56.2	035	3.7	324.2	4446
11/10/2014	62.2	135			
11/10/2014	56.5	036	5.4	515.7	4760
11/10/2014	57.5	136			
11/10/2014	57.2	037	5.6	334.7	2975

11/10/2014	60.9	137			
11/10/2014	52.3	038	5.2	260.7	2498
11/10/2014	55.3	138			
11/10/2014	50	039	6.8	268.7	1963
11/10/2014	62.4	139			
11/10/2014	60.5	040	7.5	467.7	3118
11/10/2014	50	140			
11/10/2014	75.5	041	11.2	1061.7	4760
11/10/2014	50	141			
11/10/2014	68.4	042	9.1	586.4	3215
11/10/2014	74.2	142			
11/10/2014	70.9	043	7.3	370.2	2551
11/10/2014	59.8	143			
11/10/2014	81	044	12.9	1567.8	6068
11/10/2014	68.2	144			
11/10/2014	57	045	5.9	489.4	4121
11/10/2014	57	145			
11/10/2014	56.3	046	5.5	416.3	3800
11/10/2014	64.2	146			
11/10/2014	55	047	5.6	184.5	1660
11/10/2014	56.2	147			
11/10/2014	54.5	048	6.9	218.5	1582
11/10/2014	59.9	148			
11/13/2014	54.5	049	6.7	506	3795
11/13/2014	54.6	149			
11/13/2014	56.5	050	6.3	431.1	3403
11/13/2014	54.8	150			
11/13/2014	64.8	051	7.4	472	3183
11/13/2014	73.8	151			
11/13/2014	67	052	9.8	911.1	4667
11/13/2014	76.5	152			
11/13/2014	60.5	053	5.8	290.5	2500
11/13/2014	58.5	153			
11/13/2014	57	054	7.0	471	3364
11/13/2014	75	154			
11/13/2014	56	055	6.0	460.8	3823
11/13/2014	61.5	155			
11/13/2014	54.5	056	5.8	195.6	1691
11/13/2014	58	156			
11/13/2014	74.5	057	5.8	450.7	3863
11/13/2014	60.5	157			

11/13/2014	66	058	6.9	351.7	2547
11/13/2014	57	158			
11/13/2014	57.3	059	6.1	453	3716
11/13/2014	55	159			
11/13/2014	58	060	7.5	507.1	3380
11/13/2014	54	160			
11/13/2014	57	061	5.0	442.1	4421
11/13/2014	65	161			
11/13/2014	73	062	10.0	833.8	4169
11/13/2014	69.5	162			
11/13/2014	54	063	6.0	307.4	2536
11/13/2014	55	163			
11/13/2014	57	064	5.9	477.5	4051
11/13/2014	59	164			
11/13/2014	56	065	6.5	613	4687
11/13/2014	44.5	165			
11/13/2014	57	066	6.0	370.8	3106
11/13/2014	59	166			
11/13/2014	51.5	067	6.0	334.5	2800
11/13/2014	49.5	167			
11/13/2014	59	068	6.0	458.6	3795
11/13/2014	55	168			
11/13/2014	52.5	069	7.1	353.2	2498
11/13/2014	54	169			
11/13/2014	54	070	5.4	387.4	3615
11/13/2014	56	170			
11/13/2014	58	071	7.3	454.8	3133
11/13/2014	55	171			
11/13/2014	56.5	072	6.6	281.8	2150
11/13/2014	59	172			
11/13/2014	56.5	073	6.4	378.6	2974
11/13/2014	32	173			
11/13/2014	58	074	7.6	318.1	2100
11/13/2014	57	174			
11/13/2014	66	075	9.1	411.7	2258
11/13/2014	56.5	175			
11/13/2014	55.7	076	6.0	328.3	2720
11/13/2014	61	176			
11/13/2014	57.5	077	6.1	307.3	2535
11/13/2014	58	177			
11/13/2014	68.5	078	9.5	713.3	3764

11/13/2014	55	178			
11/13/2014	56.3	079	7.1	431.6	3057
11/13/2014	50	179			
11/13/2014	52	080	6.0	462.2	3876
11/13/2014	59.5	180			
11/13/2014	59	081	7.6	741.6	4873
11/13/2014	67	181			
11/13/2014	56	082	6.3	583.9	4671
11/13/2014	56	182			
11/13/2014	56	083	7.1	490	3458
11/13/2014	51	183			
11/13/2014	57	084	7.7	490.6	3207
11/13/2014	59	184			
11/17/2014	64.2	085	8.6	590.7	3426
11/17/2014	57.5	185			
11/17/2014	57.3	086	5.6	415.8	3696
11/17/2014	58.5	186			
11/17/2014	56.8	087	7.0	376.9	2692
11/17/2014	65	187			
11/17/2014	73.6	088	7.4	639.3	2487
11/17/2014	63.2	188			
11/17/2014	71.5	089	9.6	1181.3	6151
11/17/2014	55.6	189			
11/17/2014	72.7	090	7.3	450.2	3074
11/17/2014	56	190			
11/17/2014	68.5	091	7.4	759.1	5129
11/17/2014	55.5	191			
11/17/2014	57.5	092	4.2	447.2	5366
11/17/2014	56.5	192			
11/17/2014	59.3	093	5.5	603.5	5469
11/17/2014	61	193			
11/17/2014	67.5	094	8.3	837.5	5025
11/17/2014	57.3	194			
11/17/2014	57.5	095	5.3	486.2	4557
11/17/2014	54.7	195			
11/17/2014	67.5	096	8.1	510.4	3159
11/17/2014	58	196			
11/17/2014	5.1	097	5.4	418.5	3855
11/17/2014	57.9	197			
11/17/2014	71.5	098	5.2	792.7	7700
11/17/2014	56.2	198			

11/17/2014	57.7	099	5.4	432.5	3983
11/17/2014	59	199			
11/17/2014	52.4	000-A	5.8	402.3	3448
11/17/2014	56.8	100-A			
11/17/2014	59	001-A	5.0	479.5	4795
11/17/2014	59	101-A			
11/17/2014	69.5	002-A	7.2	651.8	4227
11/17/2014	75.6	102-A			
11/17/2014	51.9	003-A	4.8	339.9	3508
11/17/2014	60.9	103-A			

Appendix 5. Results of 2014 Atlantic Salmon broodstock testing for presence of *Renibacterium salmoninarum* (Bacterial Kidney Disease) conducted by the ARL's Fish Disease Lab.

Fish Health Inspection Report-Fall, 2014

Name and Location of Fish Source		**Manager**	**Type of Water Supply**: St. Marys River water
Lake Superior State University Aquatic Research Laboratory (LSSU-ARL)		Roger Greil	**Origin of Broodstock Fish Examined:** Caught from St. Marys River and Raised at Hatchery **Type of Fish Examined:** Atlantic Salmon

Species	**Designation**	**Inspection Date**	**Pathogens Inspected for and Results**
			Rs.*
ATS	Feral Fall Atlantic Salmon Spawners (gamete samples)	**11/06/14** **11/11/14** **11/13/14** **11/17/14**	E: 27 (0); S:27 (0) E: 26 (0); S:26 (0) E: 36 (0); S:36 (0) E: 19 (0); S:19 (0)

Remarks:	**Address/phone of Contracted Fish Health Official**	**Signature of Contracted Fish Health Official**
* **Rs**:*Renibacterium salmoninarum* was tested with quantitative QELISA, which is recommended by USFWS and Michigan DNR **E**: egg; **S**: Sperm	Fish Disease Laboratory Lake Superior State University 650 W. Easterday Ave. Sault Ste. Marie, Mi 49783 **Phone: 906-635-2094**	 Jun Li, Ph.D

Appendix 6. Results of fish health inspection of 2014 Atlantic Salmon broodstock conducted by Michigan State University.

FISH HEALTH INSPECTION REPORT--FISHERIES DIVISION

MICHIGAN DEPARTMENT OF NATURAL RESOURCES

Fish Health Inspection Report

AAHL Number: 141111-3-BI-LSSU

This report is not evidence of future disease status. To determine current status, contact Fish Health Official below.

Name and Location of Fish Source:	Owner/Manager:	Inspection Date(s): Fall 2014	Type of Water Supply:
Lake Superior State University Sault Ste. Marie, MI	Roger Greil	This: 11/11/14 Prior: 11/12/13	Stream
			Origin of Fish Examined: Hatchery
		Classification: B-BK, BF	Type of Fish Examined: Salmonid

Species[1]	Designation	AAHL #	Age[1]	Number in Lot	Obtained as Eggs (E) or Fish (F) From:	Pathogens[2] Inspected for and Results[a]: *As*	*Yr*	*Rs*[b]	VHS	IHN	IPN	*Om* V[c]	WD
ATS	Feral Fall Atlantic Salmon Spawners (kidney & spleen samples)	141111-3-BI-LSSU	b	n/a	Wild	60 (1)	60 (0)	60 (0)	60 (0)	60 (0)	60 (0)	NR[d]	60 (0)
ATS	Feral Fall Atlantic Salmon Spawners (gamete samples)	141111-3-BI-LSSU	b	n/a	Wild	NR	NR	60 (0)	60 (0)	60 (0)	60 (0)	60 (0)	NR

Remarks/Recommendations:

a: Laboratory assays were conducted in accordance with the guidelines of the Great Lakes Fishery Commission (GLFC), the American Fisheries Society (AFS), and the World Organization for Animal Health (OIE).

b: The presence of *Renibacterium salmoninarum* was tested with quantitative QELISA, which is more sensitive than the direct fluorescent antibody technique; H=high, M=medium, L=low antigen concentrations.

c: *Oncorhynchus masu* virus testing is done on ovarian fluid samples only as per GLFC recommendations.

d: NR=Test not required.

Address/Phone of Contracted Fish Health Official

Aquatic Animal Health Laboratory
College of Veterinary Medicine
Michigan State University
1129 Farm Lane, Room 177K
Food Safety & Toxicology Building
East Lansing, MI 48824
PHONE: 517/884-2024; FAX: 517/432-2310

Signature of Contracted Fish Health Official

Mohamed Faisal

Mohamed Faisal, D.V.M., PhD.

[1]For juv. hatchery fish give age in months; for feral and adult hatchery fish use symbols e=eggs or fry; f=fingerlings; y=yearlings; b=older fish.

[2]See list of pathogen and spp. abbreviations (pg 2).

cc: Gary Whelan

Appendix 7. Results of fish health inspection of 2013Atlantic Salmon broodstock conducted by Michigan State University.

FISH HEALTH INSPECTION REPORT--FISHERIES DIVISION

MICHIGAN DEPARTMENT OF NATURAL RESOURCES

Fish Health Inspection Report

AAHL Number: 131112-1-BI-LSSU

This report is not evidence of future disease status. To determine current status, contact Fish Health Official below.

Name and Location of Fish Source:	Owner/Manager:	Inspection Date(s): Fall 2013
Lake Superior State University Sault Ste. Marie, MI	Roger Greil	This: 11/12/13 Prior: 11/13/12

Type of Water Supply:
Stream
Origin of Fish Examined:
Hatchery
Type of Fish Examined:
Salmonid

Classification:
B-BK

Species[2]	Designation	AAHL #	Age[1]	Number in Lot	Obtained as Eggs (E) or Fish (F) From:	Pathogens[3] Inspected for and Results[4]							
						4s	3r	8i*	VHS	BIN	IPN	Onc V*	WD
ATS	Feral Fall Atlantic Salmon Spawners (kidney & spleen samples)	131112-1-BI-LSSU	b	n/a	Wild	60 (0)	60 (0)	60 (3: 1H-2M)	60 (0)	60 (0)	60 (0)	NR[d]	60 (0)
ATS	Feral Fall Atlantic Salmon Spawners (gamete samples)	131112-1-BI-LSSU	b	n/a	Wild	NR	NR	60 (0)	60 (0)	60 (0)	60 (0)	60 (0)	NR

Remarks/Recommendations:

[a] Laboratory assays were conducted in accordance with the guidelines of the Great Lakes Fishery Commission (GLFC), the American Fisheries Society (AFS), and the World Organization for Animal Health (OIE).

[b] The presence of *Renibacterium salmoninarum* was tested with quantitative QELISA, which is more sensitive than the direct fluorescent antibody technique; H=high, M=medium, L=low antigen concentrations.

[c] *Oncorhynchus mykiss* virus testing is done on ovarian fluid samples only as per GLFC recommendations.

[d] NR=Test not required.

Address/Phone of Contracted Fish Health Official

Aquatic Animal Health Laboratory
College of Veterinary Medicine
Michigan State University
1129 Farm Lane, Room 177K
Food Safety & Toxicology Building
East Lansing, MI 48824
PHONE: 517/884-2024; FAX: 517/432-2310

Signature of Contracted Fish Health Official

Mohamed Faisal

Mohamed Faisal, D.V.M., PhD.

[1] For juv. hatchery fish give age in months; for feral and adult hatchery fish use symbols e=eggs or fry; f=fingerlings; y=yearlings; b=older fish.

[2] See list of pathogen and spp. abbreviations (pg 2).

cc: Gary Whelan

Appendix 8. Dosages for treatments of Atlantic Salmon eggs.

Saline Bath
0.75% needed
0.75/100 = 0.0075
0.0075*20L = 0.15 mL or g
0.15*1000 = 150 g needed for 20 L of H_2O

Erythromycin Treatment
2 ppm (mg/L) needed
2 mg/L*20 = 400,000 mg
400,000/0.23 = 1,739,130 (23% active)
1,739,130/10,000 = 173.9
173.9/1000 (to get to g) = 0.174g per 20 L of H_2O

Iodine Treatment
1% active
1% free iodine to get 100 ppm (mg/L) dilute 100 times
20 L = 20,000 mL
20 L*1,000 mL = 20,000 mL
20,000 mL/100 = 2,000 mL
2,000 mL*50 = 100,000 mL
100,000 / 1,000 (to mL) = 100 mL of Iodine needed for 20 L of H_2O