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Influence of Lake Erie walleye female characteristic (vitamins concentration in eggs) on survival of early stages and contribution to recruitment

Abstract

Walleye in Lake Erie is the largest freshwater sport fishery of the Great Lakes region. This fish species has a great impact on the ecosystem because of the longevity of female reproduction. The impact of this fish contributes to stabilizing the effect of ecological nutrient (vitamin) transfer, general lake health, and the dynamics of other fish species. We examined in a quantitative manner how female attributes characterized by their vitamin concentrations in eggs influence early life history characteristics of walleye progeny. We aimed to determine how an inter-annual variation in concentrations of tocopherol, thiamine, and ascorbic acid in eggs of walleye influence embryo viability, that is, survival until an advanced embryonic stage. The significant negative correlation that was found between walleye embryo survival and ascorbic acid concentration is counterintuitive as most of the evidence gathered for other fish suggests that an increase in ascorbic acid results in an increased survival. However, this finding might suggest some interaction of ascorbic acid with prooxidative toxicants. When the last 3 years of thiamine concentration in walleye eggs are compared with salmonids that were documented to result in early mortality syndrome (EMS), walleye egg thiamine is below the range in which alevin mortality of salmonids (lake trout and Atlantic salmon) has been known to occur. Further studies should also address in a more detailed manner (by including analysis in walleye larvae), the interrelationship between tocopherol and thiamine demonstrated in lake trout in our laboratory.

Introduction

This project addressed the LEPF Strategic Objective of protecting “fish spawning areas within Lake Erie and its watersheds". More specifically, we were to help “to designate special protection areas for offshore reefs…and tributaries that are essential for propagation of Lake Erie sportfish”. We examined walleye spawning stocks in terms of their viability and contribution to a “catchable” portion of population.

The proposed project explored in a quantitative manner how female attributes characterized by their vitamin concentrations in eggs influence early life history characteristics of walleye progeny. We planed to analyze data from three purported spawning groups in order to determine the relationship between their embryo viability and concentrations of vitamins, and in doing so provide information to establish their relative contributions to the Lake Erie population. Two rivers, Maumee and Sandusky, and an open water reef were the focus of research. Data related to female attributes such as weight, spawning time, egg viability, and vitamin concentrations were collected from the different spawning locations and different years (2004-2007).

This research was based on analysis of gametes from Lake Erie walleye collected between 2004 and 2006, and an additional sampling was performed in 2007. We aimed to determine how attributes of walleye females (length, weight, and age) and an inter-annual variation will influence egg characteristics such as size, fecundity, and concentrations of lipids, fatty acids, tocopherol (vitamin E), thiamine (vitamin B_1), and ascorbic acid (vitamin C), and most importantly viability (survival until and advanced embryo stage) for three purported spawning stocks (Maumee River, Western-basin reefs, and Sandusky River). We intended to examine eggs from 10 females from each location each year. Based on these finding, we envisioned the development of a predictable model to synthesize the results of this study where a correlation between concentrations of critical vitamins and survival of embryos will allow for the establishment of alternative management tactics for Lake Erie walleye, including protection of spawning locations, time of the run, or years of specific risks to reproductive stocks. Based on these findings, fisheries managers will be better able to secure long-term goals of walleye fishery in Lake Erie. The focus of this project was to develop criteria to evaluate viability of walleye progenies and identify possible mechanisms (vitamin deficiency along food chains) affecting survival of the larval stages in the wild.

Walleye in Lake Erie is the largest freshwater sport fishery of the Great Lakes region and probably in the world. This fish species has a great impact on the ecosystem and because of the longevity of female reproduction (5-10 years), the impact of this fish contributes to stabilizing the effect of ecological nutrient (vitamin) transfer, general lake health, and the dynamics of other fish species. In addition to the economic benefits of biodiversity as a component of the new approach to global change in the biosphere, it is possible to make a case for development of new environmental remediation methods. For instance, in the case of thiamin deficiency, viability of the salmonid (lake trout, Atlantic salmon) eggs can be restored by post-fertilization treatment (Lee et al. 2008). This method was not tested in walleye, and our studies provide, for the first time, some evidence of thiamine status in Lake Erie walleye, for such methods to be considered.

Objectives

We aimed to determine how an inter-annual variation in concentrations of tocopherol, thiamine, and ascorbic acid in eggs of walleye will influence embryo viability, that is, survival until an advanced embryonic stage. More specifically, we had two objectives related to the analysis that are essential for successful reproduction of walleye:

1. To analyze vitamin E, vitamin B_1, and vitamin C, and carry out experiments of fertilization and progeny viability. We were prepared to use walleye eggs stored in a “biological bank” at The Ohio State University that were collected for other investigations between 2004 and 2006. We specifically had in mind this collection of historical samples of walleye eggs for the purpose of future toxicological or genetic studies.

2. To determine if survival of embryonic stages, conditions during yolk absorption, and concentration of vitamins at the time of ovulation (just prior to fertilization) will be critical to establish inter-annual frequency and importance of “early mortality syndrome” (EMS) in Lake Erie walleye.

Materials and Methods

Our working hypothesis was that distinct walleye stocks differ in regard to female attributes, such as fecundity, egg size, and egg viability. Our focus was to quantify how female attributes influence the characteristics of early life stages of walleye groups spawning in the Western basin of Lake Erie.

Walleye were spawned to determine differences in fertilization rate, egg survival, biochemical indicators of eggs, and length of their hatched larvae. The biochemical indicators (lipids and fatty acids) were analyzed as part of the studies funded by ODNR-ODW, and will become part of further analysis where lipid fractions and individual fatty acids will be correlated with the antioxidant vitamins E and C concentrations. Consequently, the power of interpretation of the results already obtained through ODNR funding will be directly enhanced.

Present work included analyses of samples collected between 2004 and 2006, and a new collection specifically for the purpose of the present work in 2007. Eleven walleye females weighing between 932g – 4550g and four males weighing between 828g – 1853g were collected on April 9th, 2007 in the Maumee River at Fort Meigs State Memorial, Perrysburg OH. Electrofishing gear was used by the Ohio Department of Natural Resources, Division of Wildlife and fish were caught between 9 and 11 pm. The river water temperature was 6˚C.

Gametes were collected on site by stripping without anesthesia within 5-10 min after the fish were brought to the shore. Eggs were kept in individual, flat-bottom containers at 5-7˚C (eggs, 2-3 layers of eggs) or 0˚C (sperm, 1-2 mm of sperm) during transport to the laboratory in Columbus (Rinchard et al. 2005). Eggs were weighed in the field (for fecundity estimation) and subsamples (~ 5 g) from each individual female were taken and immediately frozen on dry ice on site for future analysis. The number of eggs per gram was found by weighing a subsample of each female's eggs in triplicate and then counting under the microscope. Sperm motility was evaluated at 15°C after adding hatchery water (dechlorinated Columbus city water; Rinchard et al. 2005) and recorded as relative percent of motile sperm.

We evaluated fertilization rates of walleye by mixing eggs (2 g or ~ 600 eggs) in triplicate per female with the milt mixture from the four males (25,000 spermatozoa/egg) using the dry method previously described by Rinchard et al. (2005). Sperm was diluted in Moore extender (Moore, 2003) prior to fertilization based on prior estimation of individual male sperm density (Ciereszko and Dabrowski, 1993). To prevent egg-to-egg adhesion, one minute after sperm activation (fertilization), eggs were treated with a solution of tannic acid (Sigma-Aldrich, St. Louis, MO) at 400 mg/L for 4 min with a continuous stirring (Rinchard et al. 2005). The first experiment began five hours after the eggs were stripped and was completed in three hours.

Biochemical analysis included high performance liquid chromatography (HPLC) of free thiamine and its phosphated derivatives (mono- and di-phosphate) according to the method slightly modified from Brown et al. (1998). The HPLC system consisted of a delivery system pump (110B, Beckman Instruments Inc.) equipped with a 20 µl injection loop connected to a 4.6 x 250 mm (Waters Spherisorb® 5 µm NH2, for egg) column coupled with a NH2 packed guard column. The fluorescence detector (FP-920, JASCO Co., Japan) was set at 375 nm for excitation and 430 nm for emission. Standard of thiamine and its phosphated forms were prepared. For vitamin analysis, 500 µl 2% TCA extraction solution was added to fish egg samples and the material was gently homogenized for 30 s either by hand or with a low-speed tissue grinder. The homogenized samples were placed into a boiling water bath for 5-6 min and then cooled on ice for 10 min. After cooling, the samples were supplemented by 500 µl of ice-cold 10% TCA solution, and vortexed to mix. Then, the samples were centrifuged at 14,000 x g for 15 min at 4^{o}C. The clear supernatants (800 µl) were transferred into glass test tubes (10 ml capacity). To remove TCA and lipids, the sample extracts in the test tubes were washed with 4 volumes of ethyl acetate-hexane solution (v/v, 3/2). The washed sample (roughly 500 µl volume) was then transferred into an eppendorf tube and oxidized to thiochrome by adding 25 µl of 30 mM K3Fe(CN)6. To increase pH of the sample extracts 25 µl of 0.8 M NaOH was added. Then, the oxidized sample extracts were vortexed and filtered before injection into HPLC system. When it was necessary, the sample extracts were diluted to a desired concentration. The mobile phase consisted of KH2PO4 (pH 7.5, 85 mM) with acetonitrile (v/v, 65/35).

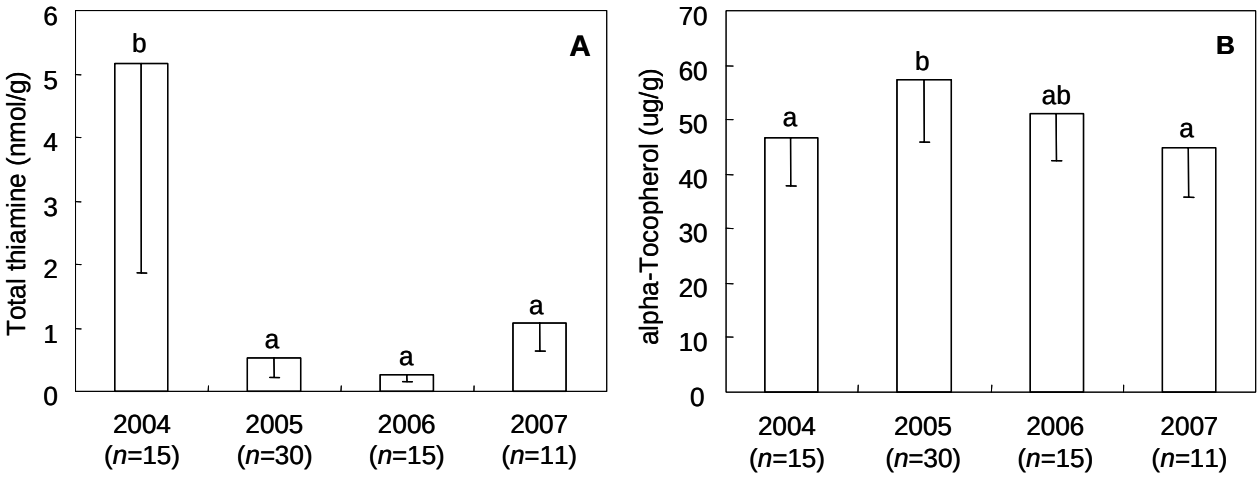
Tocopherols and ascorbic acid were analyzed as described in Lee et al. (2003). Phenomenex Luna C18(2) (4.6 x 150mm) column coupled with a C18 packed guard column were used on set at 290 nm for excitation and 325 nm for emission. Approximately, 300 mg of samples were homogenized in 4.5 mL methanol containing 1% H3PO4 and 0.45 mL methanol containing 5% pyrogallol. The homogenate was centrifuged at 5000 x g and 4˚C for 10 min, the supernatant was removed and pellet was re-suspended in order to repeat homogenization procedure. Supernatants were combined, filtered with a syringe filter and injected into a column kept at 40˚C. The mobile phase consisted of methanol, water, and H3PO4 (v/v/v, 93/6.5/0.5).

All statistical data was run through ANOVA with significance at P-value < 0.05 (SPSS V10). All data that was a percent was arc transformed before analysis.

RESULTS

Total thiamine concentrations in walleye eggs have shown enormous inter-annual variation (Fig. 1A). If the threshold for EMS in salmonids of 0.8-1.0 nmol/g holds true for walleye, three out of four years in this species put them at risk of thiamine deficiency. Our data indicates that a 10 fold variation in thiamine level can be encountered in walleye eggs in L. Erie. Thiamine pyrophosphate was the predominant form of thiamine independent of the total concentration (Fig. 2).

Fig. 1. Total thiamine (A) and α -tocopherol (B) concentration of walleye eggs in 2004-2007.



Variation in tocopherol concentrations was much less than that of thiamine over the study period (Fig. 1). There was no significant relationship between vitamin concentrations, and between tocopherol and lipid content in walleye eggs (Fig. 3), with only one exception for 2007 when tocopherol decreased as thiamine level in eggs increased.

The only significant correlation that was found between walleye embryo survival and ascorbic acid concentration is counterintuitive (Fig. 4) as most of the evidence gathered for other fish suggests that an increase in ascorbic acid results in an increased survival (Lee and Dabrowski, 2003). Other relationships seem to be biologically meaningful, however, the current data do not allow for any conclusive statement. It appears that thiamine concentrations in walleye eggs that were significantly higher than in other years did not result in embryo survival lower than 40%, whereas in other years, when thiamine concentration was below 1 nmol/g, survival declined. It is evident that multi-year variation in water-soluble (thiamine) and lipid-soluble vitamins do not follow the same trend and studies extended for longer period can provide greater insight into walleye embryonic viability. It should be emphasized that the presence and concentration of other compounds in walleye eggs than vitamins, such as toxicants (mercury, pesticides), minerals (magnesium) may be also involved in the embryo viability and needs to be examined simultaneously.

Fig. 2. Proportion of thiamine free (T-free), monophosphate (T-mp) and diphosphate (T-dp) of walleye egg in 2004-2007.

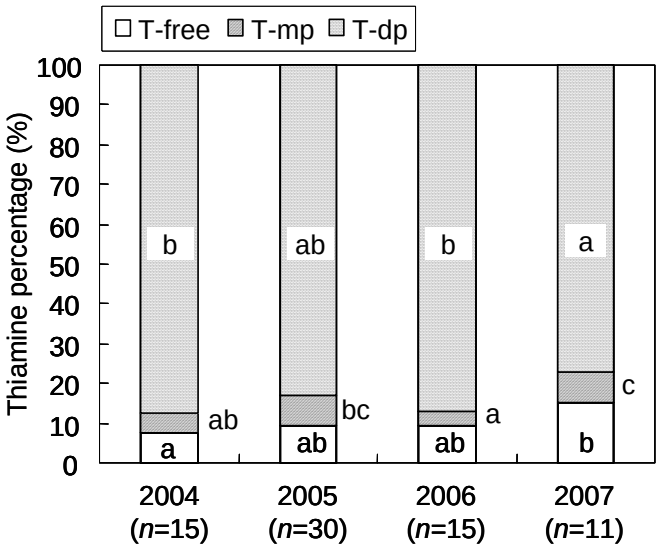


Fig. 3. Correlations between total thiamine and α -tocopherol (A) or total ascorbate (B), and between α -tocopherol and total ascorbate (C) or lipid (D) of walleye eggs (all study years combined).

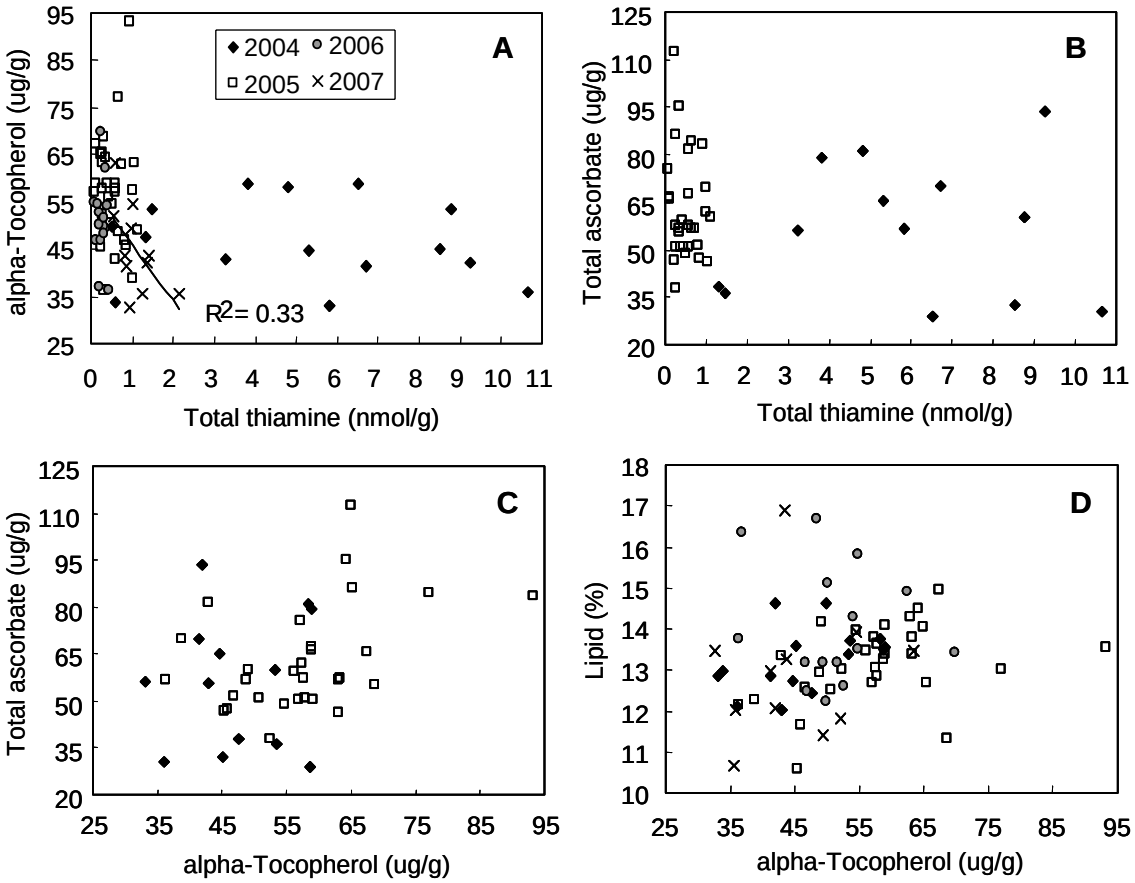
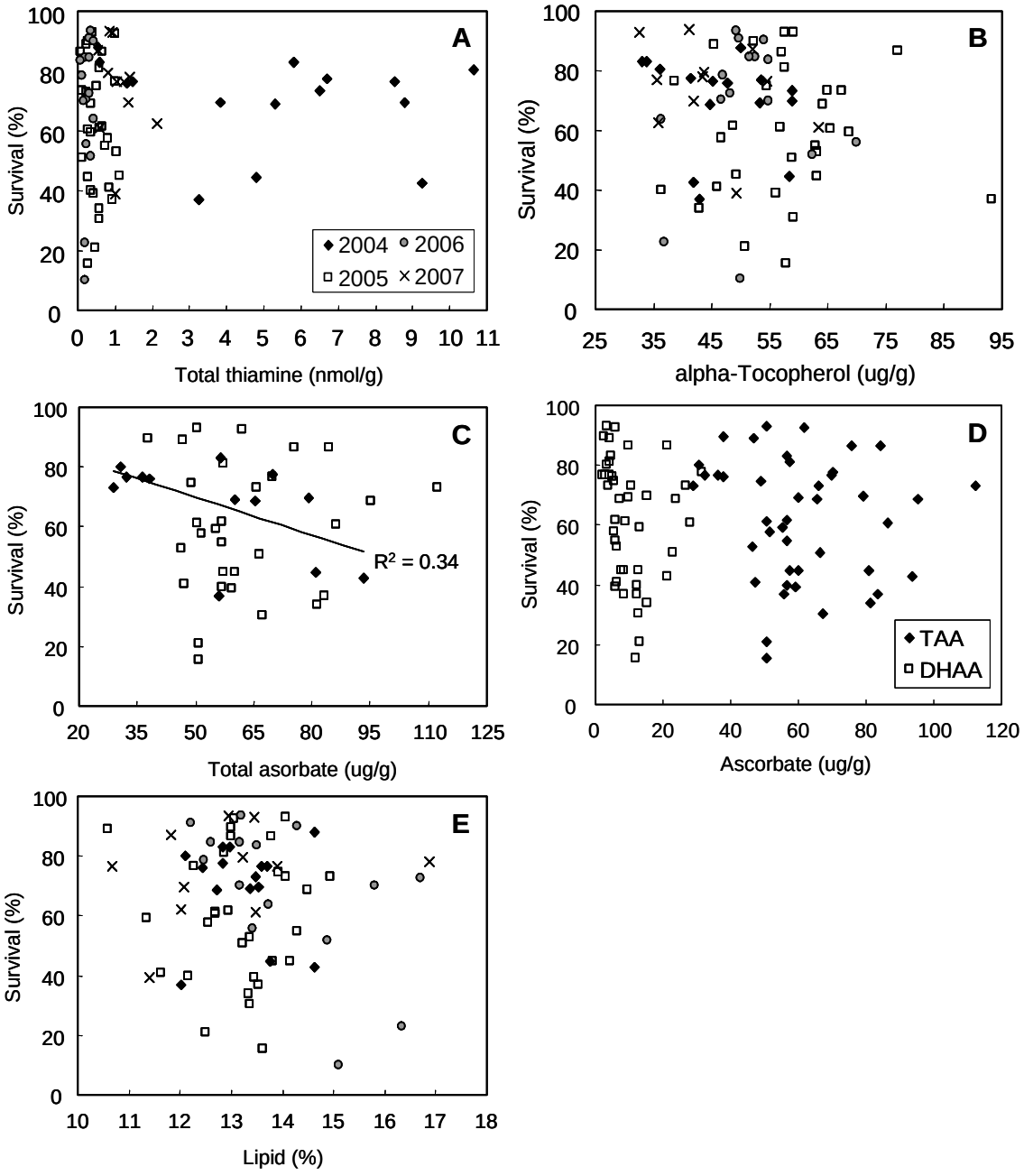


Fig. 4. Correlation between survival and total thiamine (A), α -tocopherol (B), total ascorbate (C), or lipid (E) of walleye eggs in sequential years and correlation between survival and ascorbate (D) of walleye eggs in combined years. TAA-reduced ascorbic acid, DHAA-dehydroascorbic acid.



DISCUSSION:

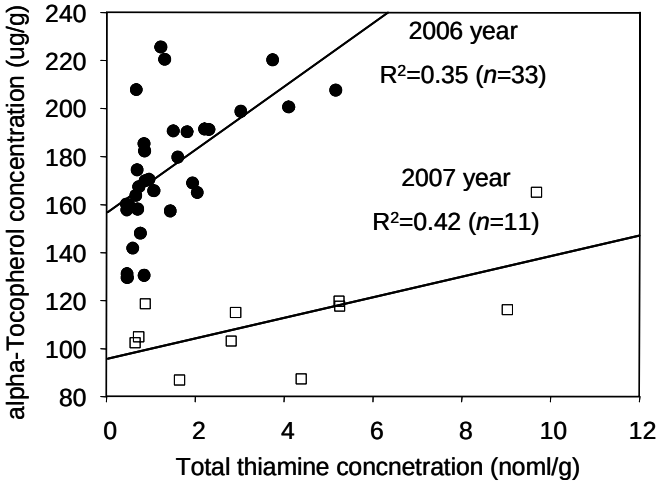
Thiamine concentrations in walleye eggs in L. Erie in the last 3 years differed considerably from data for walleye in inland reservoirs (Barnes et al. 2005; Honeyfield et al. 2007). Prior to fertilization the level of thiamine in walleye eggs from S. Dakota's lake varied between 5.18 and 7.97 nmol/g (mean values for two spawning stocks). At the southern range of walleye distribution, Honeyfield et al (2007) documented between 1.41 and 3.86 nmol/g of total thiamine in walleye eggs. Compared with salmonid data, these authors were already concerned that walleye larvae might suffer from thiamine deficiency. This concern is particularly justified in the light of the dramatic loss of thiamine during embryonic development of walleye. Barnes et al. (2005) concluded that 90% of thiamine is lost from fertilization prior to hatching. This would create conditions

of increased susceptibility to thiamine deficiency in 3 out of 4 years when walleye from Lake Erie were examined. The decrease in thiamine during embryonic development in salmonids according to Brown et al. (1998) is much less severe, 50% (46% at hatching and 72% at swim-up stage in lake trout; Lee et al. 2008).

When the last 3 years are compared with salmonid data for thiamine concentrations that were documented to result in EMS, walleye egg thiamine is below the range in which alevin mortality of salmonids (lake trout and Atlantic salmon) has been known to occur (Fitzsimons et al. 2007; Fisher et al. 1995; our own data for lake trout in Lake Michigan 2006-2007) (see also Fig. 5).

In order to make clear delineation of potential EMS phenomena, more studies are required where larval walleye at hatching is examined. It is also possible that zooplankton rich in thiamine might provide an accelerated recovery of thiamine status in larval stages of walleye. Further studies should also address in a more detailed manner (perhaps by including analysis in walleye larvae), the interrelationship between tocopherol and thiamine demonstrated in lake trout (Fig. 5). Tocopherol concentrations in eggs of walleye fall within the lower range of concentrations that is characteristic for marine fish eggs (62-151 ug/g wet weight; Yamamoto et al. 2001) and are much lower than in lake trout eggs from L. Ontario (73 ug/g; Palace et al. 1998) and L. Michigan (Fig. 5).

Fig. 5. Correlation between tocopherol and thiamine of lake trout eggs in 2006-2007 (Lake Michigan) (Lee and Dabrowski, unpublished).



Ascorbic acid concentration in walleye eggs in L. Erie falls within the range encountered in other fish in the wild (Saborowski et al. 1997).

COLLABORATION:

Dr. K. Dabrowski collaborated with Dr. Roy Stein and Mr. Jason Van Tassell, Department of Evolution, Ecology and Organismal Biology (EEOB), Ohio State University. These investigators are leaders of the project funded by ODNR entitled "Quantifying how parental attributes influence characteristics of early life stages of Ohio stocks of walleye in Lake Erie" and they provided logistic and scientific cooperation in executing the LEPF project. Dr. P. Baumann, USGS, Columbus Field Station, who is familiar with "environmental disturbances in reproduction of Lake Erie fishes", provided counsel on possible interactions of a potent antioxidant, tocopherol, with toxic compounds. Dr. Baumann and our laboratory also collaborated on related USGS project dealing with vitamin concentrations in sucker eggs from polluted rivers on the East Coast.

Ohio DNR has been involved for decades in extensive studies on walleye reproduction, propagation and restoration programs. Evaluation of viability of walleye gametes during reproduction of several spawning stocks in Lake Erie tributaries and off shore reefs was a continuous collaborative effort between the Division of Wildlife, EEOB, and our laboratory and aimed at establishing further management strategies for the protection

of spawning stocks and understanding biology of the species, its survival, migration and contribution to overall fishable portion of the population. The LEPF project complimented the Ohio Division of Wildlife research data and further analyses are planned to determine possible influence of walleye age, food (activity of thiaminase in prey fish of walleye), and spawning stocks on quality of walleye progenies.

DISSEMINATION:

The results will be presented at the Ohio Fish and Wildlife Annual meeting, American Fisheries Society annual meetings and published in a peer-reviewed journal. This study provided encouraging results that might explain variation in walleye survival during embryonic and larval stages, and consequently recruitment to young-of-the-year stock. Therefore, we will attempt to continue research by implementing methods of vitamin enrichment during embryonic stages to test the hypothesis that EMS in walleye and possibly other percids is critical to their survival in the wild. Information on the progress in establishing a link between egg quality (viability) and vitamin concentrations in walleye from Lake Erie will be published in the Ohio State University newsletter "onCampus" and the Ohio Sea Grant College Program newsletter. As with all public documents the Ohio Lake Erie Commission (OLEC) and the Lake Erie Protection Fund (LEPF) will be recognized for its support of this project. And, that the monies for the LEPF are supported by citizens of Ohio through their purchase of the Lake Erie License Plate.

ADDITIONAL BENEFITS

The project employed a graduate student (Mr. Bong Joo Lee) for 2 quarters. The graduate student was trained in handling reproduction techniques and incubation methods of walleye eggs in the laboratory. Mr. Lee gained experience in the techniques involved in vitamin E and B_1 analyses. We also provided experience to two undergraduate students who were work-study volunteers.

It is believed that suitable riverine systems now exist in Lake Erie tributaries and provide walleye spawning, however, a year-to-year variations in total or fishable stocks are still difficult to explain and predict. This project just began to explore the possible role of vitamin transfer in food chains in Lake Erie, and inter-annual variation that sets up an example of how many factors that were not researched will allow better understanding of regulatory mechanisms of population size. Furthermore, this research has the potential to develop and implement techniques that can result in successful propagation and restoration of vanishing species experiencing dramatic variations in population size due to environmental (food chain) changes. This project is crucial to the understanding of the Lake Erie ecosystem and local economy that is associated with sport fisheries which will ultimately benefit from it.

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