GREAT LAKES FISHERY COMMISSION

2004 Project Progress Report[1]

Early Mortality Syndrome Research and Information Coordination Meetings

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GREAT LAKES FISHERY COMMISSION
RESEARCH STATUS REPORT

EARLY MORTALITY SYNDROME WORKSHOP

September 8-9, 2004
Ann Arbor, MI

GLFC-Sponsored Research Coordination Meeting on Early Mortality Syndrome

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Abstract

Early mortality syndrome (EMS) is the term used to describe embryonic mortality affecting the offspring of salmonids in the Great Lakes Basin. EMS continues to an issue in the Great Lakes Basin with higher incidences observed in most monitored salmonine populations in 2003 than observed in the previous year. Work presented at the workshop can be categorized under three main topics. First, there was considerable discussion about the food web and specific food web connections that may be involved with EMS. A novel approach for describing the food web and it's compartments has been developed from social networking science. The approach was used to identify food web compartments and their connectivity for Lake Michigan. By identifying key compartments and species, the application of this type of model could change our approaches to future fishery and resource management. In addition to thiamine, other essential nutrients such as fatty acids and lipid soluble vitamins and markers of trophic status were linked to EMS. Second, work centering on fish physiology and biological processes during thiamine deficiency was presented. A target value of 4 nmol/g egg thiamine was suggested to avoid both overt mortality and secondary effect of thiamine deficiency such as prey capture, vision and growth. While chinook salmon are definitely susceptible to effects of thiamine deficiency, data presented suggest that they may have a lower thiamine requirement than other salmonids. The use of cDNA microarray technology is an exciting new approach to study EMS. It was reported that over 400 lake trout genes were modulated by thiamine deficiency. This work opens the door to possibilities such as genetic selection for fish less susceptible to thiamine deficiency. Finally, work on the relevance and significance of ecosystem thiaminase was the third main area of discussion. The significance of the discovery of thiaminase in net plankton can only be speculated at this time, but it has implications for all larval fish feeding on plankton. Factors affecting thiamine in alewife possibly include environmental stress and diet but considerable work is still required.

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Introduction

Early mortality syndrome (EMS) is the term used to describe an embryonic mortality affecting the offspring of salmonids (coho salmon, chinook salmon, Atlantic salmon, steelhead trout, brown trout and lake trout) in Lakes Michigan and Ontario and to a lesser extent Huron and Erie. Clinical symptoms of EMS include loss of equilibrium, swimming in a spiral pattern, lethargy, hyper-excitability, hemorrhage and death occurring between hatch and first feeding (Marquenski and Brown 1997; McDonald et al., 1998). The consequences of EMS are still not fully appreciated. With evidence of impacts on adults and behavior of first feeding fry it is becoming evident that the effects of thiamine deficiency may be much more significant than was originally thought.

As part of our effort to communicate current information about Early Mortality Syndrome (EMS) research to fishery managers and other researchers, we organized a workshop in Ann Arbor, MI on September 8-9, 2004. The workshop brought together 33 federal, state, provincial and tribal scientists and interested natural resource personnel to share information, present data and discuss the latest observations on EMS and other thiamine deficiencies in aquatic animals. There were 17 presentations describing the status of current EMS research. Based on the presentations, the open discussion session and literature information the working group formulated future research needs.

Incidence of EMS and thiamine status in Great Lakes Salmonines

Thiamine deficient fry mortality continues to be a concern for salmonines in Lake Michigan, Lake Huron and Lake Ontario. In the presentation by Brown et al. (page 14) mortality rates due to EMS before 1993 in Lake Michigan coho salmon offspring were around 20 %. During subsequent years the prevalence of EMS was elevated (range 35-90%) in 1993, 1996, 1999-2001 and again in 2003. (Figure 1). EMS in chinook followed a similar pattern as coho. Monitoring of chinook although has not been as extensive, the incidence of EMS was low in 1998 and 2002 at 20% and as high as 85% in 2001(Figure 2A&B). EMS in chinook salmon from Lake Huron exhibited a less dramatic rise than Lake Michigan chinook but nevertheless a significant increase in EMS for 2003. The extent of EMS mortality in lake trout from Lake Michigan has been monitored since 1975 (Figure 3). From 1982 – 2000 mortality was about 30 %. In two of the last three years EMS losses in lake trout stocks from northern Lake Michigan have exceeded 65% (Figure 3). Southern Lake Michigan lake trout also were found to have elevated incidence (50%) of EMS (Czesny et al., page 14). Incidence of EMS in lake trout has been significant in Lake Ontario. Since 1988 lake trout EMS losses have been about 50 %. (Figure 4), and in 2003 lake trout losses to EMS exceeded 75%. From these data we conclude that EMS continues to be an issue and may represent a limiting factor for natural reproduction and recruitment in Lake Michigan, Lake Huron and Lake Ontario. Werner (see page 16) reported that Lake Huron Atlantic salmon egg total thiamine averaged 2 nmol/g in groups without EMS while egg groups that produced EMS averaged 0.9 nmol/g. Fry mortality within the 12 EMS groups was greater than 70%. The number of Atlantic salmon families with EMS in 2003 (26.6%) was lower than 2001 (95%).

Morbidity and mortality in large older lake trout, steelhead and coho salmon have been observed at or below 500 pmol/g (Brown et al., 2005) In a recent chinook salmon study Sweet et al. (page 16) found 5% of small fish (~455g age 1) at or below 500 pmol/g threshold, 42% of medium fish (~ age 2), and 45% of large fish (~ age 3). None of the fish were noted to be lethargic. Given that natural reproduction has been noted in Lake Michigan chinook this data suggests that chinook may have a lower thiamine requirement than other salmonids. If true, this has significant implications for the fishery containing thiaminase positive alewife.

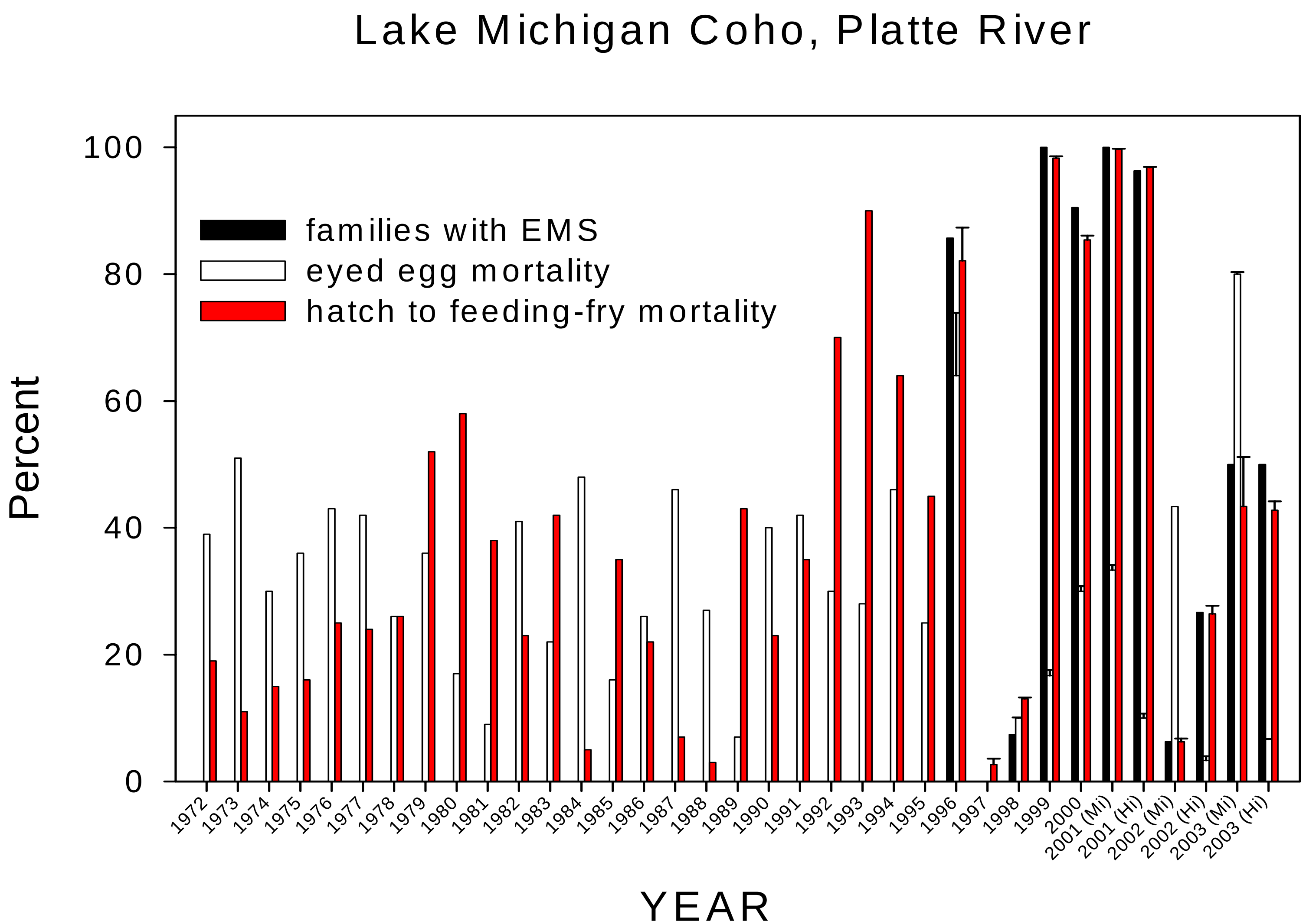

Figure 1. EMS in coho salmon in the Platte River 1972-2003 (data provided by Martha Wolgamood, Michigan DNR)

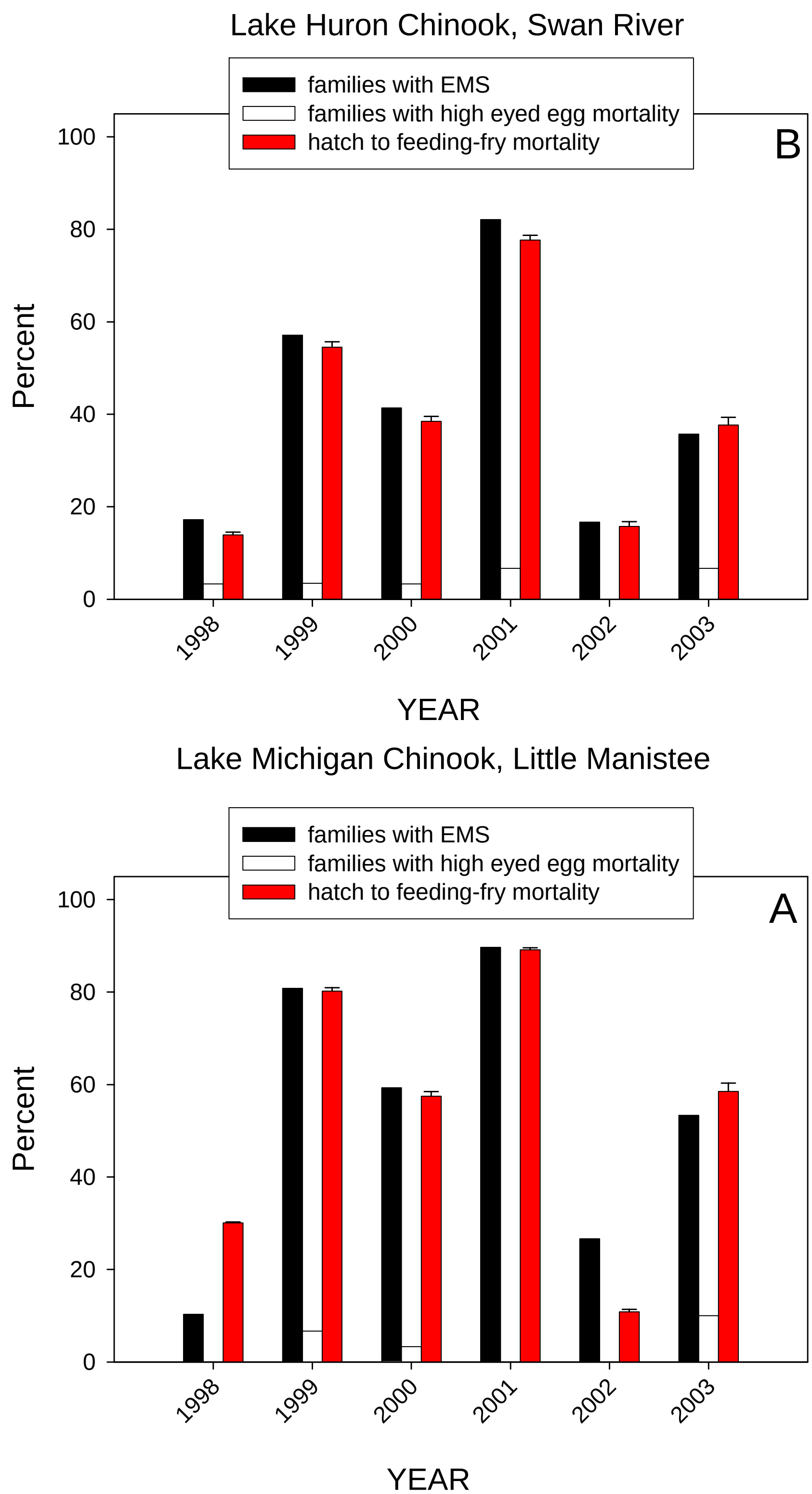

Figure 2. EMS in chinook salmon 1998-2003: A) Little Manistee River, Lake Michigan and B) Swan, River, Lake Huron (data provided by Martha Wolgamood, Michigan DNR).

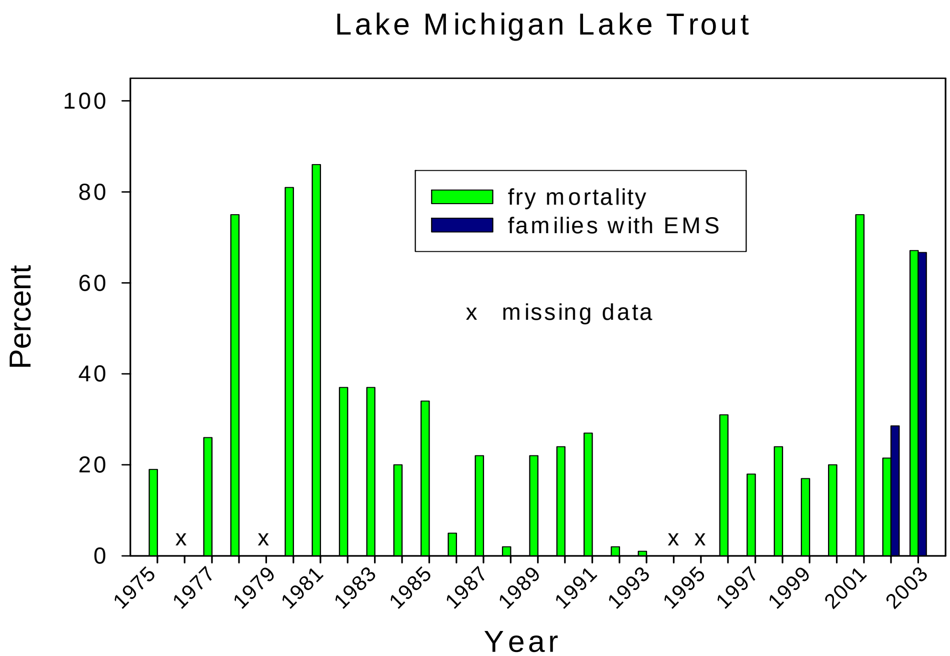

Figure 3. EMS in lake trout in Lake Michigan from 1975-2003 (data provided by Carol Edsall, USGS, Ann Arbor Lab)

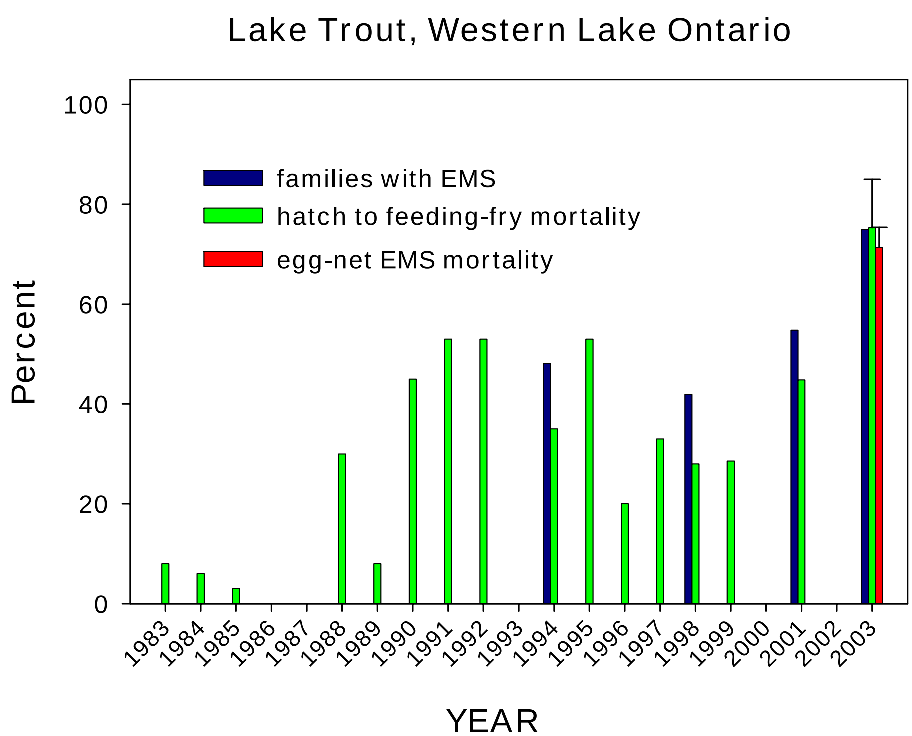

Figure 4. EMS in lake trout from Lake Ontario, 1983-2003. (data provided by John Fitzsimons, DFO)

Incidence of EMS and Thiamine Status of Walleye

Symptoms of thiamine deficiency and mortality can be induced in Lake Erie walleye fry by immersion in water with the thiamine antagonist, oxythiamine (Ketola et al. page 14). The clinical signs were generally similar to those produced by thiamine deficiency in salmonids. Concentrations of total thiamine in walleye eggs varied between lakes, years and locations. There were no significant relationships between early life survival and ova thiamine or organochlorine content in Lake Ontario stocks from the Bay of Quinte (Brown et al. page 15). Early life survival was more strongly correlated with female characteristics. Of the walleye sampled by Ketola et al. (see page 14), eggs from Otisco Lake had the lowest concentrations observed (approx 1.1 nmol/g). While these levels would be expected to induce EMS in salmonines, the concentrations observed in Otisco Lake walleye seemed to be adequate to prevent mortality due to thiamine-deficiency. Walleye from the Raisin River (see Brown et al. page 15) exhibited low egg thiamine concentrations and rearing studies should be considered.

Thiaminase in the Great Lake Ecosystem

Salmonine EMS induced by low thiamine is likely the result of a dietary predominance of prey species like alewives that are high in thiaminase (Honeyfield et al. 2005). Understanding the factors influencing thiaminase activity in alewives may be of considerable importance for managing the consequences of salmonine thiamine deficiency in the Great Lakes. Previous work has shown that the thiaminase activity of alewives exhibits considerable between and within lake variation but the causes are unknown (Fitzsimons et al., 2005; Tillitt et al. 2005). Alewives collected from western Lake Michigan during the winter when feeding activity is lowest had over twice the thiaminase activity of alewives collected during the summer when feeding activity is maximal. Recent findings suggest that stress causing factors may affect thiaminase activity in the alewives directly or indirectly through impacts on alewife physiology (Fitzsimons et al. page 17). Stress causing factors represent an important topic for further study and are the subject of current research (see Kraft et al. page 18). Furthermore, Fitzsimons et al. (page 21) reported high thiaminase activity in net plankton taken during a recent cyanobacteria bloom. However, the thiaminase in resident alewives did not change. The presence of thiaminase in net plankton was a surprise and has implication for all planktivorous fish. In a previous report Hinterkopf et al (1999) reported a correlation (R^2=0.71) between summer Microcystis blue-green algae and EMS. The prevalence of thiaminase in net plankton warrants much more thorough investigation.

In another study, Honeyfield et al (page 19) measured lipid and fatty acid content of Lake Michigan alewives and looked for relationships with thiaminase activity. While there was no relationship between total lipid content of alewife and thiaminase activity, there were correlations between thiaminase activity and several categories of fatty acids and individual fatty acids. Based on the data collected thus far, fatty acid profiles may prove to be useful to estimate thiaminase activity in alewife. Fatty acid analysis may help identify changes in the food web affecting thiaminase in alewife.

Vision, Foraging Behavior and Growth in Swim-up Fry

Previously, brain lesions in thiamine deficient fry that survived EMS were identified (see Brown et al. 2000). There was speculation that this might further reduce subsequent fry survival due to poorer fitness. Information was presented indicating that detail discrimination and motion detection in low thiamine group was significantly different between thiamine deficient and thiamine replete lake trout fry (Tillitt et al. page 21). Feeding efficiency was also reduced in fry with reduced in thiamine deficient fry. Reduction in feeding rate was most dramatic below the threshold for mortality. Moreover, a significant affect on feeding was observed in a portion of families with egg thiamine concentrations <5 nmol/gram. Approximately one-third of the families with thiamine concentrations of <5 nmol/egg-gram exhibited reduced feeding rates. This is consistent with studies investigating prey capture or predator avoidance. Fitzsimons et al. (page 21) found that the ability of lake trout fry to forage on *Daphnia* correlated with thiamine concentration in many groups, but the effect only occurred in those groups having fry thiamine concentration < 1.3 nmol (egg equivalent 2.6 nmol/g). Specific growth rate was unrelated to thiamine level in the hatchery stock evaluated but growth was directly related to thiamine concentration in three wild stocks. The growth depression followed a sigmoidal dose-response relationship with an inflection point near 4 nmol thiamine/g egg. Growth deficits resulting from the thiamine deficiency may represent a more sensitive effect of low thiamine than mortality. Given that the vast majority of egg thiamine levels in lake trout from Lake Michigan and Lake Ontario are currently below 4 nmol/g level, thiamine deficiency has the potential to seriously impair lake trout recruitment. Clearly, the risks associated with low thiamine in salmonine populations extend beyond those previously identified for EMS.

Salmonine Diet, Trophic Structure and EMS

A novel approach to modeling the food web was presented. Krause and co-workers (page 20) used a technique from social networking science. The structure of the food web was detected using two indices: weighted connectance and quantitative connectance. The structure was found to be significantly compartmentalized, where the compartments represented a range of biotic habitats. The food-web structure demonstrated changes at both the local compartment-level and at the overall structure-level after two non-indigenous species invaded. Biochemical components of the food web related to EMS were also presented. Brown et al (page 19) revised the conclusions from a previously report (Brown and Honeyfield, 2002). Egg concentrations of potential biochemical markers (stable isotopes of nitrogen and carbon, fatty acid signatures, and lipid soluble carotenoids and vitamins) are indicative of diets composed of differing food web elements or trophic structure (see Brown et al. 2005). Within all the coho and chinook salmon stocks we studied, there was a very high correspondence between EMS and low concentrations of unphosphorylated thiamine in unfertilized eggs. For salmonine stocks in the Platte River, Thompson Creek and Swan River, small but significant shifts occurred in measures of egg carotenoids, retinoids, δ15N depletion and fatty acid profiles of fish producing low EMS offspring relative to those exhibiting producing fry with EMS. Further investigations are required to determine the potential dietary sources responsible for the observed differences in the dietary biomarkers between fish with and without EMS. While the results are generally consistent with the hypothesis that a more diverse forage base may help to limit overall dietary content of thiaminase-containing species like alewife and lead to improved embryonic survival for feral salmonids this work needs to verified for other lakes and expanded with more extensive investigations.

Feral fish diet composition is useful information to fishery management. Honeyfield et al. (page 19) measured the fatty acid content of lake trout eggs from fish fed combinations of Lake Michigan alewife and bloater chub. Four individual fatty acids and the sum of omega 6 fatty acids in eggs changed linearly after one year feeding lake trout 0% to 100% alewife diets. In the second year the sum of the omega 6 fatty acids (sum w6) and 12 known individual fatty acids changed linearly as the diet went from 0 % to 100% ale. The data confirm experimentally that measuring fatty acid content of lake trout eggs may be useful for studying diet composition in lake trout.

Molecular Markers and Detection of Low Thiamine Consequences

Rise (page 22) presented data on cDNA microarray-based work identifying candidate molecular biomarkers of thiamine deficiency in lake trout eggs. There were hundreds of genes (~400) identified that are reproducibly greater than 2-fold up- or down-regulated in thiamine deficient eggs relative to thiamine replete eggs. Lake trout maternal transcripts responding to low thiamine levels include genes involved in morphogenesis, cell cycle regulation, hatching, and iron ion homeostasis. Candidate informative genes validated by quantitative RT-PCR will form a suite of molecular biomarkers that may be used to evaluate EMS susceptibility of feral and cultured ovary/egg samples. This technology has several applications and a thorough understanding of the molecular mechanisms involved in EMS may lead to the development of research tools for identifying EMS-resistant broodstock.

Flavobacterial Infections in Salmonid Fry and Fingerlings.

Flavobacterial infections in salmonine fry have become more common in the Great Lakes region. Faisal (page 23) described the etiology of swim-up fry with flavobacteriosis. Fry behavior includes spiral and erratic swimming behavior very similar to that observed in thiamine-induced EMS. Because these symptoms are similar to EMS, greater care needs to be taken when assigning cause of fry mortality. Flavobacteriosis should be considered when fry do not respond to thiamine treatment. Flavobacteriosis can be treated with antibiotics.

Updates on Work in Progress

Three laboratories reported on their planned activity. Werner (page 16) stated that purification of alewife derived thiaminase protein was successful and that antibody production was in progress. An antibody to thiaminase would expand the scope of thiaminase research. Currently the radiometric thiaminase assay measures enzyme activity not the protein per se. Hicky et al. (page 17) reported on their program planning for EMS research at the Great Lakes Science Center and progress on developing the capability to measure thiamine and stable isotopes. Finally, Kraft et al (page 18) describe plans to conduct studies to determine the effect of food web manipulation on alewife thiaminase activity. The plan employs replicated ponds treated to produce contrasting cyanobacterial primary productivity (e.g. low and abundant cyanobacteria; low and high primary productivity). Other variables potentially affecting thiaminase such as over-wintering are also planned.

Future Research Directions

As information accumulates it is important to reexamine our research priorities. In our previous EMS meeting reports (see Brown and Honeyfield 2001 & 2002), we outlined research areas identified by fishery management/biologists and scientists, considered important research needs. The present meeting and recent publications has provided new information. An updated list of research priorities is required to reflect current thinking. Determining the factors that influence thiamine deficiency and the implications of EMS to the fishery continues to be a high priority. To accomplish this goal we need a better understanding of the factors influencing the extent of the syndrome. Thiamine concentration that prevents overt mortality has been defined. We now must be concerned about thiamine concentrations that adversely affect behavior (prey capture or predator avoidance) and growth. It is important to note that thiamine deficiency is also altering adult behavior and when severe, likely affects adult survival. Thiaminase activity is highly variable in alewife. What are the factors that influence thiaminase activity in alewife? This leads to questions about basic alewife diet or adverse environmental conditions. Therefore in the larger picture, we need to have a better understanding of trophic transfer of essential nutrients and environmental conditions in the lakes that promote high thiaminase in prey species. For example, recent increases in salmonine EMS tend to parallel the patterns of abundance of dressinids in the Great Lakes. Abundant, dreissenids are known to alter nutrient dynamics in lakes and have been associated with a greater occurrence of blue-green algae. Blue-green algae are known producers of thiaminase and may represent an important source of thiaminase in the Great Lakes. It is important to understand the Great Lakes food web and the implications of disruptions by invasive non-indigenous organisms to unravel the role of alewife thiaminase and the induction of EMS in predatory salmonines.

The following is the list of research areas based on items tabled from the floor of the meeting and our assessment:

Thiaminase/Alewife

- Survey thiaminase throughout the ecosystem compartments (e.g. prevalence of thiaminase in net plankton). To determine whether there are spatial and temporal trends and their relationships among biological, physical and chemical limnological variables
- Investigate whether thiaminase within the range observed in zooplankton represents any risk to foraging juvenile fish
- Determine the relationships between alewife physiology and thiaminase activity.
- Develop more efficient, economical methods of thiaminase analysis
- Conduct alewife dietary analyses and interactions of alewife with other forage fish species
- Thiaminase in forage fish: Are there adaptive responses in forage fish to the presence of thiaminase? What is the functional value of thiaminase in alewife?

Food Web

- Application of food-web model, use a holistic approach to help identify the management factors most beneficial to natural recruitment
- Investigate relationships among recent changes in food web, invasive species, environmental factors and historical data
- Essential nutrients analysis on lower trophic levels (e.g. vitamins and lipids) of lower trophic levels
- Investigate relationship among body lipid composition, stable isotopes and expression of EMS.
- Elaborate criteria to more accurately predict feral EMS (e.g. year to year, site to site, Great Lakes versus Baltic)
- Species essential nutrient composition and partitioning within tissues and organs

Management/Monitoring

- Need for discussion of management options

- Compare essential nutrient composition (including lipids) in hatchery brood stock to feral status.
- Explore implications of greater resistance of chinook salmon to thiamine deficiency
- Establish laboratory culture techniques for algae/bacteria from lake water or from host fish.
- Explore all life-stage differences; emphasis fertilization to eye-up in coho where mortality is sometimes very high.
- Flavobacterium: investigate thiamine deficient versus thiamine replete fry.

Genetic

- Additional research into gene expression and possible tertiary responses to determine meaning of gene array data
- Develop lake trout data library to enhance discovery of potential implications of thiamine deficiency
- Gene selection criteria for EMS resistant broodstock

Information/Other

- Development of a consolidated, accessible database.
- Compile a comprehensive list of sample sources and contacts for back-testing samples
- Continue investigating the interaction between contaminants and low thiamine concentration and alterations of homeostasis in oxidative state.
- Physiology of thiamine uptake and transport in fish.

Acknowledgments

We sincerely thank the Great Lakes Fishery Commission and staff for their long-standing support of EMS research in the Great Lakes Basin. We especially want to extend our appreciation for the efforts of our speakers and other meeting attendees who took time from their busy schedules to share their work and ideas for EMS research. We thank Lisa Brown (National Water Research Institute, Burlington), Sandy Morrisson (Great Lakes Science Center, Ann Arbor) and Stephanie Sweet, (Northern Appalachian Research Laboratory, Wellsboro) for help with the meeting arrangements and recording.

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Abstracts of Presentations

Introduction: EMS in the Great Lakes

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The incidence of EMS in Great Lakes salmonids has been highly variable in the past. Therefore, it is important to continue to monitor EMS in Lake Michigan. Starting in the early 1970's, EMS was reported and has increased with time. Year to year variation in EMS that has been observed in conjunction with changes seen at the various trophic levels is one clue that may be useful for us to ultimately understand the root cause of this environmental problem. Eggs from thirty randomly selected females will be collected and reared by Martha Wolgamood, Michigan DNR, Wolf Lake State Hatchery. Percent EMS and egg thiamine were continuously recorded for Platte River Coho and Little Manistee chinook stocks. Very high levels of EMS coupled with very low thiamine levels occurred in Lake Michigan coho and chinook salmon for 1999 to 2001. The incidence of EMS dropped dramatically in 2002 with only an average of 16.4 and 26.7% of coho and chinook families, respectively, exhibiting mortality. In 2003, the incidence of EMS has increased to involve approximately 50% of reared families. EMS remains highly prevalent among Great Lakes Salmonines.

Variation in lake trout early mortality syndrome in Southwest Lake Michigan

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Self sustainable lake trout *Salvelinus namaycush* populations in Lake Michigan are a primary but unmet goal of the fisheries managers in the region. No clear cause for lack of natural recruitment has been identified for Lake Michigan lake trout. We sought (i) to determine the variability in the concentration of vitamins B1 (thiamine) in the eggs of lake trout in Lake Michigan at the time of ovulation; (ii) to quantify clinical signs of EMS (loss of equilibrium, spiral swimming, and lethargy) and compare their frequencies in Lake Michigan lake trout populations; and (iii) to correlate the frequency of EMS symptoms with levels of thiamine found in eggs from individual females. Egg thiamine concentration varied vastly among investigated females (from 0.28+0.04 to 3.83+0.61 nmol/g eggs). 17 out of 29 females sampled had initial egg free thiamine levels below 0.8 nmol/g, threshold below which variable frequency of EMS incidences was reported in lake trout from Lake Ontario. In our study, EMS occurrence among offspring of females with initial egg free thiamine levels below 0.8 nmol/g was significantly related to thiamine concentration ($r2 = 0.43$, $P<0.01$). Understanding the potential importance of EMS as a regulator of lake trout reproductive success is critical for the effective management of this native Lake Michigan fish. These findings extend our ability to interpret the role of EMS in the lake trout recruitment dynamics and will be useful to design larger scale research to investigate lake-wide variability of thiamine deficiency among Lake Michigan lake trout.

Thiamine status of walleye in Lake Erie and New York Finger Lakes

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Evaluation of the thiamine status of walleye (*Stizostedion vitreum*) in lakes having thiaminase-containing forage fishes, i.e., alewives (*Alosa pseudoharengus*) and rainbow smelt (*Osmerus mordax*) seemed important because fry of various salmonids in lakes with thiaminase-containing forage fishes manifest thiamine-deficiency early mortality syndrome (EMS). Eggs and milt were collected from walleye captured between 1996 and 2004 from various locations. After fertilization, eggs were stirred in water containing added tannic acid or bacterial protease to prevent clumping. Then eggs were transported to the laboratory and incubated until hatched at approximately 9.4°C. Between 1996 and 2002, the total thiamine in eggs from walleye captured near-shore in Van Buren Bay of eastern Lake Erie were found to vary annually from 2.1 and 6.0 nmol/g. Fry from these eggs (having a mean thiamine content of 3.05 nmol/g) were immersed in culture water alone, or with added thiamine or thiamine-antagonist, oxythiamine, and in all combinations. Results of these tests showed that oxythiamine induced mortality which was significantly reduced by immersion in thiamine. Mortality of fry immersed in thiamine alone was not significantly different from the low level of mortality observed in control fry. Signs of thiamine deficiency induced by oxythiamine included exophthalmia, pericardial edema, gaping, and lethargy. Total thiamine concentrations in eggs collected from Oneida, Conesus, and Otisco Lakes varied annually and between lakes. Oneida Lake has essentially no alewives, whereas Otisco Lake has many. Average thiamine concentrations of eggs from Oneida Lake were higher (3.4 - 8.5 nmol/g; 4 years) than those for the other lakes. Eggs from Otisco Lake had the lowest mean concentrations (1.1 - 2.6 nmol/g; 4 years). No consistent relationship was found between thiamine content of eggs from Otisco or Oneida Lake and mortality of fry or responses to 24-hour immersion of fry in thiamine. To determine to effect of thiamine content of eggs on hatchability an d survival of fry, we water-hardened eggs from ten females from Otisco Lake (1.1- 8.2 nmol/g) and from six lots of eggs from several females each from Oneida Lake (2.3 - 3.7 nmol/g) in water with and without 1,000 PPM thiamine. Results showed no significant effect of water-hardening in thiamine or thiamine content of eggs on hatchability or survival of fry. Mean concentrations of total thiamine in Lake Ontario walleye eggs were 3.3 nmol/g for five females collected in the Black River in 1997 and 2.2 nmol/g for 20 females collected in the Bay of Quinte in 2004. Based on previous studies, these levels appear to be sufficient for survival of the eggs and fry. We conclude that thiamine-deficiency early mortality (EMS) was induced in Lake Erie walleye fry by immersion in water with oxythiamine. The signs of deficiency we observed were generally similar to those seen in salmonids. Concentrations of total thiamine in eggs varied between lakes and years. Of the walleye we sampled, eggs from Otisco Lake had lowest concentrations observed (1.1 nmol/g); however, all levels observed in these lakes appeared to be adequate to prevent thiamine-deficiency EMS even though similar low levels would be expected to cause deficiency in salmonids.

Reproductive success and thiamine in Great Lakes walleye

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A controlled incubation study using gametes from a wild walleye (*Sander vitreus*) population (Bay of Quinte, Lake Ontario) in 2000 (n= 2002 (n=25 females) and 2003 (n=19 females) was conducted to assess among-female variation in offspring early-life survival in relation to ova concentrations of thiamine, planar OCs (PCDDs, PCDFs, and planar PCBs), and a suite of other maternal and ova characteristics. Planar OC concentration (TEQ as TCDD) of ova was positively related to maternal age and size, and ova lipid content. However, early life survival was unrelated to ova planar OC concentrations. Similarly, we observed no significant relationships between early life survival and ova thiamine content. Early life survival was more strongly correlated with date of spawn collection, thyroid hormone status of the ova, and ovum size. A survey of walleye stocks in Lake Ontario, (Bay of Quinte and Raisin River), Lake Erie (Grand River) and in Lake Winnipeg showed that with the exception of the Raisin River stocks ova thiamine levels were at least 2 fold that where EMS occurs in salmonines. The Raisin River stock was lower than thresholds thiamine levels where EMS is problematic in salmonines. Raisin River walleye should be investigated for the presence of EMS.

Thiamine Status of St. Marys River (MI) Atlantic salmon and progress on the development of new techniques to study EMS/TDC

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The St. Marys River has one of the only hatchery-raised returning Atlantic salmon runs in the Great Lakes. Lake Superior State's Aquatic Research Laboratory annually releases between 30-50 thousand Atlantic fingerlings and has seen spawning runs in the St. Marys River steadily increase in the last 10 years. While adult fish return to spawn, there is no evidence of natural reproduction. One of the presumed causes of this is the presence of EMS (Early Mortality Syndrome) or TDC (Thiamine Deficiency Complex) in these fish. Data will be presented on a project that correlates fry survivorship and thiamine status of eggs derived from 47 mating pairs of Atlantic salmon. In addition, a discussion of thiamine measurement techniques used, both published methods and an improved HPLC method, will be provided. Finally, an update on novel techniques that have been developed to study EMS/TDC through the purification of alewife derived thiaminase and subsequent antibody production will be provided.

Muscle and Liver Thiamine content in Lake Michigan Chinook Salmon

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Laboratory and field studies were conducted to determine the effect of winter seasonal changes in Chinook salmon thiamine status. Thiamine replete hatchery reared Chinook (32) approximately one year old (450 g) were housed at 5 C for six months in minimal light. No food was offered during the six months to simulate winter starvation. Samples for thiamine and lipid content were collected at 0, 3 and 6 months. To determine thiamine status in Lake Michigan Chinook, fish (195) were caught in spring and fall over two years. Fully replete thiamine levels in the lab-raised fish liver and muscle were approximately 25,000pmol/g and 4,000pmol/g, respectively. The liver lost half of the thiamine in the first three months, with minimal losses thereafter. The muscle lost ~170pmol/g per month. Lipid levels decreased through the study from ~8% lipids to ~1.5% lipids. Thiamine levels in the feral fish (both liver and muscle) were highest in the small fish

(~age one yr), and lowest in the medium and large (~age 3 yr) fish. Autumn thiamine levels in the liver and muscle of small feral fish were approximately 9,500pmol/g and 3,000pmol/g, respectively. Thiamine content of Lake Michigan chinook liver and muscle were significantly lower than that observed in laboratory fish. To asses risk due to thiamine deficient mortality, the percentage of fish with less than 500 pmol/g were determined. Morbidity and mortality in lake trout, steelhead and coho salmon have been observed at or below 500 pmol/g. We found 5% of small fish at or below 500 pmol/g threshold, 42% of medium fish, and 45% of large fish although no lethargic fish were noted. From this study, thiamine was shown to decrease over winter. Combining the data from this study with the observation that natural reproduction has been noted in Lake Michigan Chinook suggests that Chinook may have a lower thiamine requirement than other salmonids.

Thiamine Deficiency Complex – GLSC Involvement

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The US Geological Survey/Great Lakes Science Center is moving forward with a program utilizing an integrated approach to the investigation of trophic transfer pathways and interferences of chemical stressors in Great Lakes food webs. The effort includes both internal and external collaborations. A major project involves an investigation of the origins and transfer pathways for thiaminase in the lakes biota. A study plan and a number of proposals are being crafted for work in this area, and food web biota is being scheduled for collection for pilot studies. To support this effort, the Center is setting up facilities for thiamine assay work using the phytoflagellate Ochromonas danica, with trial data collection and fine-tuning anticipated this Fall. A collaboration with Dr. George Ketola (Tunison Laboratory of Aquatic Science) is examining the use of implanted time-release thiamine analogs to counter TDC. Collaborations with both Dr. Brian Eadie/NOAA and USEPA/ Cincinnati will bring proficiency with the stable isotope technique and fatty acid analyses, and will examine GLSC archive fish tissue composites for spatial and temporal differences related to dietary changes and major occurrences such as introduction/ban of herbicides, etc. GLSC fatty acid analysis capability is also planned, resurrecting the Center's Iatroscan technology; total lipid analysis is a current and ongoing technique.

Factors affecting thiaminase activity in alewives

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Understanding the factors influencing thiaminase activity in alewives is important for managing the consequences (larval and adult mortality along with larval growth deficits) of a salmonine thiamine deficiency in the Great Lakes. The thiaminase activity of alewives shows considerable between- and within-lake variation but the causes are unknown. We developed techniques for collection, transport and holding for alewives to experimentally assess potential influences on alewife thiaminase activity. Holding conditions affected alewife thiaminase activity but the response appeared to depend on season and stock, with elevations in activity apparently due to changes in alewife physiology rather than environmental sources. We confirmed this in a reciprocal transfer of alewives between two New York Finger Lakes having low and high thiaminase activity in resident alewives and net plankton; their was either no change in alewife thiaminase activity following transfer or activity changed opposite to that expected based on thiaminase activity of resident alewives and net plankton. We also showed that experimental diets with different lipid content did not influence thiaminase activity. A blue-green bloom in Hamilton Harbor was associated with higher thiaminase activity in net plankton but thiaminase resident alewives exhibited little response. Alewives collected from western Lake Michigan during the winter when feeding activity is lowest had over twice the thiaminase activity of alewives collected during the summer when feeding activity is maximal. Our findings suggest that stress causing factors either directly affecting the thiaminase activity in the alewives or indirectly through impacts on alewife physiology may represent important variables for further study.

Evaluating the Effects of Environment and Stressors on Thiaminase Expression in Alewife

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Our first project objective was to conduct a food web manipulation in replicated ponds with contrasting cyanobacterial primary productivity (e.g. low and abundant cyanobacteria; low and high primary productivity), then evaluate resulting thiaminase activity in alewife. Beginning in May 2004, alewives were successfully collected from Cayuga Lake and moved into ponds at the Cornell University Experimental Pond Facility. Nutrients were added to experimental ponds according to proposed treatments, and algae blooms were produced. However, due to elevated pond temperatures during July and August 2004, alewives did not survive during the initial set of experimental pond treatments. In response to this problem, three additional experiments have been initiated, as follows:

First, we established a collaboration with Mike Vanni (Miami University of Ohio), in which we used a pond experiment to evaluate the influence of contrasting environmental conditions on thiaminase expression in gizzard shad (family Clupeidae). This experiment was conducted from June – September 2004 within ponds at Miami's Ecology Research Center. The experiment consisted of moving gizzard shad from a nearby lake to 12 replicated ponds (22 x 15 x 2.5m) with four randomly assigned experimental treatments: 1) control; 2) dissolved nutrient addition; 3) allochthonous sediment addition; and 4) nutrient and sediment addition. Temperature, light and nutrient levels were monitored throughout the experiment, and water samples were also collected throughout the experiment to characterize and quantify plankton community composition in each pond. 40 gizzard shad of specified size ranges were collected for thiaminase analysis prior to being placed in the ponds, ten fish were collected every month for diet and condition analysis, and ten fish were collected for thiaminase analysis from each pond at the end of the experiment. Results from these analyses are not yet available.

Second, alewives were successfully collected from Cayuga Lake and transplanted during August into ponds at the USGS Northern Appalachian Research Facility (Wellsboro, PA). These ponds are fed by groundwater and maintain a non-lethal temperature for alewives throughout the year.

We plan to fertilize these ponds in order to produce water conditions with and without cyanobacteria blooms during summer 2005, after which alewives raised in these ponds will be evaluated for thiaminase activity.

Third, in October 2004, alewives were stocked into four ponds at the Cornell University Experimental Pond Facility in which we had previously been successful at maintaining alewives throughout the winter (during winter 2003-04). We intend to use these ponds to examine the influence of over-winter stress on thiaminase activity in alewife by maintaining two of these ponds ice-free (using an aerator) during mid-winter. Open water conditions will result in lower water temperatures, therefore alewives in these ponds should experience more stressful winter conditions – by comparison with the ice covered ponds – that could increase thiaminase activity.

Can diet-dependant factors help to explain fish-to-fish variation in EMS?

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To provide insight into the reasons why offspring of certain salmonine females exhibit early mortality syndrome (EMS) whereas others do not, we measured egg concentrations of potential biochemical markers (stable isotopes of nitrogen and carbon, fatty acid signatures, and lipid soluble carotenoids and vitamins) that are indicative of differing food web and trophic structure. To corroborate the presence of EMS, we also measured egg content thiamine vitamers. For all the coho and chinook salmon stocks we studied there was a very high correspondence between EMS and low concentrations of unphosphorylated thiamine in unfertilized eggs. For salmonine stocks in the Platte River, Thompson Creek and Swan River, small but significant shifts occurred in measures of egg carotenoids, retinoids, δ15N depletion and fatty acid profiles of fish producing low EMS offspring relative to those exhibiting EMS. Egg thiamine concentrations in chinook salmon from the Little Manistee River in the low EMS group were only marginally above the threshold for EMS induction. Along with this small thiamine differential there was no evidence of differing food web or dietary factors between low and high EMS chinook from the Little Manistee. Further investigations are required to determine the potential dietary sources for the observed differences in the dietary biomarkers between high of low EMS fish. The findings are generally consistent with the hypothesis that a more diverse forage base may help to limit overall dietary content of thiaminase-containing species like alewife and lead to improved embryonic survival for feral salmonids.

Potential for using fatty acids to predict dietary forage fish composition in lake trout eggs

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Fatty acid content of lake trout eggs was determined from fish fed combinations of alewife (ale) and bloater chub (blc) ranging for 0-100% over two years. Fatty acid methyl ester (FAME) analysis found significant difference in fatty acid content between lake trout eggs from fish fed alewife or those fed bloater chub (P<.0001). Individual fatty acids and the sum of omega 6 fatty acids changed linearly after one year (2000) in eggs from fish fed 0 % to 100% ale. In the second year (2001) the sum of the omega 6 fatty acids (sum w6) and 12 individual fatty acids were found to change linearly as the dietary ale increased form 0 % to 100%. In year one, the percentage of the variation explained by the four regression equations ranged from 19-32% (R^2). Analysis of the second year data found 18-81% (R^2) the variation was explained by the equations. The regression between the sum of w6 and alewife in the diet explained 81% of the variation (P<.0001). This work demonstrates that the amount of alewife in lake trout diet can be determined using FAME analysis of egg lipid. Potential use of this technique includes estimating lake trout dietary alewife content and tracking nutrients within the food web.

Relationship between thiaminase and fatty acid content of Lake Michigan alewife

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Thiaminase activity in Lake Michigan alewife is highly variable. Lipid and fatty acid content of Lake Michigan alewives were measured to assess the relationships among thiaminase activity, lipid content and fatty acid composition. There was no relationship between total lipid content of alewife and thiaminase activity. We did find a significant correlation between thiaminase activity and several fatty acid variables. The sum of omega six fatty acids (Sum w6) was positively correlated (r= 0.44, P< .0008) while the sum of saturated fatty acids (SAFA), was negatively correlated (r= -0.48, P. < .0002) with thiaminase activity. The following saturated fatty acids were negatively correlated with thiaminase: C15:0, C16:0, C17:0, C18:0, C.20:0, C.22:0, and C.24:0. The monounsaturated fatty acids C18:1n9t (r= -0.58, P< .0001) and C22:1n9 (r= 0.49, P. < .0002) were also correlated with thiaminase activity. The sum of the polyunsaturated fatty acids was negatively correlated with thiaminase. The fatty acids, C.20:3n3, and C.22:2 were also correlated with thiaminase (P< 0.05). Employing stepwise multivariate analysis, we found four variables that explained 66% (R^2) of the variation in thiaminase in alewife. (Sum w-6, P<.0001; SAFA, P<.0001; C18:1n9t, P<.0002; and C22:6n3, P<.0832). The source of variation in Lake Michigan alewife thiaminase remains unknown. However, it was concluded that diet or food web, lipid metabolism, and physiological response to environment such as temperature may be important factors affecting thiaminase activity in alewife.

Food-web structure of southeast Lake Michigan and its changes following two biological invasions

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We examined the food-web structure of southeast Lake Michigan and determined the changes in structure that occurred after two biological invasions, *Bythotrephes* and zebra mussels. First, we tested for compartmentalization in the structure. Compartments in food-web structure are subgroups of taxa where many, strong interactions occur within the subgroups and few, weak interactions occur between the subgroups. Theoretically, compartments increase the stability in networks, such as food webs. We used a method for detecting compartments from the social networking science. Then, we measured the structure using two indices: weighted connectance and quantitative connectance. We found that the structure was significantly compartmentalized, where the compartments represented a range of biotic habitats. The food-web structure demonstrated changes at both at the local compartment-level and at the overall structure-level after the two biological invasions. The overall structure demonstrated resistance to the invaders in compartment membership and weighted connectance. However, quantitative connectance demonstrated a detectable decline from its pre-invasion status. This decline indicated that those taxa with large biomass in the post-invasion structure had fewer effective interactions than those in the pre-invasion structure, providing fewer effective interactions as alternative pathways to absorb disturbance affects. These fewer effective interactions could act as buffers for unaffected compartments from the disturbance with fewer pathways to transfer the effects. At the local compartment-level, indices identified one invaded compartment as the weaker compartment compared to the other invaded compartment and indeed, the weaker compartment demonstrated less resistant to the invasion in its indices. More biological invasions have potential to further restructure the food web.

Influence of thiamine deficiency on larval growth, foraging, and predator avoidance in lake trout (Salvelinus namaycush)

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A diet-related thiamine deficiency increases mortality of Great Lakes salmonines during larval stages but the consequences of the deficiency on other important early life stage processes like predator avoidance, foraging efficiency and growth are unknown. Accordingly, we investigated the impacts that low thiamine may exert on swim-up fry emergence in the presence of potential predators (alewife, *Alosa pseudoharengus*, and round goby, *Neogobius melanostomus*), the ability of first-feeding fry to capture *Daphnia* and the specific growth rate of first-feeding fry in hatchery and wild stocks of lake trout (*Salvelinus namaycush*). Although alewife proved to be ineffective fry predators, the presence of a round goby significantly reduced emergence success in a hatchery stock. However, goby predation was unrelated to initial egg thiamine measurements in the families producing the swim-up fry. Foraging on *Daphnia* was correlated with fry thiamine in many groups, but it was only in groups having fry thiamine concentration < 1.3 nmol (egg

equivalent 2.6 nmol/g) where foraging was obviously depressed relative to groups with higher concentrations. Daphnia consumption in a thiamine-replete stock was density dependant whereas in a thiamine deficient group it was not. Specific growth rate was unrelated to thiamine in a hatchery stock but was related among three wild stocks where it followed a sigmoidal dose-response relationship with an inflection point near an egg thiamine concentration of 4 nmol/g. We conclude that growth deficits resulting from the thiamine deficiency may represent a more sensitive impact than mortality. Given that the majority of egg thiamine levels in lake trout from lakes Michigan and Ontario are currently below this level we suggest that the thiamine deficiency has the potential to seriously impair recruitment in these lakes.

Influence of thiamine deficiency on lake trout (Salvelinus namaycush) visual acuity and foraging ability in swim-up stage fry

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Thiamine deficiency causes early mortality syndrome (EMS) in salmonine swim-up fry. Threshold concentrations of thiamine that lead to EMS have been established for a number of species. The effects of low thiamine on physiological and behavioral functions of have not been established for any species of salmonine. Therefore, it was the objective of this study to investigate the effects of low thiamine on behavioral functions in young, post-swim-up lake trout (*Salvelinus namaycush*). We examined the potential for low thiamine to alter visual acuity, an essential component of early feeding behavior and predator avoidance skills, and we tested the effects of low thiamine on feeding efficiency in the same lake trout juveniles. Low thiamine eggs were produced by feeding broodstock lake trout diets containing thiaminase. Thiamine content of the spawned eggs ranged from 0.4 to 26.1 nmol/egg-gram. Visual acuity was determined through the behavioral optomotor and optokinetic responses of the individual fish. Feeding efficiency was evaluated on three consecutive days based on the ingestion rates of individual fish to consume *Ceriodaphnia dubia*. Both visual acuity and feeding efficiency were affected by the thiamine content of the fish. The neurobehavioral visual acuity response of the lake trout was severely affected by low thiamine, but most dramatically at thiamine concentrations less than 1 nmol/egg-gram. Feeding efficiency was also reduced at reduced thiamine content of the fish. The reduction of feeding rates was dramatic below the threshold for EMS and less dramatic, but significant, in a portion of families with thiamine concentrations of <5 nmol/egg-gram. Approximately one-third of the families with thiamine concentrations of <5 nmol/egg-gram had reduced feeding rates. The ecological significance of reduced feeding rates caused by low thiamine was not evaluated. However, the effects of low thiamine on feeding increase risks associated with low thiamine on Great Lakes lake trout population. Thus, the risks associated with low thiamine in salmonine populations extend beyond those previously determined for EMS.

Salmonid cDNA microarrays used to identify molecular biomarkers of thiamine deficiency in lake trout (Salvelinus namaycush) eggs

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Early mortality syndrome (EMS), a disease associated with low egg thiamine levels, causes embryonic mortality of Great Lakes salmonids. EMS affects strategies designed to restore and reintroduce native fish species (e.g. lake trout) in the Great Lakes. A thorough understanding of the molecular mechanisms involved in EMS will lead to the development of research tools for identifying EMS-resistant broodstock. In cDNA microarray-based experiments, the expression of thousands of genes can be evaluated simultaneously. We are using salmonid cDNA microarrays (~ 3500 genes, including 279 genes from high-complexity gonad libraries) to identify candidate molecular biomarkers of thiamine deficiency in lake trout eggs. Hundreds of genes have been identified that are reproducibly greater than 2-fold up- or down-regulated in thiamine deficient eggs relative to thiamine replete eggs. Lake trout maternal transcripts responding to low thiamine levels include genes involved in morphogenesis, cell cycle regulation, hatching, and iron ion homeostasis. Candidate informative genes validated by quantitative RT-PCR will form a suite of molecular biomarkers that may be used to evaluate EMS susceptibility of feral and cultured ovary/egg samples. This work would benefit from a lake trout egg EST (expressed sequence tag) database, since there are only 109 lake trout cDNA sequences currently in GenBank.

Flavobacteriosis causes erratic swimming behavior and mortalities in propagated fry of Great Lakes salmonids

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Flavobacterium spp. are the cause of several salmonid diseases. These include coldwater disease (*F. psychrophilum*) saddle back disease, (*F. Columnare*), and bacterial gill disease (*F. branchiophilum*). No pathogens have been linked to fry displaying the characteristic signs of early mortality syndrome such as spiral and erratic swimming behavior prior to death in swim-up salmonid fry. Most recently, we isolated *F. psychrophilum* from the brains and kidneys of propagated coho, chinook, or steelhead trout. These cases are responsive to antibiotic treatment and signs disappear following treatment. Since flavobacteria are common in the Great Lakes environment, it is important to differentiate between EMS which is thiamine responsive and infection with *F. psychrophilum* which requires antibiotic treatment.

Roster of Attendees to EMS Task Meeting, Ann Arbor, MI, September 8/9, 2004

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Agenda EMS Workshop September 8/9, 2004, Weber's Inn, Ann Arbor, MI

September 8

13:00 Introduction: EMS in the Great Lakes ………..Scott Brown, Environment Canada
13:15 Variation in lake trout early mortality syndrome in Southwest Lake Michigan…….Sergiusz Czesny, Illinois Natural History Survey
13:30 Thiamine status of walleye in Lake Ontario and New York Finger Lakes… George Ketola. USGS, Tunison Lab
13:45 Reproductive success and thiamine in Great Lakes walleye….Scott Brown, Environment Canada
14:00 Thiamine Status of St. Marys River (MI) Atlantic salmon and progress on the development of new techniques to study EMS/TDC. ….. R. Marshall Werner, Lake Superior State University
14:15 Muscle and liver thiamine in Lake Michigan chinook salmon… Stephanie Sweet, USGS, Wellsboro
14:30 Thiamine Deficiency Complex - GLSC Involvement…. James P. Hickey, USGS, Great lakes Science Lab.
14:45 Factors affecting thiaminase activity in alewives ……………….John Fitzsimons, Fisheries & Oceans Canada

15:00 Break

15:30 Pond experiments and associated efforts to evaluate thiaminase expression in alewife: A progress report……Clifford Kraft, Cornell University
15:45 Can diet-dependant factors help to explain fish-to-fish variation in EMS? …..Scott Brown, Environment Canada
16:00 Fatty acid composition of lake trout eggs fed Lake Michigan alewife and bloater chub. Dale C. Honeyfield, USGS, Wellsboro
16:15 The relationship between thiaminase and fatty acid content of Lake Michigan alewife….. Dale C. Honeyfield, USGS, Wellsboro
16:30 Food-web structure of southeast Lake Michigan and its changes following two biological invasions ……Ann Krause, Michigan State University

September 9

08:30 Influence of thiamine deficiency on larval growth, foraging, and preda tor avoidance in lake trout (*Salvelinus namaycush*)…………….. John Fitzsimons, Fisheries & Oceans Canada
08:50 Influence of thiamine deficiency on lake trout (*Salvelinus namaycush*) visual acuity and foraging ability in swim-up stage fry….Donald E. Tillitt. USGS, Columbia
09:10 Salmonid cDNA microarrays used to identify molecular biomarkers of thiamine deficiency in lake trout (*Salvelinus namaycush*) eggs……………..Matthew L. Rise, Great Lakes Water Institute, University of Wisconsin
09:50 Widespread flavobacterial infections in salmonid frys and fingerlings in Michigan…..Mohamed Faisal, Michigan State University

10:10 Break

10:30- 11:30 Group Discussion