

# **AQUATIC RESEARCH LABORATORY**

## **2017 ANNUAL REPORT**

Roger W. Greil, Manager  
Kevin L. Kapuscinski, Co-director and Assistant Professor  
Ashley H. Moerke, Co-director and Professor



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## 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.lsu.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 numerous donors, student employees, and volunteers that contribute to the success of the Aquatic Research Laboratory.



## HATCHERY OPERATIONS

### *Stocking & Disease Testing*

A total of 36,790 age-1 Atlantic Salmon *Salmo salar* were reared at Lake Superior State University's Aquatic Research Laboratory (ARL) and stocked in the St. Marys River, Chippewa County, Michigan, on 2 June 2016 (Table 1). This was our 30<sup>th</sup> year of stocking Atlantic Salmon into the St. Marys River. These fish (lot P-ATS-LL-W-14-SM-LS-LS) were stocked directly into the river at 22:15, at the water temperature at 8.5 °C. Each fish received a right pelvic (ventral; RV) clip and averaged 18.34 cm in total length and 59.83 g in weight or 16.7/kg. In the spring of 2016, 60 of these age-1 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. All fish tested negative for pathogens (Appendix 1).

The ARL's projected number of Atlantic Salmon to be stocked into the St. Marys River in June of 2017 is 28,983, as of 1 May 2017 (Table 1). Inventory of these fish was done by weight up to this point, but the fish will be hand counted as they are fin clipped for a more accurate estimate of the number stocked. Of the 28,983 to be stocked, 19,354 were reared at the ARL and 9,429 fish were transferred to the ARL from the Platte River State Fish Hatchery on 30 November 2016. As of December 2016, mean total length was 18.6 cm for the ARL fish and 16.0 cm for the fish transferred from the Platte River State Fish Hatchery; the mean for the entire lot was 17.7 cm. Prior to stocking, these age-1 fish (lot P-ATS-LL-W-15-SM-LS-LS) will receive a left pectoral (LP) fin clip and will be measured again for mean size.

Survival from the eyed-egg stage through stock-out of this 2015 lot of Atlantic Salmon, now age-1, was about 45%. This was the lowest survival we have recorded since 2008, and 80% survival is more typical since the installation of our water filtration system. Most mortality occurred at the swim-up stage, and likely resulted from not being able to run our water filtration system until 17 January 2016. The system was offline due to the unusually warm fall that delayed ice cover and severe wind and storms that kept suspended solids so high that the filters could not be used. Our inability to run the filtration system resulted in high suspended solid loads in the water, followed by unusually high numbers of the parasite *Trichodina* sp. and BKD (both diagnosed by MSU). To our knowledge, this is the first time mortality in our fish was caused by *Trichodina* sp. This problem occurred in only two of our four fry raceways, likely because the two unaffected raceways had another raceway above the raceway that acted as a "settling basin" and allowed the suspended solids to settle out before reaching the fish. We have since installed six additional filters, doubling our water filtration capacity, in an effort to avoid this problem in the future.

In the spring of 2017, 60 age-1 fish were tested for the presence of *Aeromonas salmonicida*, BKD, Infectious Hematopoietic Necrosis Virus, Infectious Pancreatic Necrosis Virus, Viral Hemorrhagic Septicemia Virus, and *Yersinia ruckeri* by MSU personnel prior to stocking. All fish tested negative for pathogens (documentation pending).

Table 1. Number and mean total length of age-1 Atlantic Salmon stocked during 1987-2017. 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               |
| 2015 | 29,880    | 164               |
| 2016 | 36,790    | 183               |
| 2017 | 28,983    | 177               |

\*Fish were held until August because they were treated for BKD and furunculosis

## *Sub-adult Rearing and Use for Education and Research*

A total of 37,573 age-0 Atlantic Salmon were moved into fry raceways in late February 2016 at the time of early feeding and reared in filtered and heated water until June. About 21,000 fry survived through mid-June of 2016, at which time they were transferred into large raceways and reared in ambient river water. On average, these fish grew about 160 mm in total length during March-December 2016 (Table 2). An additional 9,994 age-0 fish were received from the Platte River State Fish Hatchery on 30 November 2016. A total of 28,482 age-1 Atlantic Salmon were fin clipped in May 2017 and will be stocked into the St. Marys River in June 2017. During August 2016-March 2017, a total of 450 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 2016 (lot: P-ATS-LL-W-15-SM-LS-LS). The number of fish initially moved into fry raceways was 37,573.

| 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 <sup>3</sup> ) | Flow (L/min) |
|------------------------------|------------------|----------------|------------------|-------------------|--------------|------|------------------------|-----------------------------------|--------------|
| 1-Mar                        | 37,314           | 9.37           | 26.68            | 7.33              | 0.026        | 0.76 | 1.62                   | 5.34                              | 45           |
| 15-Mar                       | 31,142           | 9.19           | 28.55            | 8.21              | 0.003        | 9.06 | 16.54                  | 5.66                              | 73           |
| 29-Mar                       | 26,715           | 9.77           | 29.72            | 7.84              | 0.014        | 2.63 | 14.22                  | 5.03                              | 104          |
| 12-Apr                       | 23,899           | 9.87           | 32.40            | 8.91              | 0.025        | 1.71 | 10.54                  | 5.71                              | 104          |
| 26-Apr                       | 22,701           | 9.91           | 36.44            | 11.71             | 0.034        | 1.28 | 5.01                   | 8.25                              | 95           |
| 10-May                       | 21,790           | 11.46          | 41.73            | 16.87             | 0.037        | 1.15 | 4.01                   | 11.88                             | 95           |
| 24-May                       | 21,292           | 10.40          | 48.14            | 25.23             | 0.048        | 1.01 | 2.29                   | 17.77                             | 95           |
| 7-Jun                        | 20,936           | 9.56           | 55.55            | 38.24             | 0.059        | 0.69 | 1.67                   | 26.93                             | 95           |
| 21-Jun                       | 20,688           | 13.19          | 63.85            | 57.58             | 0.047        | 0.61 | 1.18                   | 2.64                              | 1,452        |
| 5-Jul                        | 20,593           | 16.01          | 72.89            | 85.49             | 0.042        | 0.94 | 0.46                   | 3.93                              | 1,452        |
| 19-Jul                       | 20,529           | 17.81          | 82.56            | 124.50            | 0.040        | 1.23 | 0.31                   | 4.29                              | 1,936        |
| 2-Aug                        | 20,066           | 18.91          | 92.74            | 175.15            | 0.039        | 1.56 | 2.26                   | 6.03                              | 1,936        |
| 18-Aug                       | 19,893           | 21.25          | 103.30           | 239.47            | 0.036        | 1.51 | 0.86                   | 8.25                              | 1,936        |
| 30-Aug                       | 19,672           | 20.39          | 114.11           | 321.15            | 0.038        | 1.28 | 1.11                   | 11.06                             | 1,936        |
| 13-Sep                       | 19,452           | 19.57          | 125.06           | 419.84            | 0.040        | 1.08 | 1.12                   | 14.46                             | 1,936        |
| 27-Sep                       | 19,407           | 17.80          | 136.02           | 538.57            | 0.044        | 1.00 | 0.23                   | 18.55                             | 1,936        |
| 11-Oct                       | 19,401           | 14.99          | 146.86           | 679.47            | 0.051        | 1.27 | 0.03                   | 18.10                             | 2,506        |
| 25-Oct                       | 19,399           | 12.37          | 157.47           | 840.08            | 0.060        | 1.00 | 0.01                   | 26.57                             | 2,108        |
| 8-Nov                        | 19,398           | 10.98          | 167.71           | 1017.81           | 0.065        | 0.82 | <0.01                  | 32.19                             | 2,108        |
| 22-Nov                       | 19,396           | 8.38           | 177.47           | 1208.99           | 0.081        | 0.57 | 0.01                   | 38.23                             | 2,108        |
| 6-Dec                        | 19,390           | 6.59           | 186.60           | 1408.39           | 0.095        | 0.48 | 0.03                   | 44.54                             | 2,108        |

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

Table 3. Summary data of 450 Atlantic Salmon used and sacrificed for education and research activities during August 2016-March 2017.

| Date      | Number | Size category    | Use                              |
|-----------|--------|------------------|----------------------------------|
| 8-Aug-16  | 16     | Fall fingerlings | Dr. Moerke-Senior Thesis Advisee |
| 17-Nov-16 | 60     | Broodstock       | MSU-Health Inspection            |
| 6-Feb-17  | 100    | Sac fry          | Dr. Evans-Class                  |
| 6-Mar-17  | 6      | Yearlings        | Dr. Li-Class                     |
| 20-Mar-17 | 3      | Yearlings        | Dr. Li-Class                     |
| 21-Mar-17 | 65     | Yearlings        | MSU-Health Inspection            |
| 23-Mar-17 | 200    | Swim-ups         | Dr. Evans-Class                  |

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

Personnel from the ARL, with help from student volunteers and the Michigan Department of Natural Resources (MIDNR), collected returning adult Atlantic Salmon for broodstock from 31 October 2016 to 21 November 2016. 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 12:27-16:50 on 31 October 2016 (4.38 hr), from 09:05-12:25 and 13:38-14:50 on 1 November 2016 (4.53 hr), from 09:00-11:00 and 13:05-13:50 and 14:35-15:40 on 9 November 2016 (3.83 hr), and from 10:15-10:45 on 21 November 2016 (0.50 hr), for a total of 13.25 hr. A total of 315 Atlantic Salmon were collected; 115 on 31 October, 83 on 1 November, 108 on 9 November, and 9 on 21 November. The catch rate of Atlantic Salmon was about 23.7 fish per hr (Table 4). The 186 male fish sampled averaged 65.5 cm in total length and 2.62 kg in weight, whereas the 129 females sampled averaged 64.5 cm in total length and 2.9 kg in weight. Ages ranged from 1-6, with the majority (56.5%) of all fish captured being age 3 (Table 5). The average Fulton's condition factor  $K$  for all fish was 0.9356 in 2016 (SE=0.0093), and there was no discernable trend since record-keeping began in 1990 (Figure 1). Data on individual Atlantic Salmon used as broodstock are presented in Appendix 2. Forty-nine of the 315 fish (about 16%) were reared by the MIDNR based on the presence of an adipose fin clip. About 17% 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 58%), and about 78% of all scars were type B, whereas about 21% were type A (Table 6). No species other than Atlantic Salmon were captured during broodstock netting in 2016.

Table 4. Summary data from gill-netting of Atlantic Salmon broodstock from 1990-1994 and 1998-2016.

| Year | # of fish | Mean hrs/d | # of d | Area of net (m <sup>2</sup> ) | Mean # of fish/hr | Mean # fish/d |
|------|-----------|------------|--------|-------------------------------|-------------------|---------------|
| 1990 | 46        | ----       | 23     | 47                            | ----              | 2.0           |
| 1991 | 65        | 6.5        | 23     | 47                            | 0.4               | 2.8           |
| 1992 | 19        | 6.7        | 28     | 58                            | 0.1               | 0.7           |
| 1993 | 11        | 2.5        | 18     | 56                            | 0.2               | 0.6           |
| 1994 | 18        | 2.6        | 23     | 65                            | 0.3               | 0.8           |
| 1998 | 87        | 2.6        | 17     | 47                            | 2.0               | 5.1           |
| 1999 | 49        | 3.0        | 26     | 56                            | 0.6               | 1.9           |
| 2000 | 105       | 2.8        | 18     | 47                            | 2.0               | 5.8           |
| 2001 | 116       | 2.5        | 13     | 47                            | 3.6               | 8.9           |
| 2002 | 104       | 2.7        | 13     | 56                            | 2.9               | 8.0           |
| 2003 | 158       | 2.8        | 9      | 56                            | 6.4               | 17.6          |
| 2004 | 196       | 3.1        | 14     | 56                            | 4.5               | 14.0          |
| 2005 | 210       | 4.1        | 6      | 56                            | 8.5               | 35.0          |
| 2006 | 111       | 2.7        | 6      | 56                            | 6.8               | 18.5          |
| 2007 | 276       | 2.6        | 6      | 56                            | 17.5              | 46.0          |
| 2008 | 172       | 2.8        | 4      | 47                            | 15.4              | 43.0          |
| 2009 | 140       | 4.5        | 3      | 47                            | 10.4              | 47.0          |
| 2010 | 212       | 4.8        | 3      | 47                            | 14.8              | 70.7          |
| 2011 | 240       | 4.2        | 4      | 47                            | 14.2              | 42.4          |
| 2012 | 313       | 2.0        | 6      | 47                            | 26.2              | 52.2          |
| 2013 | 378       | 3.5        | 4      | 47                            | 27.4              | 94.5          |
| 2014 | 225       | 2.9        | 2      | 47                            | 38.8              | 112.5         |
| 2015 | 348       | 4.4        | 3      | 47                            | 26.2              | 116.0         |
| 2016 | 315       | 3.3        | 4      | 47                            | 23.8              | 78.8          |



Table 5. Age of Atlantic Salmon captured in fall 2016 broodstock collection efforts.

| Age     | Type of clip | # of males | # of females | Total # of fish |
|---------|--------------|------------|--------------|-----------------|
| 1       | RV           | 2          | 1            | 3               |
| 2       | RP           | 44         | 14           | 58              |
| 3       | LV           | 95         | 83           | 178             |
| 4       | LP           | 5          | 11           | 16              |
| 5       | RV           | 2          | 1            | 3               |
| 6       | RP           | 0          | 2            | 2               |
| Unknown | AD           | 33         | 16           | 49              |
| Unknown | NC           | 2          | 1            | 3               |
| Unknown | Unknown      | 3          | 0            | 3               |
| Total   |              |            |              | 315             |

Table 6. Classification of Sea Lamprey scars observed on Atlantic Salmon captured in 2016 broodstock collection efforts. Percentages are based on fish that had scars, which was about 17% of all fish collected.

| Species | Sex    | Scar type | Scar stage | # of scars | Percent |      |
|---------|--------|-----------|------------|------------|---------|------|
| ATS     | Male   | A         | I          | 1          | 2.1     |      |
|         |        | A         | II         | 0          | 0.0     |      |
|         |        | A         | III        | 5          | 10.4    |      |
|         |        | A         | IV         | 2          | 4.2     |      |
|         |        | Subtotal  |            |            | 8       | 16.7 |
|         |        | B         | I          | 1          | 2.1     |      |
|         |        | B         | II         | 2          | 4.2     |      |
|         |        | B         | III        | 4          | 8.3     |      |
|         |        | B         | IV         | 14         | 29.2    |      |
|         |        | Subtotal  |            |            | 21      | 43.8 |
|         | Female | A         | I          | 1          | 2.1     |      |
|         |        | A         | II         | 0          | 0.0     |      |
|         |        | A         | III        | 0          | 0.0     |      |
|         |        | A         | IV         | 2          | 4.2     |      |
|         |        | Subtotal  |            |            | 3       | 6.3  |
|         |        | B         | I          | 2          | 4.2     |      |
|         |        | B         | II         | 1          | 2.1     |      |
|         |        | B         | III        | 1          | 2.1     |      |
|         |        | B         | IV         | 16         | 33.3    |      |
|         |        | Subtotal  |            |            | 20      | 41.7 |
| Total-A |        |           |            | 11         | 22.9    |      |
| Total-B |        |           |            | 41         | 85.4    |      |

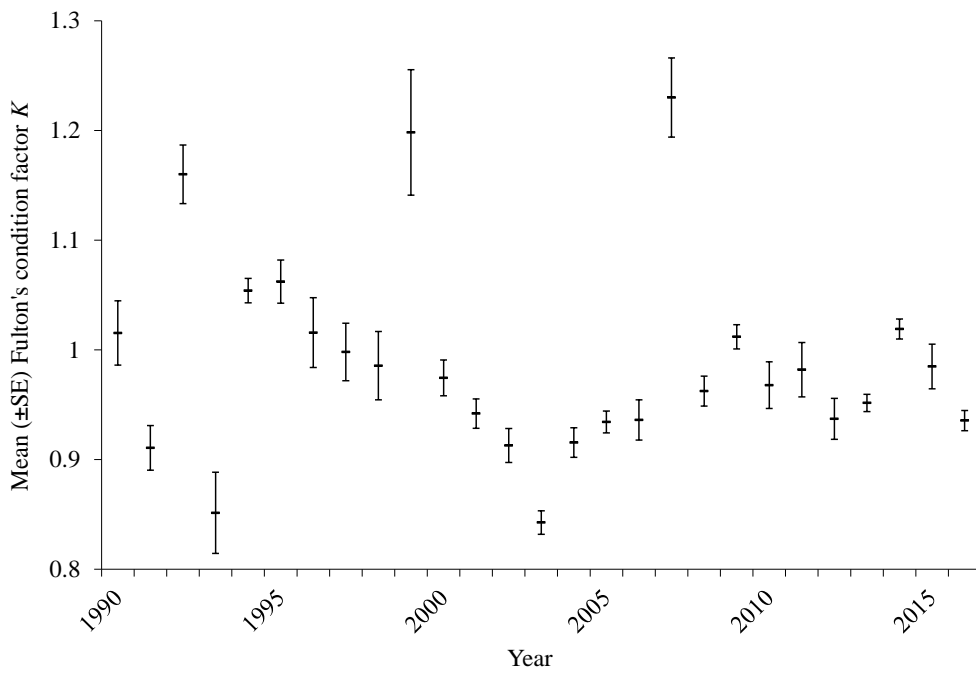


Figure 1. Annual mean ( $\pm$ SE) Fulton's condition factor  $K$  for Atlantic Salmon netted during 1990-2016.

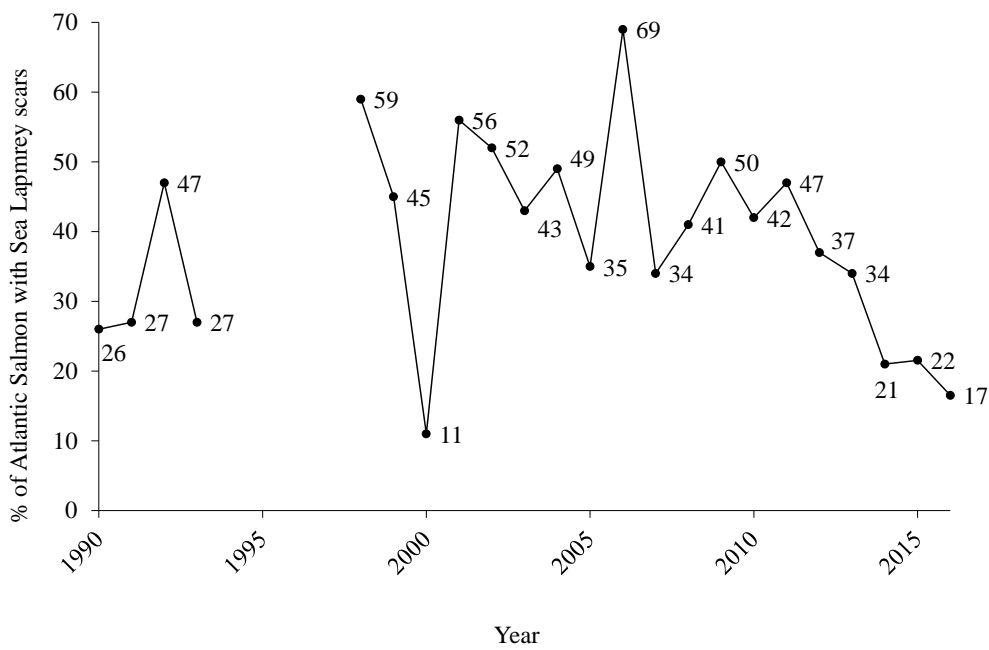


Figure 2. Percent of Atlantic Salmon broodstock that had at least one Sea Lamprey scar during 1990-1993 and 1998-2016.

Fish were held in raceways for at least one week prior to gamete collection, which occurred on 8, 10, 15, 17, 20 and 21 of November 2016. A 1 female: 1 male crossing scheme and the dry method were used during artificial spawning of 128 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 256 Atlantic Salmon brood fish tested negative for BKD (Appendix 3), fertilized eggs were mixed together according to the date of collection and placed into egg trays. Personnel from MSU arrived on 17 November 2016 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. Personnel from MSU also tested the gametes and kidney and spleen tissue from these 60 brood fish for BKD, Infectious Hematopoietic Necrosis Virus, Infectious Pancreatic Necrosis Virus, *Aeromonas salmonicida*, *Oncorhynchus masu* Virus, and Viral Hemorrhagic Septicemia Virus; all fish tested negative in gametes, while 10 tested positive for BKD in body tissues (1 medium, 9 low; Appendix 3).

We collected three age-6 fish during 2016 broodstock netting, and this was the first time we ever collected age-6 fish. All of these fish were females, and they averaged 742 mm total length and 3.8 kg in weight. One of the females had a Floy tag from a previous LSSU brood collection event, and it was determined that this female had been collected and spawned 2 years prior in the fall of 2014.

A total of 469,550 Atlantic Salmon eggs were collected in 2017 (Table 7). All fertilized eggs were treated according to MIDNR protocols, which included saline baths, erythromycin treatment, and disinfection in iodine. Dosages for each treatment are described in Appendix 4. On 5 January 2017, about 270,000 eyed eggs were transported by MIDNR personnel to the Platte River State Fish Hatchery.

For the first time, in 2016 our Atlantic Salmon were given thiamine treatments during the water hardening egg stage instead of post-hatch as was typically done in previous years. The thiamine treatments were conducted at the same time as our erythromycin bath, with both solutions present in the same bath. Thiamine baths were at a concentration of 20.4 g of 98% buffered thiamine per 20 L of filtered river water (thiamine provided by MIDNR). Our fish showed no signs of vitamin B1 deficiency once reaching the sac-fry stage, potentially due to treatments during the water hardening stage, so no additional thiamine baths were conducted.

Table 7. Summary data of egg collection efforts in 2016.

| Date        | # of pairs | Mean # of eggs/fish | Total # eggs |
|-------------|------------|---------------------|--------------|
| 8-Nov-16    | 8          | 3,853               | 30,826       |
| 10-Nov-16   | 21         | 3,087               | 64,834       |
| 15-Nov-16   | 33         | 4,202               | 138,567      |
| 17-Nov-16   | 23         | 3,972               | 91,363       |
| 20-Nov-16   | 16         | 4,080               | 65,286       |
| 21-Nov-16   | 21         | 3,741               | 78,564       |
| Grand total | 128        | 3,823               | 469,550      |

## RESEARCH ACTIVITIES (LSSU personnel listed in bold)

### *Grants & Contracts*

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**)

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**)

Acquisition of experimental tank-rack systems. The Fund for LSSU (2016; **Kapuscinski, K.L.**)

Water quality monitoring at the Hiawatha Sportsmen's Club. Hiawatha Sportsmen's Club (2016-2017; **Kapuscinski, K.L.**)

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)

Aquatic invasive species pilot studies in Michigan: *Myriophyllum spicatum* and *Didymosphenia geminata*. Michigan Department of Environmental Quality (2014-2016; **Moerke, A.H.**)

Ecological responses to restoration of flow to the Little Rapids area. Great Lakes Commission and National Oceanic and Atmospheric Administration (2014-2017; **Moerke, A.H.**)

Ontonagon River watershed freshwater mussel distribution within the Ottawa National Forest. Bond Falls Mitigation and Enhancement Fund (2017; Grant, G., sub-contract to **A.H. Moerke**)

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

Monitoring fish movement and fish condition in tributaries of Whitefish Bay. Bureau of Indian Affairs-Great Lakes Restoration Initiative Funds (2015-2016; Ripple, P., Zomer, F., **Moerke, A.H.**, **Kapuscinski, K.L.**, and **J. Li**)

Implementing Great Lakes coastal wetland monitoring. US Environmental Protection Agency (2016-2020; Uzarski, D., **Moerke, A.H.**, and 8 others)

## Peer-reviewed Publications

Chen, W., L. Yi, S. Feng, L. Zhao, **J. Li**, M. Zhou, R. Liang, N. Gu, Z. Wu, J. Tu, and L. Lin. 2017. Characterization of microRNAs in orange-spotted grouper (*Epinephelus coioides*) fin cells upon red-spotted grouper nervous necrosis virus infection. *Fish & Shellfish Immunology* 63:228–236.

Nervous necrosis virus (NNV), one of the most prevalent fish pathogens, has caused fatal disease of viral nervous necrosis (VNN) in many marine and freshwater fishes, and resulted in heavy economic losses in aquaculture industry worldwide. However, the molecular mechanisms underlying the pathogenicity of NNV remain elusive. In this study, the expression profiles of microRNA (miRNA) were investigated in grouper fin (GF-1) cells infected with red-spotted grouper nervous necrosis virus (RGNNV) via deep sequencing technique. The results showed that a total of 220 miRNAs were identified by aligning the small RNA sequences with the miRNA database of zebrafish, and 18 novel miRNAs were predicted using miRDeep2 software. Compared with the non-infected groups, 51 and 16 differentially expressed miRNAs (DE-miRNAs) were identified in the samples infected with RGNNV at 3 and 24 h, respectively. Six DE-miRNAs were randomly selected to validate their expressions using quantitative reverse transcription polymerase chain reaction (qRT-PCR), the results showed that their expression profiles were consistent with those obtained by deep sequencing. The target genes of the DE-miRNAs covered a wide range of functions, such as regulation of transcription, oxidation-reduction process, proteolysis, regulation of apoptotic process, and immune response. In addition, the effects of four DE-miRNAs including miR-1, miR-30b, miR-150, and miR-184 on RGNNV replication were evaluated, and the results showed that over-expression of each of the four miRNAs promoted the replication of RGNNV. These data provide insight into the molecular mechanism of RGNNV infection, and will benefit for the development of effective strategies to control RGNNV infection.

Crane, D.P., and **K.L. Kapuscinski**. 2017. Habitat use by age-0 Muskellunge in the upper Niagara River, New York. *Hugh C. Becker Muskellunge Symposium Proceedings*. American Fisheries Society Special Publication, Bethesda, Maryland. Pagination pending.

Studies of fine-scale habitat characteristics associated with age-0 Muskellunge *Esox masquinongy* are uncommon and those that have been conducted have relied on targeted, nonrandom sampling designs, which may bias study results. We used a random design to sample age-0 Muskellunge and shallow-water (<1.3 m) habitat features in the upper Niagara River, New York during late July through early September 2013–2015. Comparisons of habitat features between sites where Muskellunge were present and absent and Firth logistic regression were used to identify important characteristics of Muskellunge nursery locations. A total of 15 age-0 Muskellunge were collected at 11 of 295 sites sampled. *Vallisneria americana* was the dominant aquatic vegetation at 10 of 11 sites where Muskellunge were collected, and sand and mud were the dominant substrate sizes at all locations where age-0 Muskellunge were collected. The probability of age-0 Muskellunge presence was positively related to the proportion of the water column occupied by aquatic vegetation. Despite sampling nearly 300 sites, the small number of age-0 Muskellunge collected limited the types of analyses that could be performed. However, our results provide evidence that shallow-water areas with abundant *V. americana* should be

conserved or restored to provide rearing habitat for Muskellunge in the upper Niagara River. In future studies, sample sizes of age-0 Muskellunge may be increased, while maintaining a probability sampling design, by randomly sampling within predefined areas that contain habitat features identified at sites where Muskellunge were present in our study.

Farrell, J.M., Getchell, R.G., **Kapuscinski, K.L.**, and S.R. LaPan. 2017. Long-term trends of St. Lawrence River Muskellunge: effects of viral hemorrhagic septicemia and Round Goby proliferation creates uncertainty for population sustainability. American Fisheries Society Special Publication, Bethesda, Maryland. Pagination pending.

Long-term research indicates a significant and ongoing decline within the upper St. Lawrence River Muskellunge *Esox masquinongy* population. Index surveys show a sharp reduction in catch of both spawning adults and age-0 Muskellunge, and catch rates by anglers have similarly declined while harvest remains low. Other changes associated with population decline include presence of fewer female adult Muskellunge and a change in adult Muskellunge size structure (increase in proportion of fish <1,016 mm) in addition to more very large individuals >1,372 mm. A significant adult die-off occurred from 2005-2008 (103 adults recovered in US and Canadian waters) concomitant with an outbreak of Viral Hemorrhagic Septicemia (VHS). These population changes were also temporally correlated with detection and proliferation of invasive Round Goby *Neogobius melanostomus*, a known VHS virus (VHSV) reservoir, egg predator, and competitor with native fishes. Comparisons of index netting before and after VHSV and Round Goby invasions suggest a direct link to the decline, but because these are correlations, we can only explore these effects. To examine the viability of Muskellunge nursery sites, we repeated survival studies conducted in the early 1990s with experimental releases of advanced fry at four locations during 2013-2015. Findings indicate contribution to age-0 populations, but catches post-stocking (wild and stocked) were lower compared to the 1990s. We review information regarding potential stressors, including VHSV and Round Goby invasion, and conclude their combined effects have created significant uncertainty and challenges to sustainable management of the Muskellunge population. In response, the St. Lawrence River Muskellunge management plan should be updated with a focus on restoration of the declining Muskellunge stock. Recommended actions target advancing conservation and restoration of critical habitat, restoring lost sub-populations, and reducing mortality associated with angling (e.g., from handling and harvest).

Forzono, E.M., Crane, D.P., **Kapuscinski, K.L.**, and M.M. Clapsadl. 2017. Dry-weight energy density of prey fishes from nearshore waters of the upper Niagara River and Buffalo Harbor, New York. *Journal of Great Lakes Research* 43(3):215–220.

Energy density of prey fishes can affect the survival, growth, and fitness of piscivorous fishes, and these vital rates may change – for better or worse – after fish communities are altered by the establishment of new species. Invasive round goby (*Neogobius melanostomus*) and rudd (*Scardinius erythrophthalmus*) are highly abundant in the upper Niagara River and Buffalo Harbor and serve as alternative food for piscivores. However, there is a paucity of information on the energy density of native and invasive prey fishes in these waters. To better understand the energy density of available prey fishes in nearshore areas of the upper Niagara River and Buffalo Harbor, we compared the energy densities of: (1) native fishes and invasive fishes, (2) age-0 and

yearling-and-older conspecific fishes, and (3) upper Niagara River and Buffalo Harbor conspecific fishes. Fishes were collected from New York waters of the upper Niagara River and Buffalo Harbor during early August through mid-September 2013. We combusted fishes in a bomb calorimeter to determine dry-weight energy densities (J/g) for two invasive and eight native species. Energy densities were dependent on an interaction between fish species and age group, and did not differ between the upper Niagara River and Buffalo Harbor. Yearling-and-older spottail shiner (*Notropis hudsonius*) had a significantly higher energy density than all other species examined and was the only species with a significant difference in energy density between age classes. Rudd had an energy density similar to most native fishes; and, although not significantly lower, round goby energy density was lower than most native fishes. Our energy density estimates can be used to better understand mechanisms affecting growth and condition of piscivorous fishes in the upper Niagara River and Buffalo Harbor.

Ji, L., G.X. Sun, **J. Li**, Y. Wang, Y.S. Du, X. Li, and Y. Liu. 2017. Effect of dietary  $\beta$ -glucan on growth, survival and regulation of immune processes in rainbow trout (*Oncorhynchus mykiss*) infected by *Aeromonas salmonicida*. *Fish & Shellfish Immunology* 64:56–67.

The present study evaluated the effects of dietary  $\beta$ -glucan (0, 0.05%, 0.1%, and 0.2%) on growth performance after 42 days of feeding. Thereafter, rainbow trout (*Oncorhynchus mykiss*) were infected with *Aeromonas salmonicida*, and survival rates as well as the regulating processes of stress- and immune-related factors were analyzed. In general, higher dietary  $\beta$ -glucan levels obviously improved specific growth rate (SGR), weight gain (WG) and feed efficiency (FE) ( $P \leq 0.05$ ). Survival rates in  $\beta$ -glucan groups increased significantly compared with the control group after *A. salmonicida* infection ( $P \leq 0.05$ ). Serum total superoxide dimutase (T-SOD), peroxidase (POD) as well as catalase (CAT) activities, and their mRNA expressions in the head kidney of fish in the  $\beta$ -glucan groups generally increased to higher levels after infection, and more quickly, compared with in the control group. Serum lysozyme (LSZ) and its expression in the head kidney in  $\beta$ -glucan groups reached a higher peak earlier than in the control group. Serum glutamic oxalacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) levels in the  $\beta$ -glucan groups were significantly lower than in the control group ( $P \leq 0.05$ ). The peak of heat shock protein 70 (HSP70) expression in the 0.2%  $\beta$ -glucan group was higher and occurred earlier than in other groups ( $P \leq 0.05$ ). These results confirm that 0.1% and 0.2% dietary  $\beta$ -glucan are beneficial for promoting growth in rainbow trout and enhancing resistance against *A. salmonicida*. Furthermore,  $\beta$ -glucan could play an important role in regulating stress- and immune-related factors in rainbow trout to more quickly fight against bacterial infection.

Li, M., **J. Li**, and L. Sun. 2016. CsMAP34, a teleost MAP with dual role: A promoter of MASP-assisted complement activation and a regulator of immune cell activity. *Scientific Reports* 6, 39287; doi: 10.1038/srep39287.

In teleost fish, the immune functions of mannan-binding lectin (MBL) associated protein (MAP) and MBL associated serine protease (MASP) are scarcely investigated. In the present study, we examined the biological properties both MAP (CsMAP34) and MASP (CsMASP1) molecules from tongue sole (*Cynoglossus semilaevis*). We found that CsMAP34 and CsMASP1 expressions occurred in nine different tissues and were upregulated by bacterial challenge. CsMAP34 protein was detected in blood, especially during bacterial infection. Recombinant CsMAP34

(rCsMAP34) bound *C. semilaevis* MBL (rCsBML) when the latter was activated by bacteria, while recombinant CsMASP1 (rCsMASP1) bound activated rCsBML only in the presence of rCsMAP34. rCsMAP34 stimulated the hemolytic and bactericidal activities of serum complement, whereas anti-CsMAP34 antibody blocked complement activities. Knockdown of CsMASP1 in *C. semilaevis* resulted in significant inhibition of complement activities. Furthermore, rCsMAP34 interacted directly with peripheral blood leukocytes (PBL) and enhanced the respiratory burst, acid phosphatase activity, chemotactic activity, and gene expression of PBL. These results indicate for the first time that a teleost MAP acts one hand as a regulator that promotes the lectin pathway of complement activation via its ability to recruit MBL to MASP, and other hand as a modulator of immune cell activity.

**Moerke, A., C. Ruetz III, T. Simon, and C. Pringle.** 2017. Chapter 19: Macroconsumer-resource interactions pages 399-412 *in* R. Hauer and G. Lamberti, editors. *Methods in Stream Ecology*, 3<sup>rd</sup> edition, Academic Press, CA.

Macroconsumers, such as fish, decapod crustaceans, and amphibians, can be important determinants of stream structure and function. In this chapter, we present three types of manipulative field experiments to evaluate top-down effects of macroconsumers in stream food webs and illustrate the role of macroconsumers in shaping prey communities and resource availability. The most basic approach described is to use mesh enclosures and open-cage controls to test how stream invertebrate density, benthic algal biomass, and/or leaf breakdown rates respond to the absence of macroconsumers. Next, we present a more advanced approach to address similar ecological questions with electric enclosures, which reduce the cage artifacts resulting from the physical effects of cage enclosures. Finally, we describe an optional enclosure/exclosure experiment to directly manipulate macroconsumer density in mesh cages. We also discuss modifications to these three approaches to evaluate macroconsumer roles in algal- or detrital-based foods webs and in initiating trophic cascades.

Miller, L.A., Farrell, J.M., **Kapuscinski, K.L.**, Scribner, K.T., Sloss, B.L., Turnquist, K.N., and C.C. Wilson. 2017. A review of Muskellunge population genetics: implications for management and future research needs. American Fisheries Society Special Publication, Bethesda, Maryland. Pagination pending.

At the first international musky symposium held in 1984, participants recognized that management agencies needed policies for sustainable management of native Muskellunge *Esox masquinongy* stocks. Identified research needs included documenting how genetic diversity was distributed within and among natural populations and evaluating effects of management actions on diversity. Here, we summarize research over the past three decades that has addressed these needs and provided additional genetic information useful to managers. We then suggest future research directions to fill information gaps and benefit from advances in genetic marker technology and methods of statistical inference. Genetic data support the existence of distinct regional lineages associated with the upper Mississippi River, Great Lakes, and Ohio River drainages, which all likely derived from Mississippian glacial refugia. Each lineage exhibits substructure, with numerous genetically distinct subgroups influenced to varying degrees by geological history, geographic proximity, habitat connectivity, and human activities. When traits such as growth, maximum size, survival and food consumption have been compared among strains stocked into common environments, researchers have often found differences attributable



to genetic causes. Genetic evaluations of ancestry in relation to stocking have revealed a wide range of outcomes from substantial strain mixing and interbreeding to no apparent contribution to resident populations. Genetic principles and data have led to stocking guidelines for conserving within- and among-population genetic variation and avoiding artificial selection in broodstock practices. Future research should include a rangewide assessment of population genetic structure, including assessments of how stocking has affected the spatial structure of wild populations. Genetic data should be used to evaluate retention of genetic diversity in hatchery broodstocks and assess fitness effects of stocked fish on wild populations. Applications of genomic techniques can better inform managers of the genetic basis for differences in performance or adaptive traits and the potential negative consequences of imprudent management practices.

Turnquist, K.N., Larson, W., Farrell, J.M., **Kapuscinski, K.L.**, Miller, L.A., Scribner, K.T., Wilson, C.C., and B.L. Sloss. 2017. Extended Abstract: Spatial genetic structure of Muskellunge in the Great Lakes Region and the effects of supplementation on genetic integrity of remnant stocks. American Fisheries Society Special Publication, Bethesda, Maryland. Pagination pending.

Uzarski, D.G., V.J. Brady, M.J. Cooper, D.A. Wilcox, D.A. Albert, R.P. Axler, P. Bostwick, T.N. Brown, J.H. Ciborowski, N.P. Danz, J.P. Gathman, T.M. Gehring, G.P. Grabas, A. Garwood, R.W. Howe, L.B. Johnson, G.A. Lamberti, **A.H. Moerke**, B.A. Murry, G.J. Niemi, C.J. Norment, C.R. Ruetz III, A.D. Steinman, D. Tozer, R. Wheeler, T.K. O'Donnell, and J.P. Schneider. 2016. Standardized measures of coastal wetland condition: Implementation at a Laurentian Great Lakes Basin-wide scale. *Wetlands*.  
Doi:10.1007/s13157-016-0835-7

Since European settlement, over 50% of coastal wetlands have been lost in the Laurentian Great Lakes basin, causing growing concern and increased monitoring by government agencies. For over a decade, monitoring efforts have focused on the development of regional and organism-specific measures. To facilitate collaboration and information sharing between public, private, and government agencies throughout the Great Lakes basin, we developed standardized methods and indicators used for assessing wetland condition. Using an ecosystem approach and a stratified random site selection process, birds, anurans, fish, macroinvertebrates, vegetation, and physico-chemical conditions were sampled in coastal wetlands of all five Great Lakes including sites from the United States and Canada. Our primary objective was to implement a standardized basin-wide coastal wetland monitoring program that would be a powerful tool to inform decision-makers on coastal wetland conservation and restoration priorities throughout the Great Lakes basin.

### *Presentations*

**Cortell, C.J., Kapuscinski, K.L.**, and C. Kovacs. Trends in relative abundance of sport fishes of the Tahquamenon River, MI, 1993-2016. Michigan Chapter of the American Fisheries Society (Poster, 2017).

Angler attitudes towards management actions, such as stocking Muskellunge *Esox masquinongy*, and the management actions themselves have changed over the last 50 years in the Tahquamenon

River and its watershed. Regulations including increased minimum size limits and decreased bag limits over the past three decades have been put in place to conserve trophy class Muskellunge. Some anglers and sportsmen's clubs have supported these management actions, while others strongly opposed them. However, it is unknown if and how these management actions may have affected populations of other sport fishes. We analyzed data from standardized electro-fishing surveys conducted at seven locations during 1993-2016 to assess trends in population characteristics of publicly valued sport fishes. Our objectives were to determine (1) if annual catch-per-unit effort (CPUE) of Muskellunge, Northern Pike *E. lucius*, and Walleye *Sander vitreus* changed through time, (2) if the percent composition of species changed through time, and (3) in which years mean length exceeded the minimum size limit for each species. Annual mean CPUE and percent composition of each species did not differ among years. Lastly, mean length of Muskellunge and Northern Pike never exceeded current minimum length regulations, while the average length of Walleye reached or exceeded the current regulation every year since 1993. Our results suggest that increasingly restrictive regulations for Muskellunge have not adversely affected sympatric populations of Northern Pike or Walleye in the Tahquamenon River.

**Cortell, C.J., Kapuscinski, K.L.,** and C. Kovacs. Trends in relative abundance of sport fishes of the Tahquamenon River, MI, 1993-2016. Midwest Fish & Wildlife Conference (Poster, 2017).

See abstract above.

Crane, D.P., and **K.L. Kapuscinski.** 2017. Capture efficiency of a fine mesh seine in shallow riverine habitats. South Carolina Chapter-American Fisheries Society.

No abstract.

**Freebairn, M. and A.H. Moerke.** Behavioral response of Round Goby (*Neogobius melanostomus*) to auditory calls produced by reproductive con-specifics. Michigan Chapter of the American Fisheries Society (Poster, 2017).

Round Goby (*Neogobius melanostomus*) is an invasive fish species introduced into the Great Lakes in the 1990s via ship ballast waters from Eurasia. Gobies outcompete native benthic fish and alter invertebrate populations; therefore managers are seeking ways to control Goby populations. Male Goby use visual, chemical and auditory cues to attract and initiate courtship among females during their spawning season. Conspecific calls are only perceived by other Goby and therefore auditory signals could be used in the future to attempt to control Goby. We conducted preliminary tests to determine an effective distance and response time to auditory calls. An artificial river channel (6 x.7 m) was constructed in the Cheboygan River in August 2016. Male conspecific auditory calls were played using an underwater speaker at 0.5, 1.5 and 3 m. There was no significant difference in the departure time from the starting point or speed towards either speaker at 0.5, 1.5 and 3 m for females. An effective distance and response time for initiating behavioral responses towards auditory calls was not found for females. There was no significant difference in departure time, however there was a significant difference in speed at

0.5 and 1.5 m distances. These findings may indicate an effective range of 1.5 m for male Gobies to exhibit a behavioral response to auditory calls.

**Jodoin, C. and A.H. Moerke.** Assessment of Lake Sturgeon (*Acipenser fulvescens*) spawning in a St. Marys River tributary. Michigan Chapter of the American Fisheries Society (Poster, 2017).

The Lake Sturgeon (*Acipenser fulvescens*), once abundant, is now considered a threatened species in the Great Lakes. In the early 1900s, Lake Sturgeon were pushed to near extinction as a direct result of human pressures, including overfishing and habitat destruction. Currently, little is known about Lake Sturgeon spawning habitat or behavior in many areas of the Great Lakes including the St. Marys River. The objective of this study was to determine if Lake Sturgeon were using the Carp River (Mackinaw County, Michigan) as a spawning tributary to the St. Marys River. To answer this question, we assessed larval fish drift in the Carp River from May 8-June 15, 2016, corresponding to water temperatures suitable for larval sturgeon drift. In total 3,063 larval fish were collected, preserved and identified to the family in the laboratory. No larval Lake Sturgeon were captured, but other fishes that drift at similar temperatures were observed including Sculpin (*Cottidae*), Smelt (*Osmeridae*), and Suckers (*Catostomidae*). Future assessments are needed to determine if the Carp River or surrounding tributaries are being used as Lake Sturgeon spawning grounds.

**Li, J.** Effects of dietary beta-glucan on growth performance, disease resistance and immune responses in salmonid fish. The joint meetings of AFS-FHS and Great Lake Fish Commission FHC meeting and the 42<sup>nd</sup> Annual Eastern Fish Health Workshop (2017)

In the present study, we evaluated the effects of different dietary  $\beta$ -glucan dosages (0, 0.05%, 0.1% and 0.2% in regular fish feed) on growth performance, innate immunity and disease resistance against *Aeromonas salmonicida* challenge in salmonid fish. In general, higher dietary  $\beta$ -glucan levels could obviously improve specific growth rate (SGR) at  $P \leq 0.05$ . Survival rate in  $\beta$ -glucan groups increased significantly than the control group after infection by *A. salmonicida* ( $P \leq 0.05$ ). Serum superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) activities and their mRNA expression in head kidney generally increased to higher levels in shorter time than control group after infection; serum lysozyme (LSZ) and its expression in head kidney in  $\beta$ -glucan groups reached a higher peak earlier than the control group. Heat shock protein 70 (HSP70) expression and serum cortisol were also evaluated in the fish. Our results clearly confirmed that dietary  $\beta$ -glucan in 0.1% and 0.2% levels is beneficial for promoting growth and enhancing resistance against *A. salmonicida* of salmonid fish. Furthermore,  $\beta$ -glucan could play an important role in regulating stress- and immune-related factors in rainbow trout to fight against bacterial infection in a short time.

**Milan, J., Moerke, A., Kapuscinski, K., and P. Ripple.** Seasonal fish migration patterns in three Lake Superior tributaries. Michigan Chapter of the American Fisheries Society (2017).

The numerous small tributaries that feed the Great Lakes likely play an important role for sustaining lake fish populations. In the Great Lakes, there are approximately 70 species known to spawn in rivers and streams, 19 of which are suggested to be obligate stream spawners. These

tributaries provide critical habitat and nutrients for the offspring of the migratory fishes using them. As important as these tributaries appear to be, very little information exists on the extent or duration to which they are used. The objective of this study was to document the spawning migration patterns (numbers and timing) of Lake Superior fishes that use these tributaries throughout the year. To accomplish this, fyke nets were placed in three tributaries of Whitefish Bay, Lake Superior. Two nets were set biweekly back-to-back across each stream to capture in and out migrating fishes. Initial results showed 42 different species (12 migrants), with approximately 11,902 individuals captured among the three tributaries during the time of the study. The outcomes of this study will provide additional information on the importance of tributaries for Lake Superior fishes and provide baseline data to understand the potential impacts of climate change on the spawning phenology of Great Lakes fishes.

**Steinmetz, C. and A.H. Moerke.** Preliminary investigation into the effect of microbeads on growth of juvenile Atlantic Salmon. Michigan Chapter of the American Fisheries Society (Poster, 2017). Received Best Student Poster Award.

Microbeads, plastics less than 5 mm in size, are found in high concentrations (up to 1 mil/km<sup>2</sup>) in Great Lakes water as a result of plastics being washed down drains in beauty products or from degradation of larger plastic debris. Several studies have shown that microbeads are ingested by aquatic organisms and are transferred up the food web. Fewer studies have shown there are negative impacts associated and some have suggested no impacts. Very little research has been done into the effects microbeads have on the Great Lakes and its organisms. Our objective was to determine if microbeads provided to juvenile Atlantic Salmon as food affected growth. Four levels of microbeads were added to a pellet food diet and fed to juvenile Atlantic Salmon in tanks over 4 weeks. Growth, along with qualitative metrics (e.g., fish appearance) were recorded. The results showed that there was no difference in weight and length between treatment groups, indicating that microbeads did not affect growth. However, other factors such as stress and water quality were likely overriding the effects of microbeads, and therefore additional studies are warranted.

**Stutesman, J., Edwards, J., and A.H. Moerke.** Distribution and phenology of *Didymosphenia geminata* in the St. Marys River, a Great Lakes connecting channel. Midwest Fish and Wildlife Conference (Poster, 2017).

In recent decades, blooms of *Didymosphenia geminata* (Didymo), a stalk-producing diatom, have been observed more frequently in rivers throughout the world. In 2015, Didymo was documented blooming in the rapids of the St. Marys River, which was the first reported occurrence of Didymo blooms in Michigan waters. Didymo blooms can affect macroinvertebrates, fisheries, and aesthetics in streams and rivers, and therefore managers are interested in controlling these blooms. The objective of this study was to document distribution and quantify the density and blooming patterns (phenology) of Didymo in the upper St. Marys River (Sault Ste. Marie, Michigan and Ontario) in relation to water quality. Twenty-eight sites in the Eastern Upper Peninsula of Michigan were sampled for water quality and Didymo in mid-summer 2016 to document distribution. At two sites in the Main Rapids, water quality (e.g., conductivity) was measured in situ and rock scrapes were collected biweekly from May to September 2016 to quantify Didymo cell and stalk density. Didymo was present in 16 of the 28

locations sample, but presence was limited to sites ~30 km downstream of the rapids. *Didymo* was absent from tributary samples. Densities of *Didymo* cells and stalks were highest in the Main Rapids in late spring and declined throughout summer. These findings suggest that *Didymo* is currently restricted to the river, and blooms are highest in the in late spring, which may correspond to fish hatching and emergence timing. These data will serve as a baseline for monitoring *Didymo* change in Michigan waters.

**Stutesman, J., Edwards, J., and A.H. Moerke.** Distribution and phenology of *Didymosphenia geminata* in the St. Marys River, a Great Lakes connecting channel. Michigan Chapter of the American Fisheries Society (Poster, 2017).

See abstract above.

## 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 12 student projects related to the ARL and Fish Disease Lab that were completed during the 2016-2017 academic year.

Concentrations of thiamine in Atlantic Salmon eggs and sacfry from the St. Marys River, Michigan

Scott Cooper, Jun Li

The primary diet of adult Atlantic Salmon (*Salmo salar*) (ATS) consists of various baitfish species which have adverse effects to the embryonic health of ATS offspring, causing thiamine deficiency syndrome (TDS). The thiamine (vitamin B1) degrading disease causes high mortality rates among offspring resulting in overall population decline. To counteract the effects of TDS, hatcheries currently administer standard protocol treatments which involve immersing ATS sacfry in a concentrated thiamine solution however, hatcheries could potentially save valuable resources by treating an alternate life stage. In this study, thiamine levels of offspring from five pair of male and female adults were analyzed in various life stages to determine the optimal time of treatment. Samples of eggs and sacfry were collected and thiamine content was calculated using the rapid reversed-phase solid-phase extraction (RP-SPE) method. Results indicated that feral ATS offspring are thiamine deficient (<1 nmol/g) and hatcheries should revise the standard protocol to administer treatment in egg stage, which would be a convenient alternative if completed during the water hardening process.

Trends in relative abundance of sport fishes of the Tahquamenon River, MI, 1993-2016

Christopher Cortell, Kevin Kapuscinski

See abstract above.

## Comparison of fish assemblages in adventitious and non-adventitious streams

Samuel Day, Ashley Moerke

Adventitious streams are unique streams that differ from the general concept of stream networks in that they do not mark a gradual change in stream size and habitat from the mainstem. These sharp habitat differences may result in more unique fish assemblages. The objectives of this study were to determine if fish assemblages of adventitious streams differed from those of non-adventitious streams in the Pine River watershed (Eastern Upper Peninsula, MI), and if these differences could be attributed to habitat differences. There were no significant differences in Shannon-Weiner diversity values between adventitious and non-adventitious streams. Species richness and similarity varied more by stream position than stream type. However, differences in habitat, including stream width, temperature, and discharge were observed between adventitious and non-adventitious streams, whereas specific conductivity varied more by stream position than stream type. These results suggest that watershed factors (e.g., land use, surficial geology) may have a stronger influence on fish assemblage structure than stream type (i.e., adventitious or non-adventitious) in the Pine River watershed. Fisheries managers could use this information to prioritize management efforts within the watershed.

## Behavioral response of Round Goby to auditory calls produced by reproductive con-specifics

Morgan Freebairn, Ashley Moerke

Round Goby (*Neogobius melanostomus*) is an invasive fish introduced into the Great Lakes in the 1990s via ship ballast waters from Eurasia. Gobies outcompete native benthic fish and alter invertebrate populations; therefore managers are seeking ways to control Goby populations. Male Goby use visual, chemical and auditory cues to attract females during their spawning season. Conspecific calls are only perceived by other Goby and therefore auditory signals could be used in the future to attempt to control the spread of Goby. We conducted preliminary tests to determine an effective distance and the time needed to respond to conspecific calls. An artificial river channel (6 x 7 m) was constructed in the Cheboygan River in August 2016. Male conspecific auditory signals were played using an underwater speaker at 0.5, 1.5 and 3 m. There was no significant difference in the departure time from the starting point or speed towards either speaker at 0.5, 1.5 and 3 m for females. There was no significant difference in departure time for males, however, there was a significant difference in speed at 0.5 and 1.5. These findings may indicate an effective range for male Gobies to have a behavioral response at 0.5 and 1.5 m to conspecific calls.

## Developing a new methodology to detect microplastics in aquatic organisms

Rachel Frey, Ashley Moerke

Microplastic particles (<5 mm) are polluting the world's lakes and oceans, and are negatively impacting aquatic organisms that mistake them for a viable food source. Current detection methods such as dissection and microscopic analysis are time consuming and labor intensive. Here, a new methodology was investigated to detect microplastics in aquatic organisms using

fluorescence optical imaging (2D) scans. Known quantities were placed in solution and inside whole juvenile Atlantic Salmon (*Salmo salar*) mortalities then scanned using multiple fluorescence filters on a Bruker Xtreme image station. Fluorescence (integrated density) was quantified from scanned images. Polyethylene ratios were greater than one at 540 nm wavelength and polypropylene and polylactic acid ratios were highest in 430, 540 and 630 nm wavelengths. The use of optical imaging to detect plastics is promising alternative to traditional methods of detecting plastics in organisms. Method refinement and consistency is encouraged in future studies.

### Assessment of Lake Sturgeon spawning in a St. Marys River tributary

Cody Jodoin, Ashley Moerke

The Lake Sturgeon (*Acipenser fulvescens*), once abundant, is now considered a threatened species in the Great Lakes. In the early 1900s, Lake Sturgeon were pushed to near extinction as a direct result of human pressures, including overfishing and habitat destruction. Currently, little is known about Lake Sturgeon spawning habitat or behavior in many areas of the Great Lakes including the St. Marys River. The Garden River (Ontario, Canada) is the only known tributary to the St. Marys where successful Lake Sturgeon reproduction has been documented. The objective of this study was to determine if Lake Sturgeon were using the Carp River (Mackinaw County, Michigan) as a spawning tributary to the St. Marys River. To answer this question, we assessed larval fish drift in the Carp River from May 8-June 15, 2016, corresponding to water temperatures suitable for larval sturgeon drift. In total 3,063 larval fish were collected, preserved and identified to the family in the laboratory. No larval Lake Sturgeon were captured, but other fishes that drift at similar temperatures were observed including Sculpin (Cottidae), Smelt (Osmeridae), and Suckers (Catostomidae). Suckers comprised 55% of the catch, followed by Smelt at 16%, and then Sculpin at 29%. Because Lake Sturgeon are a long lived, iteroparous species with individuals that do not spawn annually, it is possible that spawning did not occur during this study year. Future assessments are needed to determine if the Carp River or surrounding tributaries are being used as Lake Sturgeon spawning grounds.

The effect of static and diminishing diet regimes on growth and survival of Cisco larvae in a culture setting

Clifford Pattinson, Kevin Kapuscinski

The Cisco (*Coregonus artedii*) is a native species of paramount importance both ecologically and economically in the Great Lakes Region. After the collapse of Alewives (*Alosa pseudoharengus*) in Lake Huron, there is now an opportunity to rehabilitate Cisco populations that were depleted via overharvest, habitat destruction, and adverse interactions with invasive species. However, little is known about raising Cisco in a hatchery environment. The purpose of my study was to determine which of the two diet regimes, static or diminishing, would result in better growth, condition, feeding efficiency, and survival of Cisco during the larval stage. Five replicates of each diet regime were maintained for three weeks post-yolk absorption. T-tests were used to determine if each performance variable differed between diet regimes. There were no significant differences between mean growth, condition or feeding efficiency. However, survival was higher

for Cisco fed the diminishing diet, likely due to decreasing water quality of the static feeding group over time. Longer term feeding trials are recommended to help further understand which feeding regime would be best for rearing Cisco.

#### Monitoring changes in lake water quality following liming and aeration at the Hiawatha Sportsman Club

Anthony Saxman, Kevin Kapuscinski

Lakes within the property of the Hiawatha Sportsman Club, located in Engadine, MI, often have water qualities that may not support fisheries desired by the club members. For example, Cranberry and Elbow lakes have low pH levels, which have been managed by adding crushed limestone. Pullup Lake has low dissolved oxygen levels, which has been managed by installation of three aerators. However, water quality monitoring has not been conducted consistently to determine if these management actions have been effective. The objectives of my study were to (1) monitor changes in pH and dissolved oxygen levels following liming at Cranberry and Elbow lakes, and (2) determine if aerating windmills effectively oxygenated the water in Pullup Lake. Water samples were conducted once a month from March, 2016 and until there was ice on the lakes. Conductivity, dissolved oxygen, pH, and temperature were measured at three depths at each location (near surface, middle, near floor). Hardness and alkalinity were also measured at the surface at all locations. The pH increased following limestone application in both lakes, but levels decreased back to previous ranges about one month after application. Oxygen levels at Pullup Lake were commonly below 5 mg/L at the bottom, which is inadequate to support desired fisheries. I recommend conducting more frequent sampling events throughout the year with less amounts of limestone being added to Cranberry and Elbow lakes and also to install more aerators to Pullup Lake in order to achieve adequate water quality for the desired fisheries.

#### Effects of temperature on egg development of Cisco

Alleigh Sexton, Kevin Kapuscinski

The Cisco (*Coregonus artedii*) is a native, zooplanktivorous fish of the Great Lakes Region that is both ecologically and economically important. The recent collapse of invasive Alewife (*Alosa pseudoharengus*) in Lake Huron has created an opportunity to rehabilitate Cisco populations; however, lack of knowledge about best practices for rearing Cisco in hatchery environments complicates rehabilitation efforts. The objective of my study was to quantify rates of development and survival of Cisco reared at 4, 6, and 8 °C. Cisco eggs were incubated in a recirculating system at one of three water temperatures (4, 6, or 8 °C), with six replicate jars per system (N=18). Mean days to the eyed stage, days to hatch, and percent survival were calculated for each temperature treatment. Times varied significantly between system for days required to eye and hatch. The treatments also showed some significant differences in the length of days that it took to hatch as well as survival. Overall, eggs in the 8 °C treatment developed the quickest to the eyed stage, hatched the earliest, and had the highest survival, which may be due to a shorter exposure time to water quality.



## Preliminary investigation into the effect of microbeads on growth of juvenile Atlantic Salmon

Courtney Steinmetz, Ashley Moerke

Microbeads, plastics less than 5 mm in size, are found in high concentrations (up to 1 mil/km<sup>2</sup>) in Great Lakes water as a result of plastics being washed down drains in beauty products or from larger plastic debris that breaks down. Several studies have shown that microbeads are ingested by aquatic organisms and are transferred up the food web. Fewer studies have shown there are negative impacts associated and some have suggested no impacts. Very little research has been done into the effects microbeads have on the Great Lakes and its organisms. Our objective was to determine if microbeads provided to juvenile Atlantic Salmon as food affected growth. Four levels of microbeads were added to a pellet food diet and fed to juvenile Atlantic Salmon in tanks over 4 weeks. Growth, along with qualitative metrics (e.g., fish appearance) were recorded. The results showed that there was no difference in weight and length between treatment groups, indicating that microbeads did not affect growth. However, other factors such as stress and water quality were likely overriding the effects of microbeads.

Does bioaccumulation of mercury differ between a hatchery-reared and wild Lake Trout in Lake Huron?

Kyle Urban, Kevin Kapuscinski

The Lake Trout (*Salvelinus namaycush*) is a top pelagic predator in the Great Lakes and an ecologically and economically important native species. In addition to being apex predators, Lake Trout have a long life span, which allows them to accumulate high concentrations of contaminants such as mercury. The objective of my study was to determine if hatchery-reared and wild Lake Trout had different concentrations of mercury at the same total length and age. Muscle tissue samples were collected in the summer of 2015 from Lake Trout in Lake Huron and analyzed for concentration of mercury using dynamic mechanical analysis. A total of 79 samples were analyzed; 47 were hatchery-reared fish and 32 were wild fish. Concentrations of mercury increased with the total length of fish, but did not differ between hatchery-reared and wild Lake Trout. My results indicate that even though hatchery-reared Lake Trout were fed a prepared diet for the first 14 months of their life, their concentrations of mercury were similar to wild Lake Trout that fed on wild prey. Lake Trout being reared in hatcheries and their prepared diets should be tested for concentrations of mercury to help better understand if they accumulate mercury from their food.

The effects of  $\beta$ -glucan on cortisol levels and hemolytic response in Atlantic Salmon

Conner Workentine, Jun Li

$\beta$ -Glucans are a group of B-D glucose polysaccharides that are found in the cell walls of plants, yeasts, and bacteria. Many studies have been done on the immunostimulating properties  $\beta$ -Glucans have for fish. The objective of this study was to find if adding different kinds of  $\beta$ -Glucans to the feed of fingerling Atlantic salmon (*Salmo salar*) would reduce the levels of cortisol in the salmon's blood over a 6 month period. Cortisol is produced by the fish when they

become stressed, meaning that the goal behind this study was to find out if  $\beta$ -Glucans in the diet of the salmon would reduce their overall stress. Atlantic salmon were raised on diets of varying amounts of two different strains of  $\beta$ -Glucans, the more widely studied yeast-derived species (Macrogard), and the newer algal-derived species (Algamune AM). When blood samples were taken from each group the data suggests that after 3 weeks there was no sign that the  $\beta$ -Glucan was benefitting the fish. After 6 weeks, however, there was obvious data to suggest that the  $\beta$ -Glucan was helping to reduce the cortisol levels in the blood. This information could prove to be useful for any hatchery setting.

# APPENDICES

Appendix 1. Results of fish health inspection of age-1 Atlantic Salmon from lot P-ATS-LL-W-14-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:** 160315-2-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:<br><br>Lake Superior State University<br>Sault Ste. Marie, MI | Owner/Manager:<br><br>Roger Greil | Inspection Date(s): Spring 2016<br>This: 3/13/16<br>Prior: 2/24/15<br><br>Classification:<br>B-BK | Type of Water Supply:<br>Stream<br>Origin of Fish Examined:<br>Hatchery<br>Type of Fish Examined:<br>Salmonid |
|---|-----------------------------------|---|---|

| Species <sup>1</sup> | Designation          | AAHL #           | Age <sup>1</sup> | Number in Lot | Obtained as Eggs (E) or Fish (F) From: | Pathogens <sup>2</sup> Inspected for and Results <sup>4</sup> |        |                |                |        |        |        |        |
|----------------------|----------------------|------------------|------------------|---------------|--|---|--------|----------------|----------------|--------|--------|--------|--------|
|                      |                      |                  |                  |               |  | As  | Fr     | Y <sup>1</sup> | B <sup>1</sup> | VHS    | IBN    | IPN    | WD     |
| ATS-LL               | P-ATS-LL-W-14-SM-LS* | 160315-2-PI-LSSU | 9                | 33,000        | E-St. Mary's River                     | 60 (0)  | 60 (0) | 60 (0)         |                | 60 (0) | 60 (0) | 60 (0) | 60 (0) |
|                      |                      |                  |                  |               |  |   |        |                |                |        |        |        |        |
|                      |                      |                  |                  |               |  |   |        |                |                |        |        |        |        |
|                      |                      |                  |                  |               |  |   |        |                |                |        |        |        |        |

|   |  |  |
|---|--|--|
| <b>Remarks/Recommendations:</b><br>Lot P-ATS-LL-W-14-SM-LS can be stocked in Michigan's public waters.<br><br>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).<br>b. The presence of <i>Renibacterium salmoninarum</i> was tested with quantitative QELISA, which is more sensitive than the direct fluorescent antibody technique. H=high, M=medium, L=low antigen concentrations.<br>c. Test not required.<br><br>* <i>Nucleosporidium salmonis</i> screening is pending. <i>N. salmonis</i> is not currently a pathogen of concern. | <b>Address/Phone of Contracted Fish Health Official</b><br>Aquatic Animal Health Laboratory<br>College of Veterinary Medicine<br>Michigan State University<br>Food Safety & Toxicology Building<br>1129 Farm Lane, room 177K<br>East Lansing, MI 48824<br>PHONE: 517/884-2024<br>FAX: 517/432-2310 | <b>Signature of Contracted Fish Health Official</b><br><br><br><br>Mohamed Faisal, D.V.M., Ph.D. |
|---|--|--|

<sup>1</sup>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.  
<sup>2</sup>See list of pathogen and spp. abbreviations (pg 2).  
 cc: Gary Whelan

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                                  | F <sup>1</sup> | As | 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) <i>Aeromonas salmonicida</i> (furunculosis) |       |                       |
| Rs                     | (BK) <i>Renibacterium salmoninarum</i> (BKD) | WD  | <i>Myxobolus cerebralis</i> (whirling disease)   |       |                       |
| Yr                     | <i>Yersinia ruckeri</i> (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           |
| COS                    | Coho Salmon                                  | FCS | Fall Chinook Salmon                              | LAT   | Lake Trout            |
| OSA                    | Other Salmonids                              | RBT | Rainbow Trout                                    | SPL   | Splake (Brook x Lake) |
| STT                    | Steelhead Trout                              |     |  | CHS   | Chinook Salmon        |
|                        |  |     |  | Mixed | Mixed species         |
|                        |  |     |  | STN   | Sturgeon              |

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.

<sup>1</sup>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.

<sup>2</sup>See list of pathogen and spp. abbreviations (pg 2).

cc: Gary Whelan

Appendix 2. Data on individual Atlantic Salmon used for gamete collection in 2016. Note: 000-099 and 000A-026A are females, 100-199 and 100A-126A are males, and fin tissue samples were collected from each.

| Date      | Fish ID # | Fin clip | Age | Total length (cm) | Weight (kg) | Tag # |
|-----------|-----------|----------|-----|-------------------|-------------|-------|
| 8-Nov-16  | 0         | RP       | 6   | 72                |             | 359   |
| 8-Nov-16  | 100       | RV       | 5   | 61                |             | 358   |
| 8-Nov-16  | 1         | LV       | 3   | 69.5              |             | 357   |
| 8-Nov-16  | 101       | LV       | 3   | 71.8              |             | 356   |
| 8-Nov-16  | 2         | RP       | 3   | 76                |             | 355   |
| 8-Nov-16  | 102       | RP       | 2   | 58.3              |             | 354   |
| 8-Nov-16  | 3         | LP       | 4   | 66.5              |             | 353   |
| 8-Nov-16  | 103       | RP       | 6   | 75                |             | 352   |
| 8-Nov-16  | 4         | LV       | 3   | 70.5              |             | 586   |
| 8-Nov-16  | 104       | LV       | 3   | 47                |             | 587   |
| 8-Nov-16  | 5         | LV       | 3   | 63                |             | 588   |
| 8-Nov-16  | 105       | RP       | 2   | 52                |             | 589   |
| 8-Nov-16  | 6         | AD       |     | 54.5              |             | 590   |
| 8-Nov-16  | 106       | LV       | 3   | 72                |             | 591   |
| 8-Nov-16  | 7         | LV       | 3   | 66.5              |             | 592   |
| 8-Nov-16  | 107       | LV       | 3   | 77                |             | 593   |
| 10-Nov-16 | 9         | RP       | 2   | 56.4              | 1.89        |       |
| 10-Nov-16 | 109       | LV       | 3   | 74                | 3.78        |       |
| 10-Nov-16 | 10        | LP       | 4   | 60                | 3.37        |       |
| 10-Nov-16 | 110       | AD       |     | 56.5              | 1.45        |       |
| 10-Nov-16 | 11        | LV       | 3   | 70                | 3.9         |       |
| 10-Nov-16 | 111       | LV       | 3   | 76                | 4.2         |       |
| 10-Nov-16 | 12        | LV       | 3   | 72                | 5.2         |       |
| 10-Nov-16 | 112       | LV       | 3   | 67                | 2.7         |       |
| 10-Nov-16 | 13        | RV       | 5   | 66.5              | 2.8         |       |
| 10-Nov-16 | 113       | RP       | 2   | 55                | 1.4         |       |
| 10-Nov-16 | 14        | LV       | 3   | 71                | 3.2         |       |
| 10-Nov-16 | 114       | RP       | 2   | 56.5              | 1.7         |       |
| 10-Nov-16 | 15        | LV       | 3   | 69                | 3.7         |       |
| 10-Nov-16 | 115       | LP       | 4   | 82                | 5.1         |       |
| 10-Nov-16 | 16        | LV       | 3   | 69                | 3.7         | 376   |
| 10-Nov-16 | 116       | RP       | 2   | 53                | 1.7         |       |
| 10-Nov-16 | 17        | LP       | 4   | 69                | 2.7         |       |
| 10-Nov-16 | 117       | LV       | 3   | 77.5              | 4.5         |       |
| 10-Nov-16 | 18        | LV       | 3   | 65                | 1.8         |       |
| 10-Nov-16 | 118       | RP       | 2   | 56                | 1.53        |       |
| 10-Nov-16 | 19        | LP       | 4   | 69                | 3.5         |       |

|           |     |    |   |       |       |
|-----------|-----|----|---|-------|-------|
| 10-Nov-16 | 119 | AD |   | 55    | 1.5   |
| 10-Nov-16 | 20  | AD |   | 51    | 1.35  |
| 10-Nov-16 | 120 | LV | 3 | 71.5  | 2.6   |
| 10-Nov-16 | 21  | RP | 2 | 51    | 1.42  |
| 10-Nov-16 | 121 | RP | 2 | 61    | 1.93  |
| 10-Nov-16 | 22  | LV | 3 | 68    | 3.12  |
| 10-Nov-16 | 122 | LV | 3 | 74    | 3.71  |
| 10-Nov-16 | 23  | AD |   | 53    | 1.7   |
| 10-Nov-16 | 123 | LV | 3 | 66.1  | 2.65  |
| 10-Nov-16 | 24  | LV | 3 | 68.5  | 4.01  |
| 10-Nov-16 | 124 | LV | 3 | 74.5  | 3.7   |
| 10-Nov-16 | 25  | AD |   | 56.4  | 1.95  |
| 10-Nov-16 | 125 | RP | 2 | 54.4  | 1.49  |
| 10-Nov-16 | 26  | AD |   | 55    | 1.46  |
| 10-Nov-16 | 126 | RP | 2 | 57.5  | 1.47  |
| 10-Nov-16 | 27  | LV | 3 | 767.5 | 3.11  |
| 10-Nov-16 | 127 | AD |   | 62.1  | 2.21  |
| 10-Nov-16 | 28  | LV | 3 | 65.5  | 2.98  |
| 10-Nov-16 | 128 | LV | 3 | 76.9  | 4.06  |
| 10-Nov-16 | 29  | LV | 3 | 70    | 3.05  |
| 10-Nov-16 | 129 | AD |   | 60    | 1.95  |
| 15-Nov-16 | 30  | AD |   | 56    | 1.6   |
| 15-Nov-16 | 130 | AD |   | 54.1  | 1.29  |
| 15-Nov-16 | 31  | LV | 3 | 60    | 2.7   |
| 15-Nov-16 | 131 | AD |   | 53.1  | 1.1   |
| 15-Nov-16 | 32  | AD |   | 49    | 1.33  |
| 15-Nov-16 | 132 | AD |   | 54.5  | 1.29  |
| 15-Nov-16 | 33  | LV | 3 | 67    | 3.66  |
| 15-Nov-16 | 133 | AD |   | 55    | 1.41  |
| 15-Nov-16 | 34  | RP | 2 | 53    | 1.51  |
| 15-Nov-16 | 134 | AD |   | 55    | 1.41  |
| 15-Nov-16 | 35  | LV | 3 | 53    | 2.15  |
| 15-Nov-16 | 135 | RP | 2 | 50    | 1.05  |
| 15-Nov-16 | 36  | LV | 3 | 71    | 3.4   |
| 15-Nov-16 | 136 | AD |   | 58.3  | 1.56  |
| 15-Nov-16 | 37  | LV | 3 | 70    | 3.7   |
| 15-Nov-16 | 137 | RP | 2 | 53.6  | 1.05  |
| 15-Nov-16 | 38  | LV | 3 | 66    | 3     |
| 15-Nov-16 | 138 | LV | 3 | 72.1  | 3.56  |
| 15-Nov-16 | 39  | LV | 3 | 65    | 2.43  |
| 15-Nov-16 | 139 | AD |   | 46.3  | 0.836 |

|           |     |      |   |      |      |     |
|-----------|-----|------|---|------|------|-----|
| 15-Nov-16 | 40  | LV   | 3 | 69   | 3.29 |     |
| 15-Nov-16 | 140 | LV   | 3 | 75.4 | 3.89 |     |
| 15-Nov-16 | 41  | AD   |   | 55   | 1.56 |     |
| 15-Nov-16 | 141 | AD   |   | 55   | 1.33 |     |
| 15-Nov-16 | 42  | LV   | 3 | 67   |      | 378 |
| 15-Nov-16 | 142 | LV   | 3 | 68.5 |      | 379 |
| 15-Nov-16 | 43  | LV   | 3 | 64.7 |      | 380 |
| 15-Nov-16 | 143 | LV   | 3 | 69.8 |      | 381 |
| 15-Nov-16 | 44  | LV   | 3 | 69.7 |      | 382 |
| 15-Nov-16 | 144 | RP   | 2 | 57.5 |      | 383 |
| 15-Nov-16 | 45  | LP   |   | 73.8 |      | 585 |
| 15-Nov-16 | 145 | AD   |   | 56.6 |      | 384 |
| 15-Nov-16 | 46  | LV   | 3 | 68.3 |      | 385 |
| 15-Nov-16 | 146 | None |   | 58.8 |      | 386 |
| 15-Nov-16 | 47  | LV   | 3 | 69   |      | 387 |
| 15-Nov-16 | 147 | LV   | 3 | 70.6 |      | 388 |
| 15-Nov-16 | 49  | LV   | 3 | 68.7 |      | 389 |
| 15-Nov-16 | 148 | LV   | 3 | 70.6 |      | 390 |
| 15-Nov-16 | 49  | LV   | 3 | 72   |      | 391 |
| 15-Nov-16 | 149 | LV   | 3 | 66.8 |      | 392 |
| 15-Nov-16 | 50  | LV   | 3 | 69.5 |      | 376 |
| 15-Nov-16 | 150 | LP   | 4 | 69.8 |      | 393 |
| 15-Nov-16 | 51  | LV   | 3 | 67.9 |      | 394 |
| 15-Nov-16 | 151 | LV   | 3 | 73.3 |      | 395 |
| 15-Nov-16 | 52  | LV   | 3 | 64.5 |      | 396 |
| 15-Nov-16 | 152 | LV   | 3 | 73   |      | 397 |
| 15-Nov-16 | 53  | LV   | 3 | 67.7 |      | 398 |
| 15-Nov-16 | 153 | LV   | 3 | 79.5 |      | 399 |
| 15-Nov-16 | 54  | LV   | 3 | 64.5 |      | 400 |
| 15-Nov-16 | 154 | RP   | 2 | 58   |      | 600 |
| 15-Nov-16 | 55  | LP   | 4 | 66.5 |      | 599 |
| 15-Nov-16 | 155 | LV   | 3 | 79   |      | 598 |
| 15-Nov-16 | 56  | RP   | 2 | 56   |      | 597 |
| 15-Nov-16 | 156 | None |   | 78   |      | 596 |
| 15-Nov-16 | 57  | RP   | 2 | 56   |      | 477 |
| 15-Nov-16 | 157 | LV   | 3 | 75   |      | 478 |
| 15-Nov-16 | 58  | LV   | 3 | 66   |      | 480 |
| 15-Nov-16 | 158 | LV   | 3 | 77   |      | 481 |
| 15-Nov-16 | 59  | LV   | 3 | 66   |      | 482 |
| 15-Nov-16 | 159 | AD   |   | 53   |      | 483 |
| 15-Nov-16 | 60  | LV   | 3 | 71   |      | 484 |

|           |     |    |   |      |      |
|-----------|-----|----|---|------|------|
| 15-Nov-16 | 160 | RP | 2 | 43.5 | 485  |
| 15-Nov-16 | 61  | LV | 3 | 67   | 486  |
| 15-Nov-16 | 161 | RP | 2 | 51   | 487  |
| 15-Nov-16 | 62  | AD |   | 54   | 488  |
| 15-Nov-16 | 162 | LV | 3 | 81   | 489  |
| 17-Nov-16 | 063 | LV | 3 | 72.5 | 0490 |
| 17-Nov-16 | 163 | LV | 3 | 76.5 | 0491 |
| 17-Nov-16 | 064 | RP | 2 | 59.5 | 0492 |
| 17-Nov-16 | 164 | LV | 3 | 74.5 | 0493 |
| 17-Nov-16 | 065 | LV | 3 | 68   | 0494 |
| 17-Nov-16 | 165 | LV | 3 | 72.1 | 0495 |
| 17-Nov-16 | 066 | LV | 3 | 68.5 | 0497 |
| 17-Nov-16 | 166 | LV | 3 | 69   | 0200 |
| 17-Nov-16 | 067 | RP | 2 | 56.4 | 0199 |
| 17-Nov-16 | 167 | LV | 3 | 71.3 | 0198 |
| 17-Nov-16 | 068 | LV | 3 | 72.5 | 0197 |
| 17-Nov-16 | 168 | RP | 2 | 54.5 | 0196 |
| 17-Nov-16 | 069 | LV | 3 | 70   | 0195 |
| 17-Nov-16 | 169 | LV | 3 | 80   | 0194 |
| 17-Nov-16 | 070 | LV | 3 | 66.2 | 0193 |
| 17-Nov-16 | 170 | LV | 3 | 69   | 0192 |
| 17-Nov-16 | 071 | LV | 3 | 69   | 0191 |
| 17-Nov-16 | 171 | LV | 3 | 75   | 0190 |
| 17-Nov-16 | 072 | LV | 3 | 74   | 0189 |
| 17-Nov-16 | 172 | LV | 3 | 70   | 0188 |
| 17-Nov-16 | 073 | LV | 3 | 74   | 0186 |
| 17-Nov-16 | 173 | LV | 3 | 65   | 0185 |
| 17-Nov-16 | 074 | LV | 3 | 73.2 | 0184 |
| 17-Nov-16 | 174 | LV | 3 | 75.2 | 0183 |
| 17-Nov-16 | 075 | RP | 2 | 51.6 | 0182 |
| 17-Nov-16 | 175 | LV | 3 | 68   | 0181 |
| 17-Nov-16 | 076 | LP | 4 | 76   | 0180 |
| 17-Nov-16 | 176 | AD |   | 56.2 | 0179 |
| 17-Nov-16 | 077 | LV | 3 | 65.4 | 0178 |
| 17-Nov-16 | 177 | RP | 2 | 64.2 | 0177 |
| 17-Nov-16 | 078 | LV | 3 | 70.4 | 0176 |
| 17-Nov-16 | 178 | LV | 3 | 73.2 | 0225 |
| 17-Nov-16 | 079 | LV | 3 | 57.9 | 0224 |
| 17-Nov-16 | 179 | AD |   | 70   | 0223 |
| 17-Nov-16 | 080 | LP | 4 | 65   | 0222 |
| 17-Nov-16 | 180 | LV | 3 | 73   | 0221 |



|           |       |    |   |      |      |
|-----------|-------|----|---|------|------|
| 17-Nov-16 | 081   | LV | 3 | 64   | 0422 |
| 17-Nov-16 | 181   | LV | 3 | 70   | 0220 |
| 17-Nov-16 | 082   | LP | 4 | 71   | 0219 |
| 17-Nov-16 | 182   | LV | 3 | 74   | 0218 |
| 17-Nov-16 | 084   | LV | 3 | 73   | 0216 |
| 17-Nov-16 | 184   | LV | 3 | 75   | 0215 |
| 17-Nov-16 | 086   | LV | 3 | 70   | 0213 |
| 17-Nov-16 | 186   | LV | 3 | 65   | 0212 |
| 17-Nov-16 | 087   | RP | 6 | 76   | 0211 |
| 17-Nov-16 | 187   | LV | 3 | 76   | 0210 |
| 20-Nov-16 | 88    | LV | 3 | 73   | 209  |
| 20-Nov-16 | 188   | RV | 5 | 77   | 208  |
| 20-Nov-16 | 89    | AD |   | 56   | 207  |
| 20-Nov-16 | 189   | AD |   | 50   | 206  |
| 20-Nov-16 | 90    | LV | 3 | 66   | 205  |
| 20-Nov-16 | 190   | LP | 4 | 49   | 204  |
| 20-Nov-16 | 91    | LV | 3 | 70   | 94   |
| 20-Nov-16 | 191   | RP | 2 | 36   | 203  |
| 20-Nov-16 | 92    | LV | 3 | 68.5 | 202  |
| 20-Nov-16 | 192   | AD |   | 55   | 201  |
| 20-Nov-16 | 93    | LV | 3 | 65   | 82   |
| 20-Nov-16 | 193   | LV | 3 | 79   | 83   |
| 20-Nov-16 | 94    | AD |   | 53   | 84   |
| 20-Nov-16 | 194   | RP | 2 | 56.5 | 85   |
| 20-Nov-16 | 95    | LP | 4 | 69.5 | 86   |
| 20-Nov-16 | 195   | RP | 2 | 56   | 87   |
| 20-Nov-16 | 96    | LV | 3 | 64.5 | 89   |
| 20-Nov-16 | 196   | LV | 3 | 70   | 90   |
| 20-Nov-16 | 97    | RP | 2 | 50.5 | 91   |
| 20-Nov-16 | 197   | AD |   | 55   | 92   |
| 20-Nov-16 | 98    | LV | 3 | 69   | 93   |
| 20-Nov-16 | 198   | AD |   | 57   | 94   |
| 20-Nov-16 | 99    | LP | 4 | 68   | 95   |
| 20-Nov-16 | 199   | RP | 2 | 61.5 | 96   |
| 20-Nov-16 | 00-A  | LV | 3 | 65   | 97   |
| 20-Nov-16 | 100-A | AD |   | 56   | 98   |
| 20-Nov-16 | 01-A  | LV | 3 | 69.5 | 99   |
| 20-Nov-16 | 101-A | LV | 3 | 66.5 | 100  |
| 20-Nov-16 | 02-A  | LV | 3 | 62   | 175  |
| 20-Nov-16 | 102-A | RP | 2 | 50.5 | 174  |
| 20-Nov-16 | 03-A  | RV | 5 | 60   | 173  |

|           |       |    |   |      |     |
|-----------|-------|----|---|------|-----|
| 20-Nov-16 | 103-A | RP | 2 | 53.5 | 172 |
| 21-Nov-16 | 004-A | LV | 3 | 69   | 171 |
| 21-Nov-16 | 104-A | LV | 3 | 71   | 170 |
| 21-Nov-16 | 005-A | AD |   | 53   | 169 |
| 21-Nov-16 | 105-A | LV | 3 | 77   | 168 |
| 21-Nov-16 | 006-A | LV | 3 | 72.5 | 167 |
| 21-Nov-16 | 106-A | LV | 3 | 76   | 166 |
| 21-Nov-16 | 008-A | LV | 3 | 64   | 164 |
| 21-Nov-16 | 108-A | RP | 2 | 57.5 | 163 |
| 21-Nov-16 | 009-A | LV | 3 | 56.5 | 162 |
| 21-Nov-16 | 109-A | LV | 3 | 76.5 | 161 |
| 21-Nov-16 | 010-A | AD |   | 51   | 160 |
| 21-Nov-16 | 110-A | LV | 3 | 71.5 | 159 |
| 21-Nov-16 | 011-A | LV | 3 | 67   | 158 |
| 21-Nov-16 | 111-A | RP | 2 | 52   | 157 |
| 21-Nov-16 | 012-A | LV | 3 | 68   | 156 |
| 21-Nov-16 | 112-A | LV | 3 | 60   | 155 |
| 21-Nov-16 | 013-A | LP | 4 | 69   | 154 |
| 21-Nov-16 | 113-A | AD |   | 51   | 153 |
| 21-Nov-16 | 014-A | LV | 3 | 71.5 | 152 |
| 21-Nov-16 | 114-A | LV | 3 | 72   | 151 |
| 21-Nov-16 | 015-A | LV | 3 | 68.5 | 226 |
| 21-Nov-16 | 115-A | LV | 3 | 72   | 227 |
| 21-Nov-16 | 017-A | RP | 2 | 52.9 | 229 |
| 21-Nov-16 | 117-A | RP | 2 | 47.5 | 230 |
| 21-Nov-16 | 018-A | RP | 2 | 50   | 231 |
| 21-Nov-16 | 118-A | LV | 3 | 73.5 | 232 |
| 21-Nov-16 | 019-A | LV | 3 | 65.5 | 233 |
| 21-Nov-16 | 119-A | LV | 3 | 79   | 234 |
| 21-Nov-16 | 020-A | LV | 3 | 66   | 235 |
| 21-Nov-16 | 120-A | LV | 3 | 64   | 237 |
| 21-Nov-16 | 021-A | RP | 2 | 55.3 | 238 |
| 21-Nov-16 | 121-A | AD |   | 67.8 | 239 |
| 21-Nov-16 | 022-A | AD |   | 57.6 | 240 |
| 21-Nov-16 | 122-A | RP | 2 | 53.3 | 241 |
| 21-Nov-16 | 023-A | LV | 3 | 68.8 | 243 |
| 21-Nov-16 | 123-A | RP | 2 | 58.1 | 244 |
| 21-Nov-16 | 024-A | LV | 3 | 67.3 | 245 |
| 21-Nov-16 | 124-A | LV | 3 | 70.3 | 246 |
| 21-Nov-16 | 025-A | RP | 2 | 51   | 247 |
| 21-Nov-16 | 125-A | LV | 3 | 76.4 | 248 |

|           |       |    |   |      |     |
|-----------|-------|----|---|------|-----|
| 21-Nov-16 | 026-A | LV | 3 | 71.6 | 249 |
| 21-Nov-16 | 126-A | LV | 3 | 73.6 | 250 |

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Appendix 3. Results of fish health inspection of 2016 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: 161115-1-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:<br><br>Lake Superior State University<br>Sault Ste. Marie, MI | Owner/Manager:<br><br>Roger Greil | Inspection Date(s): Fall 2016<br>This: 11/17/16<br>Prior: 11/18/15<br><br>Classification:<br>B-BK, BF | Type of Water Supply:<br>Stream<br>Origin of Fish Examined:<br>Hatchery<br>Type of Fish Examined:<br>Salmonid |
|---|-----------------------------------|---|---|

| Species <sup>1</sup> | Designation   | AAHL #           | Age <sup>1</sup> | Number in Lot | Obtained as Eggs (E) or Fish (F) From: | Pathogens <sup>2</sup> Inspected for and Results <sup>3</sup> |        |                 |        |        | VHS    | IHN             | IPN    | Om V <sup>4</sup> | WD |
|----------------------|---|------------------|------------------|---------------|--|---|--------|-----------------|--------|--------|--------|-----------------|--------|-------------------|----|
|                      |   |                  |                  |               |  | As  | Yr     | Rs <sup>5</sup> |        |        |        |                 |        |                   |    |
| ATS                  | Feral Fall Atlantic Salmon Spawners (kidney & spleen samples) | 161115-1-BI-LSSU | b                | n/a           | Wild                                   | 60 (4)  | 60 (0) | 60 (10: 1M-9L)  | 60 (0) | 60 (0) | 60 (0) | NR <sup>4</sup> | 60 (0) |                   |    |
| ATS                  | Feral Fall Atlantic Salmon Spawners (gamete samples)          | 161115-1-BI-LSSU | b                | n/a           | Wild                                   | NR  | NR     | 60 (1: 1L)      | 60 (0) | 60 (0) | 60 (0) | 60 (0)          | NR     |                   |    |

|  |   |  |
|--|---|--|
| Remarks/Recommendations:<br>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).<br>b. The presence of <i>Renibacterium salmoninarum</i> was tested with quantitative QELISA, which is more sensitive than the direct fluorescent antibody technique; H=high, M=medium, L=low antigen concentrations.<br>c. <i>Oncorhynchus masu</i> virus testing is done on ovarian fluid samples only as per GLFC recommendations.<br>d. NR=Test not required. | Address/Phone of Contracted Fish Health Official<br>Aquatic Animal Health Laboratory<br>College of Veterinary Medicine<br>Michigan State University<br>1129 Farm Lane, Room 177K<br>Food Safety & Toxicology Building<br>East Lansing, MI 48824<br>PHONE: 517/884-2024; FAX: 517/432-2310 | Signature of Contracted Fish Health Official<br><br>Mohamed Faisal<br><br>Mohamed Faisal, D.V.M., PhD. |
|--|---|--|

<sup>1</sup>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.

<sup>2</sup>See list of pathogen and spp. abbreviations (pg 2).

cc: Gary Whelan

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 |    |    |  |  | VHS | IHN | IPN | Om V | WD |
|---------|-------------|--------|-----|---------------|--|-------------------------------------|----|----|--|--|-----|-----|-----|------|----|
|         |             |        |     |               |  | As                                  | Yr | Rs |  |  |     |     |     |      |    |
|         |             |        |     |               |  |                                     |    |    |  |  |     |     |     |      |    |
|         |             |        |     |               |  |                                     |    |    |  |  |     |     |     |      |    |
|         |             |        |     |               |  |                                     |    |    |  |  |     |     |     |      |    |
|         |             |        |     |               |  |                                     |    |    |  |  |     |     |     |      |    |
|         |             |        |     |               |  |                                     |    |    |  |  |     |     |     |      |    |
|         |             |        |     |               |  |                                     |    |    |  |  |     |     |     |      |    |
|         |             |        |     |               |  |                                     |    |    |  |  |     |     |     |      |    |
|         |             |        |     |               |  |                                     |    |    |  |  |     |     |     |      |    |
|         |             |        |     |               |  |                                     |    |    |  |  |     |     |     |      |    |

SUPPLEMENTAL INSPECTION INFORMATION

| Date | Species | Lot # | Findings |
|------|---------|-------|----------|
|      |         |       |          |
|      |         |       |          |
|      |         |       |          |
|      |         |       |          |
|      |         |       |          |

|   |   |                           |                     |  |
|---|---|---------------------------|---------------------|--|
| <b>Pathogen Abbreviations</b>                   |   |                           |                     |  |
| IHN Infectious Hematopoietic Necrosis Virus     | IPN Infectious Pancreatic Necrosis Virus            |                           |                     |  |
| VHS Viral Hemorrhagic Septicemia Virus          | As (BF) <i>Aeromonas salmonicida</i> (furunculosis) |                           |                     |  |
| Rs (BK) <i>Renibacterium salmoninarum</i> (BKD) | WD <i>Myxobolus cerebralis</i> (whirling disease)   |                           |                     |  |
| Yr <i>Yersinia ruckeri</i> (ERM)                | X Various (see remarks box)                         |                           |                     |  |
| Y Various (see remarks box)                     | Z Various (see remarks box)                         |                           |                     |  |
| <b>Species Abbreviations</b>                    |   |                           |                     |  |
| 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                             |   |                           |                     |  |

<sup>1</sup>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.

<sup>2</sup>See list of pathogen and spp. abbreviations (pg 2).

cc: Gary Whelan

#### Appendix 4. Dosages for treatments of Atlantic Salmon eggs.

##### **Saline Bath**

0.75% needed

$$0.75/100 = 0.0075$$

$$0.0075 * 20L = 0.15 \text{ mL or g}$$

$$0.15 * 1,000 = 150 \text{ g needed for 20 L of H}_2\text{O}$$

##### **Erythromycin Treatment**

2 ppm (mg/L) needed

$$2 \text{ mg/L} * 20 = 40,000 \text{ mg}$$

$$40,000/0.23 = 1,739,130 \text{ (23\% active)}$$

$$1,739,130/10,000 = 173.9$$

$$173.9/1,000 \text{ (to get to g)} = 0.174\text{g per 20 L of H}_2\text{O}$$

##### **Iodine Treatment**

1% active

1% free iodine to get 100 ppm (mg/L) dilute 100 times

$$20 \text{ L} = 20,000 \text{ mL}$$

$$20 \text{ L} * 1,000 \text{ mL} = 20,000 \text{ mL}$$

$$20,000 \text{ mL}/100 = 2,000 \text{ mL}$$

$$2,000 \text{ mL} * 50 = 100,000 \text{ mL}$$

$$100,000 / 1,000 \text{ (to mL)} = 100 \text{ mL of Iodine needed for 20 L of H}_2\text{O}$$

##### **Thiamine Treatment**

1,000 ppm (mg/L) needed

20.4 g of 98% active, buffered thiamine added per 20 L of H<sub>2</sub>O