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BOARD OF TECHNICAL EXPERTS
RESEARCH TASK REPORT

REPORT ON EARLY MORTALITY SYNDROME WORKSHOP

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GLFC-BOTE TASK

Research Coordination Meeting on Early Mortality Syndrome

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ABSTRACT

Early mortality syndrome (EMS) is the term used to describe an embryonic mortality affecting the offspring of salmonids in the Great Lakes Basin. Recently, the incidence of EMS in Lake Michigan coho has declined from peak years but continues to be problematic for some coho stocks as well as other salmonid species. In Lake Ontario EMS in lake trout has diminished fry production by more than one-third over the past 10 years. Stocks of Atlantic salmon from the New York Finger Lakes and the Baltic Sea also exhibit a similar early life-stage mortality, called Cayuga syndrome and M74, respectively. Low egg thiamin levels and enhanced survival following thiamin treatments are common characteristics of EMS, Cayuga Syndrome and M74. In May 1999 a workshop was held in Ann Arbor, MI which brought together 37 federal, state, provincial and tribal scientists and interested resource personnel to share information, present data and discuss the latest observations on EMS and other early life stage moralities. Twenty-two speakers presented the breath of current EMS research. Based on the open discussion session at the end of the meeting and published data, future research needs were formulated and put forward. Most significant developments in recent EMS research include: 1) the demonstration that EMS-like symptoms can be induced in the laboratory by a thiamin deficiency or by the application of thiamin antagonists; 2) the confirmation that thiamin in forage fish is adequate to meet thiamin requirements but that thiaminase activity can be highly variable within a species (temporal and geographic variability) as well as differing among the prey species which elaborate the enzyme; and 3) the implication that inadequate egg thiamin may be associated with sub-lethal and/or interactive effects that extend beyond initial overt fry mortality.

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BACKGROUND

Early mortality syndrome (EMS) is the term used to describe an embryonic mortality affecting the offspring of salmonids (coho salmon, chinook 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, hyperexcitability, hemorrhage and death occurring between hatch and first feeding. Before 1993 mortality rates due to EMS in Lake Michigan coho salmon offspring were around 20 %. Beginning in 1993 EMS mortality in coho salmon rose dramatically (60-90 %). In 1994 and 1995, the extent of EMS was lower (30-40 %) but exceeded 70 % in 1996 (Figure 1). Recently, EMS in Lake Michigan coho has declined but continues to be problematic for some coho stocks (Wolgamood et al., page 16) as well as other salmonid species (Edsall et al., page 16). The extent of EMS mortality in lake trout from Lake Ontario has not been monitored as extensively but has ranged between 8 and 53% over the past 10 years (Fig.1; Fitzsimons et al., 1999). Stocks of Atlantic salmon from the Finger Lakes and the Baltic Sea also exhibit a similar early life-stage mortality, called Cayuga syndrome (Fisher et al., 1996) and M74 (Börjeson & Norrgren, 1997), respectively. Low egg thiamin levels and enhanced survival following thiamin treatments are common characteristics of EMS, Cayuga Syndrome and M74.

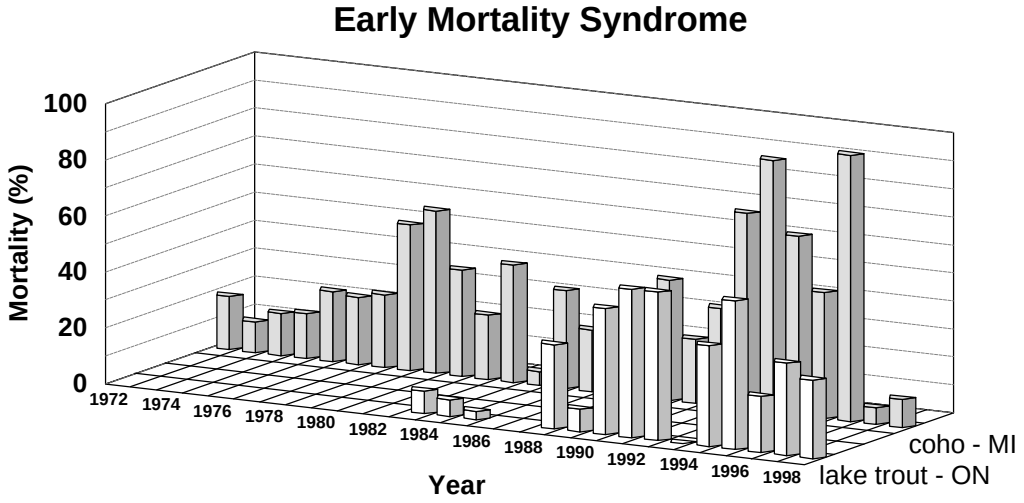

Figure 1. EMS mortality in coho salmon from Lake Michigan (MI), 1972-1998 and in lake trout from Lake Ontario (ON), 1981-98. (*Coho data from J. Hnath and M. Wolgamood, Michigan DNR and lake trout data from John Fitzsimons, Fisheries & Oceans, Canada).*

The Great Lakes Fishery Commission (GLFC) has sponsored two previous workshops held in Ann Arbor, MI (July, 1994) and Romulus, MI (February 1995) on Early Mortality Syndrome (EMS) and co-sponsored a special symposium at the 1996 American Fishery Society (AFS) Meeting. The previous workshops proved to be instrumental planning exercises that collated and disseminated knowledge about the EMS and developed research priorities. The first workshop firmly established the therapeutic potential of thiamin treatments. The second provided the direction that implicated thiamin deficiency as a key factor in EMS. The research guidance developed at the two workshops formed the backbone of the presentations at an AFS Symposium on EMS held in Dearborn, MI (August 1996). The AFS Symposium led to a

special publication focusing on EMS (McDonald et al.1998). The May 1999 Ann Arbor workshop was organized to again bring together federal, state, provincial and tribal scientists and interested resource personnel to share information, present data and discuss the latest information on EMS and other early life stage mortalities. Twenty two speakers presented the breath of current EMS research. The presentation by Brown et al. (page 15) provided an overview of the EMS research initiative sponsored by the Great Lakes Fishery Trust. The following is a synopsis of the presentations and concluding statements.

WORKSHOP SYNOPSIS

In the past two years, there have been three significant developments in EMS research on Great Lakes species. These developments include:

- the demonstration that EMS-like symptoms can be induced in the laboratory by a thiamin deficiency or by the application of thiamin antagonists;
- the confirmation that thiamin in forage fish is adequate to meet thiamin requirements but that thiaminase activity can be highly variable within a species (temporal and geographic variability) as well as differing among the prey species which elaborate the enzyme;
- implication that inadequate egg thiamin may be associated with sub-lethal and/or interactive effects that extend beyond initial overt fry mortality.

Incidence of EMS/M74

As noted, it is important and essential to monitor the incidence of EMS on a family specific basis to facilitate studies on cause-effect relationships because EMS is female specific with the progeny from different females exhibiting differing amounts of EMS. As part of the on-going Michigan DNR hatchery program and EMS studies under the Great Lakes Fishery Trust (GLFT), Martha Wolgamood et al. (page 16) presented data showing that the incidence of EMS in Lake Michigan coho is now lower than high incidences observed in 1996 but does varies by sub-population. Coho salmon taken at the Platte River exhibited less than 15% EMS mortality in 1997 and 1998 but Thompson Creek Coho exhibit 27 % EMS mortality. The suggestion that the prey fish composition differs between the sub-populations warrants study that is more detailed. There was higher incidence of EMS in chinook salmon from Swan River, Lake Huron (16.7%) relative to those collected from the Little Manistee River in Lake Michigan (6.7 %). Results from monitoring lake trout in Lake Michigan showed that EMS did not correlate with biological measurements such as fertilization rates or hatching rates. Fry groups that developed EMS generally had total thiamin levels less than 1 nmol/g (Edsall et al., page 16). The overall incidence of EMS in lake trout from Lake Michigan for 1998 was 28%.

In the Baltic Sea, data from Sweden on M74 showed that incidence had declined to 7% in 1998 but increased to 35% in the 1999-year class (Norrgren & Börjeson, page 14). Finnish investigations of Baltic salmon stocks (Vuorenin et al., page 14) showed that the incidence of M74 varied depending upon the geographical location of the salmon.

Thiamin Treatment of EMS

Investigations aimed at improving the thiamin treatment protocols have continued. Monthly thiamin bath treatments (300-350 ppm for 30-45 min) given to Skamania steelhead broodfish did not appear to result in any significant increase in egg thiamin concentrations (Marcquenski et al., page 24). Thiamin baths adjusted to either pH 6.8 or 7.2 had similar egg uptake of thiamin during water hardening treatments. Data on thiamin levels in the eggs held in flowing water after bath treatment show that egg thiamin declines considerably following treatment. A 48-hour wash period is necessary before evaluating the uptake efficiency of thiamin following bath treatments at water hardening. This has been corroborated in lake

trout eggs where 2 hour thiamin bath (750 ppm) indicated very high initial levels (>100 nmol/g) but produced a sustained net increase of about 1 nmol/g thiamin (Honeyfield and Brown, unpublished data). Ketola et al. (page 20) investigated the effectiveness on intraperitoneal thiamin injections in female Atlantic salmon. Injection of females with 7 mg thiamin/kg body weight 14-23 days before spawning resulted in physiological increases in egg thiamin content and reduced offspring mortality from 98.6 % to 2.1 %.

Experimental Induction of EMS

Understanding cause/effect and interactive relationships in EMS has been impeded by the lack of a suitable laboratory model for the condition. Information reported by Honeyfield (page 25) and Fitzsimons et al. (page 25) shows that there has been important progress on the experimental induction of EMS in the laboratory. Feeding lake trout a diet low in thiamin in combination with added bacterial thiaminase produced the clinical symptoms of EMS in the offspring (Honeyfield, page 25). Histopathology on the fry exhibiting EMS-like symptoms showed the presence of focal necrotic brain lesions. This pathology is consistent with brain lesions observed in Baltic salmon exhibiting M74. Norrgren and Börjeson (page 14) reported a high incidence of necrotic cells in certain parts of the brain and depletion of the glycogen stores in the liver. In lake trout, feeding the experimental diet also lowered egg thiamin concentrations to levels similar to those observed in feral fish displaying EMS. The feeding experiment by Honeyfield (page 25) represents the first clear demonstration of overt EMS in lake trout under laboratory conditions. Fitzsimons et al. (page 25) showed that egg injections of the specific thiamin antagonists (pyrithiamin and oxythiamin) were also capable of producing EMS-like symptoms. They also reported that lake trout sac-fry from Lake Ontario and other thiamin deficient stocks were almost a thousand-fold more sensitive to oxythiamin than Lake Manitou even though their background thiamin levels only varied by four-fold. This observation suggests that that there are unanswered questions about the factors affecting the sensitivity of thiamin deficient stocks.

Thiamin in Prey Species

Studies were conducted to establish the conditions required to preserve the thiamin content of sampled alewife. To prevent losses when sampling for total thiamin, it was found that the samples need to be placed on dry ice within 20 minutes of capture, regardless of holding temperature between 4 and 21°C (Wright et al., page 18). In Lake Michigan, thiamin concentrations in alewife differed with respect to location and season (range 4-13 nmol/g). Alewife collected in the southern part of the Lake Michigan contained about half the thiamin as those in the northern areas. Of the species examined, rainbow smelt contained low thiamin concentrations (1-2 nmol/g) that marginally exceeded levels recommended for salmonid growth (Brown et al., page 19). Data from the Baltic forage species showed that thiamin content of Baltic sprat was 7 nmol/g. Thiamin content of Baltic herring ranged from 6-9 nmol/g in age 1-2 year class and 9-10 in 3 year class (Vuorinen et al., page 15).

Thiaminase in Prey Species

Zajicek et al. (page 19) reported that the amount of thiamin degrading activity in the two exotic forage fish species (alewife and smelt) was up to a hundred times the activity observed in the native bloater species (alewife, 6.6 nmol/g; rainbow smelt, 2.6 nmol/g; bloater chub, 0.02 nmol/g). The thiaminase content of alewife also showed a four-fold between lake variability. Moreover, Honeyfield (page 28) reported that two microbial strains of thiaminase positive bacteria were isolated from alewife viscera; *Bacillus thiaminolyticus* and an un-named *Bacillus* species, thereby suggesting a possible source of thiaminase in these prey species.

Other Food Web Relationships

There are changes to the primary production and tropic structure in progress in the Great Lakes caused by zebra mussels (Cook & Lozano, page 21). If alewife thiaminase levels are altered by these changes in the food web food base and salmonids have no alternative food sources to alewife, progress toward natural reproduction of salmonids could be delayed by continuation or increase in incidence of EMS. Hinterkoff et al. (page 17) correlated information on plankton species over a 10 year period (1983 -1992) in Lake Michigan with EMS. There were some good correlations between changes in abundance of planktonic food items and historical incidences of EMS, however, the biological significance of these relationships is presently unclear. Between 1993 and 1998 EMS in Lake Michigan coho underwent dramatic changes and comparison of this more recent data with abundance of planktonic species may provide greater insight. Associations between incidence of EMS and planktonic species capable of producing thiaminase (e.g. some blue-green algae) and salmonid prey species warrants further investigation. No data were presented but the topic of thiaminase in blue-green algae and other algal toxins also represent aspects under discussion in Scandinavian investigations on M74 (Leif Norrgren, Swedish University of Agricultural Sciences, Uppsala, Sweden, personal communication).

Contaminants

A direct link between a specific contaminant and the development of EMS has not been demonstrated in North American studies (reviewed by Marquenski and Brown 1997; Honeyfield et al. 1998). In Swedish studies on M74, the role of toxicants is also unclear and no single candidate has been linked to the syndrome (see Norrgren & Börjeson, page 14). However, Finnish researchers reported that higher muscle concentrations of dioxin-like planar halogenated hydrocarbons (PHHs) in female salmon from the River Simojoki stock were coincident with the start of M74-mortality in offspring (Vuorenin, page 15). Cook & Lozano (page 21) point out that attempts to detect a direct chemical toxicity relationship for EMS have only been examined over short time periods within a relatively narrow range of chemical exposure concentrations. Thus, the studies are unlikely to identify the potential for the various chemicals to act synergistically on the thiamin sensitivity of sac fry.

Based on laboratory tests, present concentrations of PHHs in Great Lakes lake trout are insufficient to cause overt mortality in the developing embryos. However, the clinical pathology of PHH-induced toxicity and that of EMS does share similarities (Tillitt et al., page 19). These two stressors (elevated PHHs and low thiamin) may interact with some common biochemical/physiological pathways and as such may exacerbate the effect of each other in Great Lakes salmonids. By examining the temporal relationship between the incidence of pathology such as tissue-specific apoptotic cell death and the onset of EMS, the early stages of toxicity that occur prior to gross pathological effects may be isolated (Whyte et al., page 27). In support of the hypothesis of some shared pathways, the non-lethal symptoms of PHH toxicity were consistently greater in embryos with low thiamin. Wright et al. (page 26) reported that in thiamin deficient lake trout embryos, the number of embryos exhibiting startle response was similar to results in thiamin sufficient embryos, however, the magnitude of the response was significantly decreased. Furthermore, TCDD adversely affected both the overall number of embryos exhibiting startle, and the magnitude of the response.

Other Vitamins and Biochemical Markers

Swedish studies show that both thiamin and perhaps astaxanthin constitute important factors during the development of M74 and that the carotenoids constitute a valuable marker for the presence of M74. Norrgren and Börjeson (page 14) described the long standing correlation between roe color and the risk of developing M74 in Baltic salmon. These observations are supported both by ocular determination of the roe color and by biochemical analysis of astaxanthin concentrations. Gluthionine synthase and gluthionine reductase activity was increased in M74 affected fish. Similarly, M74 affected fish from Finland showed a

high yolk-sac fry mortality related to a low thiamin content and the pale color of eggs. In North American, changes in egg color, carotinoid content and antioxidants have not been routinely assessed. In 1994, lake trout displaying EMS in Lake Ontario showed few differences in these parameters when compared to cohorts that did not exhibit EMS. While differences in egg color have not always proven predictive of EMS for Lake Michigan salmonid stocks, hatchery personnel noted that relative to previous years there were dramatic color and size differences in females whose offspring did not exhibited EMS in 1997. These observations suggest a major shift in coho diet for this year class. Chemical analyses of tissues and eggs will provide corroborative evidence of dietary shifts.

Preliminary studies aimed at investigating maternal thiamin dynamics have begun with research on a potential estrogen-dependent thiamin carrier protein such as found in other species. These studies were proposed to determine whether there are potential problems with parental thiamin transport to eggs. So far, initial attempts to isolate a thiamin carrier protein have been unsuccessful and more fundamental approaches are needed to investigate the issue (O'Connor et al., page 27).

Research with Other Top Predators

The extent that alewife represent a major diet item and the role that thiamin deficiencies might play in the reproductive success of other top predators such as walleye is under investigation. Observations by Ketola et al. (page 22) in walleye fry from Lake Erie at the Tunison Laboratory (1997) showed a 10% incidence of mortality associated with clinical signs similar to those induced by the thiamin antagonist, oxythiamin. This is perhaps suggestive of a low incidence of early mortality syndrome. Ketola et al. (page 22) reported mean total thiamin concentrations in eggs from walleye captured in 1996, 1997 and 1998. Total thiamin values ranged from 1.4 to 11.8 nmol/gram. Invasion of alewife into Tennessee reservoirs has been associated with impaired walleye recruitment and studies are currently underway to examine the potential role that low levels of thiamin plays relative to other factors influencing embryonic survival (Vandergoot et al., page 24). Walleye egg thiamin concentration from Center Hill, Tellico, Upper Dale Hollow and Lower Dale Hollow Reservoirs were 1.4, 2.3, 3.1 and 4.5 nmol/g, respectively

Historical Perspectives on Salmonid EMS

In a retrospective analysis of historical information, Ketola et al. (page 20) note that in addition to the other factors (e.g. dams, over-fishing) often associated with Atlantic salmon extirpation from Lake Ontario and Cayuga Lake, their disappearance also coincides with the entrance of alewives to the systems in the 1800s. Thiaminase containing alewives may have induced egg thiamin deficiency, increased fry mortality in the predatory salmon and contributed to their extirpation.

Fitzsimons et al (page 19) compared the historical information for EMS incidence in Lake Michigan coho salmon with alewife trawl abundance (CPUE) and reported that EMS was weakly (r_s=-0.37) correlated with alewife catch. Because coho salmon continued to feed on the alewives even at low levels of abundance, these results suggest the possibility that qualitative changes in alewife themselves may contribute to EMS.

Cook & Lozano (page 21) reviewed the history of lake trout in Lake Ontario. Before 1930, lake trout catches and fry recruitment was good suggesting that there was significant recruitment from native stocks. Due to unsustainable commercial fishing and sea lamprey predation, lake trout populations declined rapidly in 1940s and 1950s. According to models using environmental dioxin levels to estimate levels in sac-fry , by 1950 dioxin toxicity to sac fry, independent of other stressors was probably sufficient to eliminate lake trout recruitment. In the post 1970 dioxin/PCB recovery period, slow progress toward natural reproduction and survival of early life stages has been observed with limited year old lake trout from natural reproduction evident after 1995.

SUMMARY OF THE MEETING DISCUSSION

The workshop participants spent three to four hours discussing the results, following the formal presentations. The information presented below is a compilation of notes and bullet items that were listed by the participants.

1. EMS continues to impact salmonid populations in the Great Lakes basin. There have been recent declines in the incidence of EMS in Lake Michigan coho salmon, but it is clear that sub-populations of coho and other species still exhibit significant EMS mortality. EMS continues to impair hatchery production and probably hinders the establishment of self sustaining salmonid populations by compromising early life stage survival and recruitment.

2. In salmonid embryos, EMS is a result of thiamin deficiency. Three pieces of evidence support this conclusion: a) thiamin is therapeutic and water hardening eggs in a thiamin bath, injecting female broodstock prior to spawning with thiamin or treating sac-fry with thiamin prevents death; b) treating of normal healthy offspring with thiamin antagonists (e.g. oxythiamin or pyrithiamin) result in their expressing EMS-like clinical symptoms; c) and low thiamin diets containing bacterial thiaminase fed to lake trout broodstock result in low egg thiamin and the off-spring exhibiting the EMS-like symptoms similar to those observed in feral fish.

3. Thiamin concentrations measured in eggs immediately following treatment at water hardening are not representative of internal thiamin concentration. This fact accounts for some of the variability between exposure levels and effect on EMS reported in previous investigations and suggests that thiamin treatment procedures of eggs may require further refinement. Small increases in thiamin near the threshold (1 nmol/g) are biologically significant for reducing EMS and this low uptake is sufficient to enhance survival.

4. Forage fish body stores of thiamin were found adequate to meet dietary thiamin requirements for growth of salmonids. Thiamin content of prey fish is somewhat variable between lakes and between species, presumably reflecting the differing nutritional status of these animals.

5. Thiaminase activity in salmonid prey was variable. Variation was observed across lakes, within alewife and rainbow smelt and the activity varied with time of year that samples were collected. Although the source for the variation is currently unknown, these differences may account for some of the observed temporal, regional and species variability in the extent of EMS. Comparison of present and past thiaminase activity data is difficult because there were differences in the methods used to determining thiaminase activity. Thiaminase I activity requires both thiamin and co-substrate to be functional and present assays use optimal levels of both substrate co-substrate thereby giving higher results than previously reported.

6. Food web factors that may influence forage thiaminase activity are not well defined. The primary prey fish associated with mortality syndromes have thiaminase activity; alewife and rainbow smelt in North America and clupeids (herring family) in Baltic region. However, the factors responsible for the observed variability in enzyme activity need further study. Information to be gained from stable isotope analysis of food web items was discussed.

7. No single contaminant has been directly linked to EMS but attempts to detect direct relationships have only been examined over short time periods and within a relatively narrow range of chemical exposure concentrations. Therefore, this limits our potential to detect chemicals, which act synergistically on the thiamin sensitivity of sac fry.

8. The sub-lethal consequences of low thiamin on fry survival, recruitment and long-term viability have not been assessed.

9. Although criteria are available for the identification of EMS in different species, an unambiguous set of criteria explicitly identifying EMS from other early-life stage problems is lacking. For example, characteristic histopathology has received limited attention in North American stocks and biochemical mechanisms and key disease or physiological processes (e.g. apoptosis) have not been identified.

10. Top piscivore predators are involved. So far, all known cases of thiamin responsive syndromes (EMS, M74, Cayuga syndrome) involve salmonids. Recent studies in walleye suggest a low incidence of early-life stage mortality similar to mortality induced by thiamin antagonists. Egg thiamin concentration varies in walleye and it is plausible that this variability may be related to a diet of alewife in those waters (Lake Erie and Tennessee reservoirs). Walleye recruitment in Tennessee reservoirs has declined following alewife introductions and these situations could provide useful models to investigate alewife/walleye interactions.

11. Examine stocks across a range of natural (e.g., some stocks of Lake Ontario rainbow trout and chinook) to identify potential differences in corresponding EMS could help identify critical factors and the relative importance of EMS.

12. Although the issue of sperm viability has not been identified as a problem in feral fish where fertilization occurs under hatchery conditions, male reproductive competence in the wild fish experiencing EMS has received little attention.

13. A regularly updated web site would facilitate communication among researchers, managers and fishers. There is a site for M74 (http://w1.185.telia.com/~u18503767/m74eng.htm). A synopsis of a recent review by Marcquenski and Brown (1997) is posted on the Great Lakes Fishery Commission site (http://www.glfc.org/fishmgmt/emsib.htm).

14. These syndromes (EMS, M74, Cayuga syndrome) have the potential to bring the affected stocks to extinction. Presently we note that not all stocks are afflicted to the same degree and that the effects of these syndromes on unmonitored stocks are less clear. However, given that offspring mortality of an individual female can reach 100%, it is assumed that complete loss of specific stocks is possible.

SUMMARY OF RESEARCH NEEDS

The following ten areas of research needs were drafted from the meeting presentations, the discussions during the meeting and considerations of existing published data.

1. Sub-lethal consequences of low thiamin on fry survival, recruitment and long-term viability need to be assessed. Feral fry that survive the acute stages of thiamin deficiency may have transitory or permanent behavior or metabolic difficulties. Does low thiamin decrease the ability to avoid predators or to capture food items? The present levels of dioxin-like contaminants are unlikely to cause acute mortality; however, there are potential synergistic interactions between these contaminants and low thiamin that may impact behavioral or other responses and lead to poor survival and inadequate recruitment. The sub-lethal consequences of low thiamin also raise concerns about when thiamin treatment should be occurring: at water hardening or when symptoms are observed in fry?

2. There is a need to know more about thiaminase. The source of the variability in thiaminase activity within salmonid prey is unknown. Is the thiamin/thiaminase content of forage fish is influenced by nutritional status of the animals? Food web factors influencing forage thiaminase activity are not well defined. Investigations are needed to determine the source of enzyme activity. Both endogenous physiological factors (e.g. stress & condition) and exogenous sources (e.g. bacteria, blue-green algae, exotic species) may influence elaboration of enzyme activity in forage species. The possible role contaminants or environmental factors to affect thiaminase activity are a big question. Research is needed to determine if annthropogenic chemicals (e.g. triazine herbicides) or natural factors act as nucleophile/base-co-substrate and thus enhance thiaminase activity or generate endogenous thiamin antagonists.

3. Thiamin treatment procedures for eggs, fry and adult need to be further refined (a related issue is the treatment of egg/fry to prevent sub-lethal consequences).

4. Hatchery personnel have a need for a rapid predictive test prior to or at spawning to determine whether thiamin treatment will be necessary to maintain hatchery production.

5. To facilitate better understanding of cause and effect relationships and to identify stocks at risk there is a need to continue monitoring of EMS in the various salmonid populations in the Great Lakes basin.

6. There is a need to provide an unambiguous set of criteria to identify EMS across species and to identify key disease mechanisms, characteristic histopathology, biochemical mechanisms, physiological processes (e.g. apoptosis) and behavioral responses that result in EMS mortality across species. This includes studies on routine thiamin uptake, metabolic pathways and characterization of the processes involved with prey digestion, and absorption of thiamin as well as its transport and deposition into the developing ovary.

7. The information obtained from stable isotope and carotinoid analysis in salmon tissues and food web items will help to delineate potential differences in predator-prey relationships between stocks expressing different levels of EMS. This information would address inferences that Lake Michigan coho not exhibiting EMS were primarily utilizing benthic organisms rather than alewives and smelt as a food source in 1997.

8. Studies to determine contaminant interactions with low thiamin are warranted. Thiamin is a cofactor of thiamin containing enzymes, but thiamin may have other biochemical functions. We do no know for example whether thiamin forms an abduct during the metabolism of contaminants. In addition to the direct effect of thiamin deficiency, we do not know whether there are possible interactions with other vitamins, especially the antioxidant vitamins and biochemicals (Vitamin E, Vitamin A, beta-carotene, astaxanthin, ascorbate, and gluthionine). A related issue is programmed cell death (apoptosis).

9. There is a need to determine whether EMS extends to other top predator species such as walleye.

10. Sperm production, quality and viability in thiamin deficient males are unknown. Is there a problem?

ACKNOWLEDGMENTS

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Villella for the general support that they provided for the meeting. We especially appreciate the efforts of all the speakers and other meeting attendees who took time from their busy schedules to share their work and ideas for EMS research. In particular, we acknowledge Leif Norrgren and Pekka Vuorinen for taking the time to travel to our meeting, sharing current information about M74 in the Baltic and providing advice for EMS researchers to consider.

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ABSTRACTS OF PRESENTATIONS

SUMMARY OF M74 RESEARCH IN SWEDEN - LEIF NORRGREN[1] AND HANS BÖRJESON[2]

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During the recent decades the M74 syndrome affecting the Baltic salmon has been in the focus of diseases causing reproduction disturbances among fish species in the Baltic Sea. Major efforts have been made in description of pathogenesis and possible causes of M74 including genetics, pathogens, feeding habits, toxicants and nutritional factors. Furthermore, the impact of M74 on wild spawning populations has been determined. Major macroscopical pathological findings during the propagation of the disease include darkening of the skin, a pale liver and spleen, retardation of the yolk-sac resorption and neurological disturbed behavior. The most significant histopathological observations are a high incidence of necrotic cells in certain parts of the brain and depletion of the glycogen stores in the liver. No correlation between genetic factors or involvement of pathogens has been found. The salmon does not appear to have changed its feeding habits during recent decades, the period when the M74 incidence has accelerated. A decline in recruitment of wild spawning salmon caused by extensive fishing and M74, make these populations threatened to an extent higher than that of hatchery raised populations. Already in 1977, a correlation between roe color and the risk of developing M74 was noted. This has later been confirmed both by ocular determination of the roe color and by biochemical analysis of astaxanthin concentrations. Due to results from measurements of thiamin levels in salmonids from the Great Lakes suffering from EMS, initiatives were taken to determine also thiamin in tissues from Baltic salmon and in their progeny. The results show a suppression in total thiamin levels in salmon producing progeny developing M74 and a threshold value of 0.4 nmol thiamin/g roe has been proposed. The role thiamin during development of M74 has also been confirmed by prophylactic treatment of either maturing females or their progeny, showing very positive results on survival. The potential role of toxicants involved in the M74 syndrome is still somewhat obscure. Classical environmental toxicants, i.e. PCBs and DDT, show a general decline over the last decade in several aquatic organisms of the Baltic Sea, i.e. herring and seal. However, during the same time period increasing concentrations have been recorded for others, i.e. brominated flame retardants. The incidence of M74 reached a peak during the early 1990s with occasional moralities in some rivers at 90%. During 1997-98 the mortality decreased and the lowest incidence since 1984 was recorded in 1998 with a total mean mortality of 7% estimated from 1071 family groups. This positive trend shifted again during 1999 when the total mean mortality due to M74 was approximately 35%. In conclusion, the Swedish studies show that both thiamin and astaxanthin constitute important factors during the development of M74 and that the carotenoids constitute a valuable marker to M74. The role of toxicants is obscure and no single candidate can be linked to the syndrome. More extensive chemical and biological studies on toxicants should be performed in the future, including also evaluation of candidates not previously evaluated, i.e. algae toxins. In order to provide more information of the etiology of M74 we are presently applying different models all aiming at experimentally inducing M74. These are:

- microinjection of thiamin antagonists in combination with prooxidants in newly fertilized salmon eggs.
- toxicological evaluation of algae extracts on yolk-sac fry.
- long-term studies on farmed salmon by providing low thiamin feed contaminated with the thiamin antagonist amprolium in combination with PCBs.

SUMMARY OF M74 RESEARCH IN FINLAND - PEKKA J. VUORINEN

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In salmon from the River Simojoki which empties into the Gulf of Bothnia, the proportion of M74-females has fluctuated from 12 to 92% and the mean annual yolk-sac fry mortality from 14 to 76% since the end of the 1980s. Both variables peaked from 1992 - 1994. In salmon from the River Kymijoki which flows into the Gulf of Finland, the mean yolk-sac fry mortality from 1995 - 1998 was 35 - 56%, the values for 1998 being higher than those in the R. Simojoki salmon. The timing of the mortality of yolk-sac fry varies between batches from about the second quarter of the yolk-sac phase until near the completion of yolk absorption. Particularly in the latter offspring there are partial mortalities. High yolk-sac fry mortality is related to low free and total thiamin content and the pale color of eggs. Significant mortalities occur when the free thiamin content of eggs is below about 1 nmol/g. In sea trout some unconfirmed observations of M74-like mortalities have been reported. Coincident with the start of M74-mortality the concentrations of dioxin-like organochlorines (OCs) increased in the muscle of female salmon from the R. Simojoki. In forage species of salmon, Baltic herring and sprat, which have comprised about 90% of the feed of salmon since the 1960s, OC concentrations in lipid weight basis increase strictly with age. As growth rate and mean size at age of herring and sprat have decreased since the early 1980s, salmon is bound to feed on older prey than before. The thiamin contents did not vary between herring and sprat and between different age classes, the mean total thiamin being about 7 nmol/g in all groups. The individuals in different salmon stocks seem to have different migration patterns which probably give rise to differences in their diets. This might be one reason for individual differences in the M74 prevalence of feral females.

INVESTIGATING SOLUTIONS TO THE EARLY MORTALITY SYNDROME - SCOTT B. BROWN[1], DALE C. HONEYFIELD[2], JOHN FITZSIMONS[3] AND DONALD E. TILLITT[4]

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Managing for sustainable salmon and trout fisheries in Lake Michigan is threatened by the occurrence of Early Mortality Syndrome (EMS), a syndrome that reduces fry survival. Early Mortality Syndrome affects the offspring of feral coho and chinook salmon, lake trout, steelhead and brown trout in Lakes Michigan and Ontario and to a lesser extent Huron and Erie. Since the mid-1980's the mortality of eggs collected from feral Lake Michigan coho increased steadily from around 20% to more than 80% by 1993. Subsequently, thiamin immersion of eggs and sac-fry was shown to be an effective treatment to reverse EMS. Treating developing coho eggs with thiamin is the way that management agencies provide enough eggs to meet angler demand for coho salmon in Lake Michigan when EMS is prevalent. Without this intervention, EMS would have the potential to extirpate the affected feral species.

The Great Lakes Fishery Trust has sponsored a project that investigates four primary areas critical to the understanding of EMS in the Great Lakes and its treatment: 1) quantification of thiaminase/thiamin content in the food web of Lake Michigan salmonids; 2) determination the extent of EMS in coho, chinook, and lake trout from Lake Michigan; 3) improved procedures for thiamin therapy; and 4) development of a laboratory model for EMS. The aim is to provide a better understanding of the factors, which control or

precipitate EMS and to seek the most effective thiamin treatment methodology to maintain hatchery production. A quick screen technique to measure egg thiamin levels will provide hatchery personnel a tool to decide which eggs require treatment will be developed. Once the causes and pathway of the thiamin deficiency in Great Lakes salmonid eggs are identified, fishery agencies will have more information to use in the management the Great Lake fish community in order to minimize salmonid egg losses due to EMS. Methodology and analytical techniques from this project may also prove useful in the investigation of recent recruitment problems observed in other species such as walleye. Research results will be distributed to management agencies and the public through news releases and existing information networks such as the Great Lakes Fishery Commission news letter Forum, and the Great Lakes Sport Fishing Council. Major findings will also be published in scientific journals.

INCIDENCE OF EARLY MORTALITY SYNDROME IN LAKE MICHIGAN COHO AND CHINOOK SALMON IN 1998 - MARTHA WOLGAMOOD[1], SCOTT BROWN[2], JOHN FITZSIMONS[3], DON E. TILLITT[4], DALE C. HONEYFIELD[5], SUSAN V. MARQUENSKI[6]

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Fry mortalities due to early mortality syndrome (EMS) continue to be a problem in Lake Michigan. The cause of EMS remains unclear, but low egg thiamin content is associated with the problem. In a project funded in 1998 by the Great Lakes Fisheries Trust Fund, the incidence of EMS in coho and chinook salmon was monitored to compare with existing data. From each river (Platte River, Michigan; Root River, Wisconsin; and Thompson Creek, Michigan) eggs were collected from thirty coho females. Additionally, eggs were collected from thirty chinook salmon from the Little Manistee River (Lake Michigan) and from Swan River (Lake Huron). A sample of unfertilized eggs from each female was frozen for thiamin analysis. In 1998, the incidence of EMS in coho was 6.7% in Platte River, 13.3% in Root River and 26.7% in Thompson Creek with total thiamin in eggs averaging 3.46, 2.63, and 1.98 nmol/g, respectively. In Little Manistee and Swan River chinook, EMS was 6.7 and 16.7% with total thiamin averaging 1.87 and 3.47 nmol/g, respectively. In 1997 and 1998 the incidence of EMS was less in the Platte River and Root River coho (0.0 – 25.0%) when compared to the previous three years when it ranged from 60-80%. This data shows that the incidence of EMS in Lake Michigan coho varies by sub-population and may indicate that the composition of their forage is different.

BIOLOGICAL MONITORING OF EARLY LIFE STAGES OF LAKE MICHIGAN LAKE TROUT (*SALVELINUS NAMAYCUSH*) FROM 1996-1998 - CAROL COTANT EDSALL, JEFFREY D. ALLEN, AND LYNN M. OGILVIE

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Lake Michigan lake trout collected near Sturgeon Bay, Wisconsin have been monitored for fertilization, survival to hatch, abnormalities, and survival of fry to swim-up at the Great Lakes Science Center in Ann Arbor, Michigan. A total of 73 lots of eggs have been collected in the last three years. Subsamples of those

lots were frozen for thiamin (Dale Honeyfield's laboratory) and contaminant analyses (Don Tillitt's laboratory). Average fertilization rates varied from 86.7% in 1996, 73.7% in 1997, and 49.9% in 1998. Survival to hatch based on fertilized eggs was highest in 1996 at 91.3% followed by 80.4% in 1997 and lowest in 1998 at 71.9%. Nineteen of 69 lots of fry showed the distinctive signs of Early Mortality Syndrome (EMS); survival was less than 5% for 9 lots and ranged from 15.6% to 87.2% for the remaining 10 groups. The first signs of the syndrome appeared two weeks after normal fry were transferred to glass tanks but sometimes did not develop until 4-5 weeks after transfer; all fry that developed the syndrome died over a 14 to 28 day period after showing signs of the syndrome. Fry survival to 75 days post-hatch was 98 to 100% for those lots that did not show EMS. EMS does not correlate with biological measurements such as fertilization rates or hatching rates. Lots of fry that developed the syndrome generally had total thiamin levels less than 1 nmol/g. Since EMS does not seem to be declining in Lake Michigan lake trout, research needs to continue to find the cause of the syndrome in which multiple factors are probably involved.

ABUNDANCE OF PLANKTON SPECIES IN LAKE MICHIGAN AND INCIDENCE OF EARLY MORTALITY SYNDROME FROM 1983 TO 1992 - JOY P. HINTERKOPF[1], D. C. HONEYFIELD[1], J. MARKAREWICZ[2] AND T. LEWIS[2]

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Zooplankton and phytoplankton contribute to the food web, but it is not known if changes at this trophic level directly affect the incidence of early mortality syndrome of salmonids in Lake Michigan. To determine if a relationship existed, plankton abundance and biomass were correlated with the incidence of EMS in Lake Michigan coho salmon, and lake trout from 10 year data previously reported (Makarewicz et al., 1998; Makarewicz, et.al.,1995; Fitzsimons et al., 1999). Incidence of EMS in lake trout increased with total spring daphnia abundance ($r = 0.73$) and with summer abundance of Daphnia pulicaria, an exotic zooplankton present from 1978 to 1987, ($r = 0.684$), but incidence of lake trout EMS was inversely related to the summer abundance of Daphnia galeata mendotae ($r = - 0.354$). Conversely the incidence of EMS in coho increased with an increase in the summer abundance of Daphnia galeata mendotae ($r = 0.689$). The incidence of EMS in lake trout decreased with expanding abundance of Bythotrephes, an exotic zooplankton identified in Lake Michigan in 1986, ($r = -0.57$); no relationship was found between incidence of EMS in coho and abundance of Bythotrephes. The summer abundance of Diaptomus minutus was positively correlated with EMS in coho ($r = 0.623$), but negatively correlated with EMS in lake trout ($r = -0.260$). Within phytoplankton data, the incidence of EMS in cohos was magnified as spring abundance of all blue-green algae increased ($r = 0.363$), and in particular with an increase in summer Microcystis, a thiaminase-containing genera of blue-greens, ($r = 0.84$); incidence of EMS decreased with increasing summer abundance of green algae ($r= -0.231$). An inverse relationship existed between incidence of EMS in lake trout and abundance of both blue-green (spring $r = -0.348$), and green algae (summer $r = -0.659$). Pyrrophyta spring abundance was positively correlated with EMS in cohos ($r = 0.619$), but negatively correlated with EMS in lake trout ($r = -0.641$). Pyrrophyta species are known to contain toxins (some marine species cause red tides, freshwater species associated with fish kill) and possibly alkaloids. The plankton data analysis shows no consistent patterns for the incidence of EMS in both lake trout and coho. Coho salmon are known to change prey items while lake trout prey items tend not to change. The relationships between incidence of EMS in coho with blue-green algae, and with Pyrrophyta species warrant further investigation.

Makarewicz, J., et al. 1998. Changes in phytoplankton Size-class Abundance and Species Composition Coinciding with Changes in Water Chemistry and Zooplankton Community Structure of Lake Michigan, 1983 to 1992. J. Great Lakes Res. 24(3): 637-657

Makarewicz, J., et.al.1995. A Decade of Predatory Control of Zooplankton Species Composition of Lake Michigan. J. Great Lakes Res. 21(4): 620-640

Fitzsimons, J., et al. 1999. A Review of Early Mortality Syndrome (EMS) in Great Lakes Salmonids: Relationship with Thiamin Deficiency. Ambio. 28(1): 9-15

EFFECTS OF TEMPERATURE AND TIME ON THIAMIN LEVELS IN ALEWIVES COLLECTED FROM LAKE MICHIGAN - GREG WRIGHT[1], M. VILLELLA[2], J.L. ZAJICEK[3], J.FITZSIMONS[4], D. C. HONEYFIELD[5], D.E. TILLITT[3] AND SCOTT B. BROWN[2]

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Alewife (*Alosa pseudoharengus*) were collected to evaluate how different handling and processing conditions may affect prey thiamin/thiaminase measurements. Fish were captured by trawl and reference samples of live fish were quick-frozen on dry ice immediately following capture. Other samples were maintained on ice (4 °C) or in ambient lake water (21.5 °C) for up to 5 h prior to quick freezing on dry ice. Thiamin concentrations were determined by a specific HPLC procedure that separated phosphorylated and unphosphorylated thiamin forms. Thiamin levels proved more stable than expected for a species which elaborates thiaminase. Total thiamin levels for samples immediately frozen on dry ice averaged 13 nmol/g and consisted of 70, 14 and 16% of thiamin pyrophoshate (TPP), thiamin monophosphate (TMP) and unphosphorylated thiamin (FT), respectively. After 20 min at 21°C thiamin concentration averaged 12 nmol/g and the was 60% TPP, 22% TMP and 18% FT. There were significant differences between the samples placed on dry ice and those samples held at 4°C on wet ice. After 5 h total thiamin concentrations had declined by 25 % in fish held either at 21.5 or 4°C and the proportion of thiamin as FT had increased to 28% of total. We conclude that prolonged sampling times and warm temperatures caused shifting between the various thiamin forms and some net losses in total thiamin. When testing fish for total thiamin the samples need to be placed on dry ice within 20 minutes of capture, regardless of holding temperature between 4 and 21°C.

SPECIES, AGE, LOCATION, AND TEMPORAL TRENDS IN THIAMINOLYTIC ACTIVITY MEASURED IN FORAGE FISH OF GREAT LAKES SALMONIDS - JAMES L. ZAJICEK[1], G. WRIGHT[2], J. D. FITZSIMON[3], SCOTT B. BROWN[4], D. C. HONEYFIELD[5], M. HOLEY[6] AND D.E. TILLITT[1]

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2) COTFMA, Hessel, MI, USA
3) Fisheries & Oceans, Bayfield Institute, Burlington, ON, Canada
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Thiamin degrading enzymes are contained in exotic forage fish species in the Great Lakes. Alewife (*Alosa*

pseudoharengus) and smelt (*Osmerus mordax*) are two such species and the thiaminolytic activity present in these fish is thought to be a prominent factor in the development of thiamin deficiency in certain salmonids. Therefore, the objective of our studies was to measure temporal, age, and location- related trends in the thiamin degrading activity of alewife, smelt, and bloater chub (*Coregonus hoyi*) from Lake Michigan and the Finger Lakes of New York, USA. We measured thiaminolytic activity in the fish tissues with a radiometric assay. The assay conditions were optimized which required addition of a base co-factor, nicotinic acid, to achieve maximum catalytic activity. The amount of thiamin degrading activity in the two exotic forage fish species (alewife and smelt) was up to a hundred times the activity observed in the native bloater species. Additionally, there were four fold differences in the thiaminolytic activity measured in fish from different lakes. The seasonal, temporal, and age-related differences in thiamin degrading ability of forage fish species in the Great Lakes will be important to ascertain the role of this factor in the development of early mortality syndrome.

THIAMIN LEVELS IN FORAGE SPECIES FROM THE GREAT LAKES - SCOTT B. BROWN[1], G. WRIGHT[2], M. VILLELLA[1], J.L. ZAJICEK[3], J.FITZSIMONS[4], D.C. HONEYFIELD[5] AND D.E. TILLITT[3]

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Previous work has demonstrated an association between low egg thiamin concentrations and a high embryonic mortality called Early Mortality Syndrome (EMS) in several salmonid species from the Great Lakes area. We collected samples of the major salmonid prey species (alewife, *Alosa pseudoharengus*; rainbow smelt, *Osmerus mordax*; bloater chub, *Coregonus hoyi*) to assess whether spatial, temporal, and species differences in their thiamin content may represent a potential contributing factor to the low egg thiamin. Prey were captured by trawl and samples of live fish were quick-frozen on dry ice. Thiamin concentrations were determined by a specific HPLC procedure that separated phosphorylated and unphosphorylated thiamin forms. Thiamin concentrations in alewife (4-13 nmol/g) and bloater (5-10 nmol/g) exceeded levels required for salmonid growth by at least 7-fold. Thiamin concentrations in alewife differed with respect to location and season. Alewife collected in the southern part of the Lake Michigan contained about half the thiamin as those in the northern areas. Of the species examined, rainbow smelt contained the lowest thiamin concentrations (1-2 nmol/g) that marginally exceeded levels recommended for salmonid growth. If alewives are the major forage for salmonids in Lake Michigan, it is unlikely their thiamin content the reason for the low egg thiamin levels associated with salmonid EMS.

FACTORS ASSOCIATED WITH TEMPORAL VARIATION IN EMS - JOHN D. FITZSIMONS[1], SCOTT B. BROWN[2], D.C. HONEYFIELD[3], D.E TILLITT[4], J. ZAJICEK[4], AND J.G. HNATH[5]

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Salmonid species in Lake Ontario and Lake Michigan show similar patterns of temporal variability of EMS. Variation of EMS for Lake Michigan coho salmon was weakly and negatively correlated with alewife trawl abundance (CPUE) ($r^2 = -0.37$). EMS was also correlated with alewife CPUE for Lake Ontario ($r^2 = -0.65$) but not Lake Michigan ($r^2 = 0.34$) lake trout. The reason for this difference may be the linkage between alewife consumption and EMS and the lack of an alternate food source to alewives for Lake Ontario lake trout. Temporal variation in EMS in Lake Michigan coho salmon seems most likely attributable to qualitative changes in their alewife diet. Coho salmon continue to feed on alewives at low levels of abundance and may increase ration size or frequency to compensate for reduced prey availability. The qualitative characteristics of alewives at low abundance most likely implicated with increases in EMS are tissue levels of thiaminase, thiamin and lipid. Of these factors thiaminase may be the most significant although it may interact in as yet unknown ways with the other factors.

THIAMIN REMEDIATION IN ATLANTIC SALMON AND NEW INSIGHTS ON THEIR EXTIRPATION - H. GEORGE KETOLA[1], PAUL R. BOWSER[2], GREGORY A. WOOSTER[2], LESLIE R. WEDGE[3] AND STEVEN S. HURST[3]

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Early mortality in fry (called Cayuga syndrome) of Atlantic salmon, *Salmo salar,* from Cayuga Lake (New York) is associated with low levels of thiamin. We injected four gravid female Atlantic salmon with physiological saline (PS) and six gravid females with PS + thiamin (7 mg/kg weight) 14-23 days before eggs were stripped, fertilized, and incubated in individual lots. Analyses showed that eggs from control and treated salmon contained 1.1 and 1.6 nanomoles thiamin /gram, respectively. Observations of these eggs showed that thiamin injections had no significant effect on the percentage of eggs that hatched. Starting between 700 and 800 Celsius degree-days post-fertilization, control fry (saline) showed signs of Cayuga syndrome and a 45% incidence of mortality, in contrast to 1.9% mortality in fry that received thiamin. By the end of the study, 1,078 degree-days post-fertilization, mean mortality of control fry was 98.6%, while that for thiamin-injected salmon was 2.1%. This study showed that thiamin injections of pre-spawning female salmon from Cayuga Lake increased thiamin content of their eggs and prevented the Cayuga syndrome and subsequent mortality of fry.

Historically, over-fishing, pollution, and building of dams and barriers to spawning migration have been suggested as likely causes of the decline of the Atlantic salmon in Lake Ontario and Cayuga Lake. However, based on these findings and other reports, we suggest another possible contributing cause of the extirpation of landlocked Atlantic salmon in Lake Ontario and some other inland waters of New York. The completion of the Erie Canal connecting the Mohawk River to Lake Ontario, in the early 1800's permitted the entrance of alewives *Alosa pseudoharengus*. These fish gradually became established in large numbers as a new forage species in Cayuga Lake and Lake Ontario. Alewives contain thiaminase and may have induced thiamin deficiency in eggs and increased mortality in fry of the predatory salmon, contributing to their extirpation.

DIAGNOSIS OF 20TH CENTURY LAKE ONTARIO LAKE TROUT POPULATION TRENDS IN A CONSTANTLY CHANGING, MULTI-STRESSOR ENVIRONMENT - PHILIP M. COOK AND STEVEN J. LOZANO

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Early mortality syndrome (EMS) in Great Lakes salmonids is thought to reduce recruitment through a thiamin deficiency in embryos that is related to dietary input of thiaminase by the female. This may be complicated for lake trout in Lake Ontario by a dietary dependence on alewives which may lose nutritional value while declining in numbers and size in response to predation pressure and changes in food supply (Fitzsimons et al. 1999). A chemical etiology for EMS has not been indicated from correlations of bioaccumulative chemical residues in feral female fish or their eggs with incidence of EMS. However, these attempts to find a direct chemical toxicity relationship for EMS have only been examined over short time periods with a relatively narrow range of chemical exposure concentrations and are probably not sensitive to potential chemical co-factor effects on thiamin sensitivity of sac fry.

Direct toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and other Ah receptor agonists to lake trout embryos and associated sac fry mortality through the blue-sac syndrome contributed to the decline of Lake Ontario lake trout populations at mid-century; the extinction of the species by 1960; and the difficulty in establishing natural reproduction by large numbers of lake trout stocked since 1970. Despite the historical presence of many other stressors on lake trout populations (predation by humans and sea lamprey, alewife, other chemicals, etc.) throughout the 20th century, this conclusion is possible because of the well characterized mechanism of action and dose response relationship for lake trout embryos exposed to TCDD; the determination of past exposure levels from the sediment record; and the concordance of the toxicity predictions with Lake Ontario lake trout monitoring data throughout the century.

Is EMS in Lake Ontario lake trout a recent problem or has the potential for EMS existed for a long time, perhaps undetected because of lack of sensitive monitoring and periods in which no reproduction was possible? Analysis of very large Lake Ontario lake trout population levels during the period of 1880-1930, when millions of stocked fry were stocked with many appearing to have survived to successfully reproduce, suggests that lake trout reproduction and recruitment was very good and probably both blue sac syndrome and EMS did not cause significant early life stage mortality during that time. When fry stocking declined, the lake trout population declined rapidly, primarily due to unsustainable commercial fishing rates exacerbated by sea lamprey predation. By 1950, dioxin toxicity to sac fry, independent of other stressors, appears sufficient to have eliminated lake trout recruitment. In the post-1970 dioxin/PCB recovery period slow progress toward natural reproduction and survival of early life stages has been observed with year old lake trout from natural reproduction evident after 1995.

Ecological changes in Lake Ontario affecting the alewife are a present concern because they may modify the incidence of EMS in lake trout in the future. For example, Lozano et al. (1999) have reported on rapidly declining populations of benthic macroinvertebrates in conjunction with the increase in *Dreissena bugensis*, a non-native mussel, which appears to survive in deeper, colder, softer sediment regions of Lake Ontario than *Dreissena polymorpha*. The loss of *Diporeia* from large areas of Lake Ontario benthos, in response to the loss of planktonic food, reduces a key food source for sculpin and young lake trout. The zooplankton food base for alewives may be similarly altered due to the primary production and trophic structure changes in progress in Lake Ontario caused by *Dreissena*. If alewife thiaminase levels are increased by reduction of the zooplankton food base and lake trout have no alternative food sources, progress toward natural reproduction of lake trout could be delayed by continuation or increase in incidence

of EMS.

Fitzsimons, J.D., S.B. Brown, D.C. Honeyfield and J.G. Hnath. 1999. A review of early mortality syndrome in Great Lakes salmonids and its relationship with thiamine. Ambio 28: 9-15

INTERACTIONS BETWEEN LOW THIAMIN CONTENT AND CONTAMINANTS ON EARLY MORTALITY IN LAKE TROUT -DONALD E. TILLITT[1], J. A. ALLERT[1], P.J. WRIGHT[1], D.C. HONEYFIELD[2] AND M. HOLEY[3]

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Lake trout (*Salvelinus namaycush*) embryos are known to be sensitive to the lethal effects of polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and planar PCBs (pPCBs), collectively referred to as planar halogenated hydrocarbons (PHHs). The concentrations of PHHs in Great Lakes lake trout are currently not great enough to cause overt mortality in the developing embryos based on laboratory tests. However, the symptoms of PHH-induced toxicity and that of early mortality syndrome (EMS) share some similarities. These two stressors (elevated PHHs and low thiamin) may interfere with common biochemical and physiological pathways and as such may be exacerbating the effect of one another in Great Lakes salmonids. Our studies were designed to determine the potential for interactions between thiamin deficiency and PHH toxicity on the development of lake trout embryos. Eggs low in thiamin were dosed with PHHs in a graded fashion, incubated, and monitored for stage-specific mortality, gross pathologies, and symptoms of EMS. No change was observed in the dose-response curves toward PHH-induced mortality between low thiamin and thiamin supplemented batches of embryos. However, the non-lethal symptoms of PHH toxicity were greater in embryos with low thiamin. The ecological significance of the increased sensitivity toward PHH-induced gross pathologies among low thiamin individuals is not understood at this time. We are currently investigating effects of these combined stressors on the ability of larval and juvenile fish to capture prey and avoid predation.

RECENT STUDIES ON THIAMIN RESPONSE IN ATLANTIC SALMON AND THIAMIN STATUS OF WALLEYE AND OTHER FISHES - H. GEORGE KETOLA[1], DIETER W. BUSCH[2], SANDRA LARY[2], SANDRA KEPPNER[2], LES WEDGE[3], KEN OSIKA[4], WILLIAM CULLIGAN[5], DONALD EINHOUSE[5], RICHARD COLESANTE[6] AND ANDY GREULICH[7]

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Cayuga syndrome is an early mortality in fry of Atlantic salmon Salmo salar from Cayuga Lake (New York) associated with low levels of thiamin in eggs shown to be prevented by intraperitoneal injections of gravid female Atlantic salmon with thiamin 14-23 days prior to spawning. A follow-up study demonstrated that eggs from gravid females injected between 3 and 28 days prior to spawning had

markedly differing results. Injections from 3 to 11 days before spawning had little impact on early mortality in fry; whereas eggs from females injected between 24 and 28 days prior to spawning prevented early mortality in fry. In a previous study, injections made 14 to 23 days before spawning were effective. Previous analyses of salmon eggs suggested that thiamin injections administered just before spawning did not sufficiently increase thiamin content of the egg to impact fry health and survival. Therefore, thiamin injections made between 14 and 30 days prior to spawning may be the most effective for Atlantic salmon.

Preliminary analyses of salmon tissues showed that the predominant form of thiamin in green eggs was free thiamin (53-83%). The levels of thiamin monophosphate were considerably higher than those of thiamin pyrophosphate. In kidney and muscle, the levels of phosphate forms were greater than the free form of thiamin. Analyses of thiamin in eggs from chinook salmon (*Oncorhynchus tshawytscha*), coho salmon (*O. kisutch*), rainbow trout (*O. mykiss*), walleye (*Stizostedion vitreum*) , and perch (*Perca flavescens*) captured in 1996-8 from Lake Ontario, Cayuga Lake, Lake Erie, and other lakes were made to better understand the impacts of thiaminase in forage fishes. Total thiamin (free thiamin + phosphate bound forms) in eggs of four chinook and four coho salmon captured in 1997 from Lake Ontario was 1.54 ands 1.21 nanomoles/gram, respectively. None of the eggs from four chinook were less than 1 nanomole/gram while three lots of the coho eggs were less than 0.9 nanomoles/gram. Coho fry held at the New York State Department of Environmental Conservation (NYS-DEC) Salmon River Hatchery frequently experience "roll-over," lethargy and mortality that is relieved by treatment of water with thiamin (1,000 ppm for one hour). Little "roll-over" has been observed in chinook salmon fry in the Salmon River Hatchery. Mean total thiamin in eggs of four rainbow trout captured in 1998 from Cayuga (Lake) Inlet was 1.0 nanomoles/gram. Fry of that spawning run held in the NYS-DEC hatchery experienced a high incidence of lethargic behavior, "rolloever," and mortality that was relieved by several thiamin treatments of water. Similar observations were made in fry from the 1999 spawning run of trout held in the laboratory and hatchery. This thiamin-responsive lethargy and mortality represent a recent phenomenon not previously seen in Cayuga Lake rainbow trout that are routinely stripped of eggs for hatchery propagation by the NYS-DEC.

Mean total thiamin concentrations in eggs from six walleye captured in 1996 from eastern basin of Lake Erie and Oneida Lake (New York) were 2.7 and 7.1 nanomoles/gram, respectively. Previous observations of walleye fry from Lake Erie at the Tunison Laboratory (1997) revealed about a 10% incidence of mortality associated with signs similar to those induced by oxythiamin (an anti-thiamin) suggesting a low incidence of early mortality syndrome possibly induced by consumption of thiaminase-containing forage fishes in the eastern basin. The forage base for walleye in Oneida Lake is mainly yellow perch which do not contain appreciable amounts of thiaminase activity, thus explaining the greater levels of thiamin found in walleye eggs from Oneida Lake. Mean total thiamin concentrations in eggs from six walleye captured in 1997 from eastern and western Lake Erie were 2.1 and 3.1 nanomoles/gram, respectively. Eggs from six walleye captured from Lake Winnipeg (Manitoba) also had a mean total thiamin content of 3.1 nanomoles/gram. The forage fish in these lakes include few alewives but numerous rainbow smelt (*Osmerus mordax*), also known to contain thiaminase. Eggs of thirteen walleye captured in 1998 from eastern Lake Erie contained an average of 6.0 nanomoles of total thiamin/gram and ranged from 1.4 to 11.8 nanomoles/gram. Variability of thiamin content of walleye eggs was generally high with coefficients of variability ranging from 13.9 to 48.6%. Statistical analyses of the data showed that thiamin levels were significantly greater in the eggs from Oneida Lake and the 1998 eggs from eastern Lake Erie than eggs from other sources. It is not known if this change in thiamin content of eggs is associated with sampling variability or a recent change in forage of walleye in the eastern basin of Lake Erie from 1997 to 1998. The predominant form of thiamin in the eggs from eastern Lake Erie in 1998 was thiamin pyrophosphate while free thiamin was the least prevalent form.

Total thiamin in eggs of six perch captured in western Lake Erie averaged 3.3 nanomoles/gram and ranged from 1.4 to 4.5 nanomoles/gram suggesting varying intakes of thiaminase-containing forage fishes.

WALLEYE RECRUITMENT FAILURE IN TENNESSEE RESERVOIRS - CHRIS S. VANDERGOOT[1], PHILLIP W. BETTOLI[1] AND DALE C. HONEYFIELD[2]

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Walleye recruitment was monitored in Dale Hollow reservoir, Tennessee, between 1988 and 1993 by aging sagittal otoliths. Dale Hollow reservoir supported a self-sustaining walleye population during the 1960's and 1970's. However, natural recruitment of walleye declined after alewife were introduced in 1976. Between 1981 and 1986, walleye recruitment ceased. Since 1987, walleye year classes have formed only in years when the Tennessee Wildlife Resource Agency and Kentucky Department of Fish and Wildlife Resources stocked walleye fry or fingerlings. Similar patterns of walleye recruitment failure following alewife introduction have been observed in other Tennessee reservoirs. In 1999, we collected 16 spawning walleye from Dale Hollow and analyzed their eggs for thiamin levels. Preliminary findings indicated Dale Hollow walleye might be thiamin limited. An extensive survey of alewife abundance and walleye recruitment in five Tennessee reservoirs is scheduled for the 1999 and 2000 field seasons.

RESULTS OF VARIOUS THIAMIN TREATMENTS ON EGGS AND BROODFISH TO AMELIORATE A VITAMIN DEFICIENCY - SUSAN V. MARCQUENSKI[1], RANDY LINK[2], AND STEVE FAJFER[3]

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From September 1997 through January 1998, monthly thiamin HCl bath treatments (300-350 ppm for 30-45 min.) were given to Skamania steelhead broodfish at the Kettle Moraine Springs hatchery in an effort to avert thiamin deficiencies in the fish and their gametes. A subgroup of the fish was not treated with thiamin HCl, but was handled similarly in all other respects. Skamania steelhead are stocked as yearlings in Lake Michigan and return at age 48 months or older to streams in August/September where they are captured, transferred to the Kettle Moraine Springs hatchery and held until they spawn in January/February. Thiamin concentrations were measured in eggs from five females that received the monthly treatments and from two females that were not treated Although the number of fish sampled is small, there does not appear to be any difference in thiamin concentrations of eggs from either group of parents (thiamin transport did not occur). However, anecdotal accounts suggest that broodfish survival and quality was higher in tanks receiving the thiamin treatment.

In 1997, eggs from ten Seeforellen brown trout females at the Wild Rose hatchery were water hardened in 750 ppm thiamin HCl at pH 6.8 or 7.4 for 2 hours to see if pH had an effect on the uptake of thiamin and whether the slightly alkaline pH degraded thiamin at a faster rate. After water hardening, eggs were rinsed briefly in hatchery water and snap frozen on dry ice. Egg levels of thiamin increased 100X compared to eggs water hardened in water. Egg thiamin levels were similar irrespective of the pH of the thiamin solution, and thiamin did not degrade appreciably over a 5 hour period in solutions at either pH. We

recommend adjusting the pH to 7.4 using 10 N NaOH when water hardening eggs in 750 ppm thiamin to minimize the difference in pH between the treatment water and hatchery water supply.

In 1998, eggs were handled similarly, but a subset was held in flowing water for 48 hours after being water hardened in 750 ppm thiamin HCl at pH 7.2 to see if the high thiamin values were due to residual thiamin on the egg surface rather than levels within the egg. Thiamin concentrations in eggs that were rinsed briefly in hatchery water were similar to those measured in 1997. Thiamin levels in the eggs held in flowing water for 48 hours showed a net increase of about 1 nmol/g thiamin versus the hundred-fold increase in the eggs that were briefly rinsed. We conclude that residual thiamin on the egg surface contributed most to the extremely high thiamin levels measured initially. We recommend that eggs be held in flowing water for at least 48 hours after water hardening when evaluating the uptake of thiamin during egg treatments.

SUCCESSFUL EXPERIMENTAL REPRODUCTION OF EARLY MORTALITY SYNDROME IN LAKE TROUT - DALE C. HONEYFIELD

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To determine if EMS could be generated as a result of a thiamin deficiency, studies were conducted in the laboratory using hatchery reared lake trout free of exposure to environmental contaminants or other environmental factors. Treating eggs or affected fry with thiamin dramatically reduces EMS mortality. Conversely, treating normal fry that are thiamin replete with thiamin analogs (oxythiamin, pyrithiamin) results in fry with signs and symptoms of EMS. In this study adult lake trout (*Salvelinus namaycush*) were fed either a low thiamin basal diet or a semi-purified casein/gelatin diet prepared with bacterial thiaminase. Thiamin concentration of eggs collected from fish fed diets containing bacterial thiaminase were the low and similar to egg thiamin values from feral lake trout. Total egg thiamin ranged from 0.25 to 4.70 nmol/g of egg. Free egg thiamin ranged from 0.006 to 3.05 nmol/g. Fry mortality ranged from 100 percent to zero. Observed signs of EMS in lake trout swim-up fry include a loss of equilibrium, lethargy, erratic swimming when disturbed with a settling to the bottom and laying over onto their side once swimming has ceased. Fry with these signs generally died. In affected fry, yolk sac absorption was reduced. Mild hemorrhaging in yolk sac, gill and eye regions could be observed. This is the first report of EMS like fry mortality reproduced under laboratory conditions in lake trout. It is concluded that reduced eggs concentrations produced by feeding a diet low in thiamin in combination with bacterial thiaminase resulted in clinical EMS.

USE OF THIAMIN ANTAGONISTS TO CAUSE A THIAMIN DEFICIENCY - JOHN D. FITZSIMONS[1], SCOTT B. BROWN[2], H.G KETOLA[3], G.W. WOOSTER[4], B. WILLISTON[1]

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Synthetic thiamin antagonists (e.g. pyrithiamin, oxythiamin) have been used to induce thiamin deficiency in fish. Although EMS has been associated with low egg thiamin concentrations it is not clear that a thiamin deficiency is the sole cause. We tested whether antagonists that are specific to thiamin could induce EMS

and assessed the effect of background thiamin level, species and time of exposure on sensitivity. Pyrithiamin was a more potent antagonist than oxythiamin when given to sac-fry. Lake trout sac-fry from Lake Ontario were almost a thousand-fold more sensitive to oxythiamin than Lake Manitou even though their background thiamin levels only varied by four-fold. For stocks having similar background thiamin levels (Lakes Ontario and Erie, Seneca Lake) sensitivity to oxythiamin was similar. Atlantic salmon with similar background thiamin levels exhibited a similar sensitivity to oxythiamin. The effects of oxythiamin were similar to the clinical signs of EMS. The effects of oxythiamin when administered at fertilization were restricted to the period after hatch causing a dose-dependent decline in yolk-sac utilization. As no dose-dependent declines in tissue thiamin levels were detected in oxythiamin exposed groups it seems likely that oxythiamin exerts its effect by inhibition of thiamin-dependent enzymes.

PRE-HATCH STARTLE RESPONSE AND HATCHING SUCCESS IN LAKE TROUT - PEGGY J. WRIGHT AND DON TILLITT

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The startle response in fish mediates the ability to react quickly to environmental stimuli, including the presence of predators. Most teleost startle responses are triggered by input from large Mauthner-type neurons, which appear early in embryogenesis, and are characterized by a short-latency, C-type fast-start acceleration. Embryos are capable of coordinated startle responses while in the egg capsule. In pre-hatch embryos part of the startle response function is the distribution of the hatching enzyme, essential for the successful emergence from the egg chorion. In later stages, the startle response plays a role in predator avoidance. Using an experimental design encompassing several environmentally relevant stressors on salmonid reproduction including 2,3,7,8-TCDD exposure and thiamin deficiency, the startle response in rainbow trout (Oncorhynchus mykiss) and lake trout (Salvelinus namaycush) eggs was evaluated using a tactile stimulus probe. 2,3,7,8-TCDD adversely affected both the overall number of embryos exhibiting startle response (LD50 and LD100 doses), and the magnitude of the response (LOAEL, LD50, and LD100 doses). The startle response to tactile stimulus was inversely proportional to half-hatch mortality in embryos injected with LD50 and LD100 TCDD doses. In thiamin deficient lake trout embryos (hatchery and Lake Michigan sources), the number of embryos exhibiting startle response was similar to results in thiamin sufficient embryos, however the magnitude of the response was significantly decreased. Because startle response is an essential part of swimming and escape behavior in lake trout, a decrease in the magnitude of the response due to environmental stressors such as contaminants and nutritional deficiencies may affect the ability of lake trout fry in the wild to feed and avoid predation.

PROGRESS IN ISOLATING A THIAMIN CARRIER PROTEIN FROM FISH - DANIEL V. O'CONNOR[1] AND DALE C. HONEYFIELD[2], JOHN P. GIESY[3], ALAN BLAKENSHIP AND MINGUA NIE

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Egg thiamin concentration has been shown to be directly related to incidence of early mortality syndrome in Great Lakes salmonids. Transport of thiamin into the egg is thought to be mediated through thiamin carrier protein synthesized simultaneously with estrogen induction of vitellogenesis but a specific thiamin carrier protein has not been reported for fish. Thiamin carrier proteins have been reported for many species from bacteria to amphibians, birds, plants and primates. We are currently attempting to determine if a thiamin carrier protein is present in trout and salmon. Initially the isolation techniques used to isolate the thiamin carrier protein in other species were evaluated. To date the procedures outlined for the avian model used by Muniyappa and Adiga (1979) to isolate a thiamin carrier protein in fish have been followed. In our first attempt with blood serum from hatchery lake trout serum that had been diluted with 5 volumes of buffer was centrifuged to remove debris and the supernatant loaded on a DEAE column to remove lipoproteins. The resulting eluant was mixed with a dextran/charcoal to remove residual thiamin from the carrier protein. All protein loaded on the column remained bound to the dextran/charcoal. In subsequent experiments with rainbow trout that had been treated with estrogen, purification procedures incorporated ammonium sulfate and ultra centrifugation steps before adding the clarified serum on an affinity (thiamin linked to NHS-activated sepharose) column. Our initial attempts to isolate a thiamin carrier protein have been unsuccessful, therefore we propose to apply a more fundamental approach.

Muniyappa, K., and P.R. Adiga. 1979. Isolation and characterization of thiamine-binding protein from chicken egg white. Biochem. J. 177:887-894.

EARLY MORTALITY SYNDROME AND TISSUE-SPECIFIC APOPTOTIC CELL DEATH DURING EMBRYONIC DEVELOPMENT IN LAKE TROUT (*SALVELINUS NAMAYCUSH*) FROM LAKE MICHIGAN. - JEFF J. WHYTE, J.A. ALLERT, AND D.E. TILLITT

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The gross pathological symptoms of early mortality syndrome (EMS) in Great Lakes salmonids with low thiamin concentrations in their eggs resemble those seen in fish embryos exposed to planar halogenated hydrocarbons (PHHs). This suggests a common mode of toxicity for these stressors. Laboratory exposures of Medaka (*Orizias latipes*) embryos to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin resulted in apoptotic cell death in the embryonic vasculature, neural and digestive tissues during development. This same tissue-specific pattern of programmed cell death was also observed in Atlantic salmon (*Salmo salar*) embryos from the New York Finger Lakes experiencing Cayuga syndrome and from the Baltic Sea exhibiting M74. These disorders are highly similar to EMS. This indicates that apoptotic cell death in specific embryonic tissues may result from disruption of a pathway common to both thiamin utilization and PHH metabolism. The objective of the present study is to determine the temporal and tissue-specific nature apoptotic cell death during development in lake trout (*Salvelinus namaycush*) from Lake Michigan. Trout from this region exhibit low egg thiamin levels, elevated PHH concentrations, and a high incidence of EMS. Our preliminary results reveal that apoptotic cells are readily apparent in gill and neural tissue of

developing lake trout from Lake Michigan, supporting the previous laboratory and field investigations. These findings are currently being verified histologically. By examining the temporal relationship between the incidence of tissue-specific apoptotic cell death and the onset of EMS, our studies will isolate the early stages of toxicity that occur prior to gross pathological effects. Results will be related to thiamin and PHH concentrations in eggs and tissue-specific induction of CYP1A activity, an indicator of PHH exposure. Determining if the pathological symptoms of EMS are preceded by programmed cell death in critical tissues during development will contribute greatly to an understanding the cellular etiology of this disorder.

ROLE OF STRESS, GUT MICRO FLORA, IMMUNE FUNCTION AND ALEWIFE ON THIAMINASE - DALE C. HONEYFIELD

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Alewife are known to contain thiaminase activity. No definitive rational has been put forward to explain biological role of thiaminase in alewife. The majority of the thiaminase activity has been reported to be associated with the viscera. The assumption has been that thiaminase is a product of alewife protein synthesis. This presentation has two parts. First, to report the isolation of thiaminase producing bacteria from viscera of alewife and second to present a hypothesis that includes stress, immune function, gastrointestinal micro flora, thiaminase activity in relationship to early mortality syndrome. Two microbial strains of thiaminase positive bacteria were isolated from alewife viscera. *Bacillus thiaminolyticus* and an un-named *Bacillus* species were cultured and identified by fatty acid methyl ester analysis. This suggests that thiaminase in alewife may be a product of bacterial synthesis rather than alewife protein synthesis. The current thinking is that an animals immune system allows or regulates a quasi symbiotic relationship between the intestinal micro flora and the host and that differences in populations of intestinal micro flora exist among animal families as a result. Stress has been shown to increase host cortisol levels and cortisol is known to reduce the immune response. Combining this information one is lead to the following. If an alewife is chronically stressed, basal cortisol levels would increase. This would lead to a reduced immune response toward gut micro flora including thiaminase producing bacteria. Thus proliferation of gut bacteria would increase thiaminase production above that of a non-stressed alewife. Since intact proteins cross the gut membrane of fish (which is not true in mammals), it is theoretically possible for alewife levels of thiaminase to increase. Whether whole body levels or just gut concentration of thiaminase increases is not known but consumption of a stressed alewife by a salmonid would result in higher burden of thiaminase. Current studies are finding differences in thiaminase levels in alewife from different lakes. Changes in gut bacteria populations that affect thiaminase has not been determined. The potential for thiaminase to come from blue-green algae also needs to be investigated as another external sources of thiaminase. In conclusion thiaminase producing bacteria have been isolated from alewife and the hypothesis put forward to explain the observed increased incidence of EMS in salmonid stocks by alterations in thiaminase in alewife.

ROSTER OF ATTENDEES TO EMS SYMPOSIUM, ANN ARBOR, MI, MAY 17TH & 18TH, 1999

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