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Reproductive Effort and Lipid Dynamics of the Emerald Shiner (Notropis atherinoides) in the Upper Niagara River, New York

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Reproductive Effort and Lipid Dynamics of the Emerald Shiner (*Notropis atherinoides*) in the Upper Niagara River, New York

by

Christopher Allen Osborne

An Abstract of a Thesis
in
Biology

Summited in Partial Fulfillment
of the Requirements
for the Degree of

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State University of New York
College at Buffalo
Department of Biology

ABSTRACT OF THESIS

Reproductive Effort and Lipid Dynamics of the Emerald Shiner (*Notropis atherinoides*) in the Upper Niagara River, New York

Life history theory predicts that reproductive characteristics of organisms will be shaped by biotic and abiotic factors to maximize their overall fitness. In this study, I investigated how growth, reproductive effort, and lipid dynamics vary ontogenetically and seasonally for emerald shiners (*Notropis atherinoides*) in the upper Niagara River. Growth rates were highest in age 2 shiners and lower in age 1 and age 3 individuals. Evidence of reproduction was found beginning at age 1, and reproductive investment as measured by ovarian lipid content was lowest in age 1 and age 2 individuals and greatest in age 3 fish. All age classes exhibited significant changes in somatic lipids across sampling seasons, with minima in spring and maxima in fall. Fulton's condition factor K did not accurately represent variation in somatic lipid reserves that were found in this study, and therefore this index is not likely to be an accurate measure of overall health and robustness for this species. My findings suggest that emerald shiners exhibit a life history strategy where somatic growth is prioritized over reproduction until their third year of life, when large investments into reproduction result in significantly reduced growth rates. Although prior studies have examined growth and reproductive traits of the emerald shiner, my study is the first to document significant changes in lipid dynamics across seasons and age groups in this native keystone species.

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Introduction: Life History Theory and Reproductive Effort

The fitness of an organism is often measured as its ability to produce healthy and fertile offspring (Fisher, 1958). Life history theory is a branch of evolutionary biology that examines reproductive traits of organisms and attempts to predict how these traits may be shaped by natural selection under different environmental conditions. Life history studies focus on how specific processes such as growth, development, and maintenance both affect and are affected by reproduction and its respective physiological and energetic requirements. The study of specific trade-offs between these physiological processes in the presence of environmental factors such as predation and nutrient availability have brought to light the complexity and diversity of life histories utilized by living organisms.

For an organism to provide its progeny with a chance of survival, sufficient energy and resources must be allocated to reproduction. The amount of energy allocated to reproduction from all utilized sources is known as reproductive effort (Fisher, 1958). However, due to the fact that the energy and resources available to an organism are finite, the provisions made to reproduction often come at a cost in that any time, energy, or nutrients that are allocated to reproduction are diverted from somatic growth or maintenance. If a reproducing adult does not obtain enough nutrients to satisfy the costs of reproduction, growth, and maintenance, then a trade-off between the different processes must occur. For organisms that are capable of reproducing multiple times before dying, the cost of this trade-off can be carried on to future reproductive events. This occurs if the cost of a trade-off between reproduction and maintenance is high enough to make the parent less capable of collecting or storing provisions for successive reproductive events (Calow, 1973). In this sense, the amount of energy and resources an

organism can allocate to a reproduction is influenced not only by current availability of resources but also the amount allocated to preceding reproductive events.

Understanding how various factors may affect an organism's ability to allocate energy to reproduction provides insight into how natural selection shapes life history strategies over time. Exactly when and how much energy a parent dedicates to a single reproductive event can vary greatly based on factors such as habitat, age of sexual maturation, photoperiod, condition of the parent, and nutrient availability (Calow, 1973; Pianka and Parker, 1975; Reznick, 1985). For example, fishes that mature at a young age typically have lower reproductive effort compared to those that mature later in life, and reproductive effort tends to increase with decreased stress (Post and Parkinson, 2001; Roff, 1991).

Semelparous organisms (i.e., those reproducing only once in a lifetime) are able to divert a large majority of their energy and nutrients to reproduction, greatly undersupplying somatic demands since they die shortly after they reproduce (Diana, 1995). For example, northern populations of the American Shad (*Alosa sapidissima)* will utilize up to 80% of their energy reserves during a spawning event, resulting in high fecundity but also death of the parent fish (Glebe and Leggett, 1981). However, for iteroparous organisms (i.e., those that reproduce more than once in a lifetime), it can be detrimental to invest too heavily in a single reproductive event because the parent could be left in a weakened state, either reducing its ability to reproduce again or to survive environmental threats (e.g., predation or thermal fluctuation). However, if a parent does not allocate enough provisions to the current reproductive event, then the chances of recruitment are greatly diminished. Ideally, an iteroparous organism will supply its current reproductive event with enough provisions to promote recruitment of the brood stock while reserving enough energy to maintain a level of health sufficient to survive to reproduce again. If

this balance is not met, then the total number of offspring produced by a parent during the remainder of its life (i.e., its reproductive value) is reduced. In theory, natural selection would favor individuals that are physiologically capable of finding this balance, even under varying environmental limitations (Clutton-Brock, 1984). Indeed, organisms have developed finely balanced allocation regimes that prioritize energy allotment between reproductive success and post-spawn survival depending on specific environmental conditions. (Calow, 1973). For example, in many fishes a strong positive correlation between reproductive effort and mortality has been documented (Gunderson, 1997). Factors such as body size, food availability, temperature, predation, sex, age, and population densities have been shown to influence the relative amount of energy used for reproduction vs the amount used for processes that directly promote individual survival (Kamler, 2012; Pangle and Sutton, 2005; Roff, 1991).

Investigating Reproductive Effort and Lipid Dynamics in the Emerald Shiner

In this study, I examine reproductive effort and lipid dynamics in the emerald shiner (*Notropis atherinoides*) from the upper Niagara River, NY. Although various aspects of the reproductive biology of the emerald shiner have been studied previously, reproductive effort and energy allocation strategies in a lotic system have not previously been documented. Moreover, this species can serve as a representative organism to investigate how reproductive energy allocation of an iteroparous, small bodied fish in a temperate river varies seasonally and across age classes. In the sections that follow I provide a general overview of topics relating to reproductive effort and lipid dynamics in fishes and describe what is known regarding these topics for the emerald shiner.

Length-weight relationships

The relationship between length and weight in fishes is influenced by genetics, environmental conditions, age, and sexual maturity. As fishes age, their growth is partially governed by allometric relationships of body size and metabolism that apply to poikilotherms in general, meaning that metabolic rate, and therefore, the growth rate is expected to slow as a fish increases in age and size (Clutton-Brock, 1984). Fishes also exhibit indeterminate growth, meaning that length and weight increase throughout an individual's life and growth does not end when sexual maturity is reached. Fishes often exhibit a sigmoidal growth curve, where growth is slow early in life and then increases rapidly before slowing again following sexual maturity. This growth pattern appears to be highly conserved across evolutionary time (Clutton-Brock, 1984; Kamler, 2012; Pianka, 1976) and is well-described by the von Bertalanffy growth model:

$$L_t = L_{max}\,(1\text{-}e^{-kt})$$

where L_t is body length at time t, L_{max} is the asymptotic or maximum length for the species, and k is a species-specific growth constant that typically varies between 0.1 and 1.0. Higher values of k correspond to a higher growth rate.

Most variation in length-weight relations between species of fish can be explained by genetic factors, while variation between populations of conspecifics and between individuals in a population are generally due to non-heritable factors such as food availability, temperature, reproductive stage, age, predation, and competition pressures (Kamler, 2012; Post and Parkinson, 2001; Roff, 1991). For these reasons, length and weight measurements can be used to create reliably predictive models and condition indices by which the health of separate populations of conspecifics and the health of individuals within a population can be compared.

Fulton's condition factor K is a metric of robustness that uses a fish's length and weight, where greater weight at a given length is interpreted as better health (Froese, 2006). Fulton's K is calculated as:

$$K = (W/L^3) \times 100{,}000$$

where W = weight (g) and L = length (mm).

Fulton's K works as a condition index by assuming that differences in weight are the result of differences in some form of stored energy (e.g., lipids or protein). Several studies where proximate measures of tissue composition were taken along with Fulton's K have found this to be a reliable assumption (Encina and Granado-Lorencio, 1997; Schultz, 1999). However, other studies have found that Fulton's K is not always a reliable measure of physiological condition. For example, Sutton et al. (2000) found that in wild Atlantic salmon (*Salmo salar*), Fulton's K remained relatively constant while percent fat and whole body dry weight fluctuated. Similarly, Pangle and Sutton (2005) found that in lake herring (*Coregonus artedii*), Fulton's K and lipid content were only strongly correlated when the ratio of crude-lipid to whole-body water content was high. These findings suggest that values of Fulton's K may remain high even during periods in which lipid stores are low due to increases in whole-body water content, buffering changes in body mass.

Seasonal and age-related changes in condition have been studied in emerald shiners, and values for Fulton's K typically range from 0.19 – 0.40 in juveniles and 0.68 – 0.85 in adults (Atkinson et al. 2015; Flittner, 1964; Fuchs, 1967; Hayer et al. 2014; Schaap, 1989). However, no studies have examined how well Fulton's K represents energy status or physiological health in emerald shiners. Given that emerald shiners are a key forage prey fish (Cochran, 2016;

Courtney and Blokpoel, 1980; Follett, 1957), it is important to determine if Fulton's K is a suitable metric for health in this species.

Lipid dynamics

In temperate environments, productivity is known to be highly variable between seasons. As a result, organisms in these environments have developed the ability to store excess energy obtained during times of high productivity (i.e., in summer and fall) in order to meet metabolic demands when food is scarce (i.e., during winter). How much energy is stored, when reserves are built-up or depleted, and in what form it is stored are all important life history characteristics (Diana, 1995; Post and Parkinson, 2001). Among temperate fishes, the ability to store energy in the form of lipids appears to be a particularly strong determinate of survival and reproductive success. As such, distinct seasonal patterns of lipid storage and depletion have arisen in these regions. Typically, lipid stores are depleted during spawning events when lipid is used to fuel the physiological and behavioral demands of reproduction, and also during the winter months when food availability is low. Lipid stores are then replenished in summer and/or autumn when temperature and food availability are high (Encina and Granado-Lorencio, 1997; Schultz, 1999; Tytler and Calow, 1985). For example, Madenjian et al. (2000) examined seasonal variation in lipid content of a number of Great Lakes fishes and found that in alewives (*Alosa pseudoharengus*), rainbow smelt (*Osmerus mordax*), and deepwater sculpin (*Myoxocephalus thompsoni*), the highest lipid content occurred in the fall while the lowest lipid levels were found in summer, presumably after spawning had occurred.

The relationship between body size and percent lipid composition has been studied in many fishes (Diana, 1995; Tytler and Calow, 1985). In general, percent lipid composition increases with body size. For semelparous fishes this trend continues until death (Diana, 1995).

However, in iteroparous fishes, the onset of reproductive activity appears to change this relationship. After reaching sexual maturity, iteroparous fishes will often begin allocating a portion of assimilated energy to reproduction, resulting in a reduction in the energy available to store as lipids (Diana, 1995; Martin et al. 2017; Tytler and Calow, 1985). Indeed, somatic lipid stores and measures of reproductive investment (e.g., gonad weight, gonad energy density, and gonad lipid content) typically exhibit a strong inverse relationship in fishes (Lochmann et al. 2007; Martin et al. 2017). These same seasonal and ontological changes in lipid storage appear to be common in small bodied cyprinids (Encina and Granado-Lorencio, 1997; Lochmann et al. 2007; Rabito Jr., and Heins, 1985; Schultz, 1999) but have not been studied in the emerald shiner.

Fecundity and egg quality

Reproductive effort can be measured as fecundity, which is the number of eggs produced by a female during a single reproductive event. The potential energetic cost of egg production often varies with body size in fishes (Clutton-Brock, 1984; Tytler and Calow, 1985). For example, if it requires less energy per gram of fish for larger individuals to produce a single egg, then the production of 50 eggs by a small female would come at a greater cost than the same task would for a larger female (Reznick, 1985). For these reasons, reproductive effort in fishes is usually expressed as the gonadosomatic index (GSI), which is the wet weight of the gonad divided by the wet weight of the entire fish multiplied by 100 (Schreck and Moyle, 1990). This allows for the level of investment into egg development to be standardized to account for body size of the fish.

Previous studies provide some measures of fecundity for emerald shiners from lake populations. For example, fecundity of emerald shiners ranged from 1,040 – 3,054 eggs per

female in Dauphin Lake, Manitoba (Schaap, 1989), from 2,200 – 4,365 eggs per female in Lake Erie (Flittner, 1964), and from 868 - 8,733 eggs in Lake Simcoe, Ontario (Campbell and MacCrimmon, 1970). However, these fecundity values were gathered by counting only opaque (i.e., post-vitellogenic) eggs present in the ovaries at the time of capture. Due to the fact that these fish are likely to exhibit asynchronous egg development (Schaap, 1989), the number of yolk-bearing eggs present in an ovary at any one time may not represent the total number of eggs to be spawned (Blazer, 2002). Thus, in emerald shiners it is more appropriate to enumerate all eggs that have begun the maturation process and have left the oogonial nest (Babin et al. 2007) as this is likely a better representation of total fecundity (Blazer, 2002). In addition to fecundity, egg quality can also be a measure of reproductive effort. The quality of eggs in a given ovary can be determined in several ways. For example, egg size has been shown to be a strong indicator of egg quality in fishes (Brooks et al. 1997). Another measure of egg quality is the amount of lipid stored in each egg. Percent lipid content of eggs has been shown to have a positive correlation to reproductive success and larval survival in fishes (Brooks et al. 1997; Kamler, 2012).

Variation in reproductive effort between age classes

Ontogenetic changes in reproductive effort and physiology are well documented in a variety of animals (Charlesworth and Leon, 1976; Calow, 1973). One common trend in iteroparous animals is to exhibit an increase in reproductive effort with age (Pianka and Parker, 1975). This is believed to be due to the fact that as an organism ages and nears senescence, the probability of successfully reproducing again decreases (Charlesworth and Leon, 1976). For example, Mills (1987) found that two-year-old common minnows (*Phoxinus phoxinus)* that had reproduced exhibited significantly higher measures of reproductive effort, lower post-spawn condition, and lower winter survivability than one-year-old individuals that had reproduced for

the first time. This indicates that the older fishes allocated a larger proportion of their available resources to reproduction, resulting in a greater cost to somatic development and maintenance. In some cases, however, the relationship between age and reproductive effort is not so obviously correlated. This is due to the fact that aging often results in changes in size, feeding ability, and energy storage capacity, all of which can have independent or compounding effects on measures of reproductive effort (Reznick, 1985).

Summary

A primary objective of this study was to determine to what degree reproductive effort and lipid dynamics vary among emerald shiners that are preparing for their first spawning season (age 1), their second spawning season (age 2), and a third spawning season (age 3). To do this, I measured reproductive effort as GSI, relative ovarian lipid investment, average egg size, and fecundity. Another objective of this study was to determine how different levels of reproductive effort may influence the health of spawning fish. For this part of the study, I examined how levels of investment correlate with measurements of parental health (e.g. growth rate, Fulton's condition factor K, and percent somatic lipid stores) to provide insight into the variability in cost of reproduction on the growth, development, and robustness of adult female fish (Pianka and Parker, 1975).

Methods

Fish Collection

Starting on June 2015 and continuing through October, 123 female emerald shiners were collected via electrofishing during biweekly visits to 11 sites along the upper Niagara River, Buffalo, New York (Fig. 1). Once collected, fish were immediately euthanized via overdose with MS-222 and then stored on ice to prevent degradation of tissues. Chilled fish were brought directly to the lab for processing. The whole body wet weight *W* (measured to the nearest 0.01 g) and the total length (measured to the nearest 1.0 mm) of each fish were recorded. Total length was measured from the tip of the snout to the end of the caudal fin; caudal fins were dorsoventrally compressed to get the most accurate total length of each fish.

Gonadosomatic Index (GSI)

For each sampling location, the sex of individual fish was determined via dissection and observation of gonads (see Appendix A for detailed dissection description) until five females were identified. In the genus *Notropis*, testes are generally smaller, have a smooth texture, and are white in color; ovaries are generally larger, have a globular texture, and are more yellow in color (Rabito Jr. and Heins, 1985). Once sex was determined, the ovaries were carefully removed and any visceral fat attached to the exterior of the ovaries was removed and placed back in the body cavity of the fish; all males were discarded and not analyzed further. The ovaries were blotted with a paper towel to remove surface moisture and weighed. After weighing, one of each pair of ovaries was placed in a 2 ml vial containing a mixture of methanol and BHT (an antioxidant), the air in the vial was displaced with nitrogen to prevent degradation of fatty acids, and each vial was placed in a -20°C freezer until ovarian lipid extractions were performed. The second ovary was immediately used to determine fecundity and egg characteristics (see below).

The remainder of the fish carcass (whole fish minus the ovaries) was then wrapped in cellophane and aluminum foil and stored at -20°C for future use.

The whole body wet weight of the fish and its respective ovary weight were then used to calculate the GSI value of each fish. GSI was calculated as follows:

$$\boldsymbol{GSI = \frac{Wet\ Weight\ of\ Gonad\ (g)}{Whole\ Fish\ Wet\ Weight\ (g)} \times 100}$$

Egg Counts and Egg Size

One ovary from each female was used to determine relative fecundity (i.e., number of eggs present), average egg size (average diameter in mm), and developmental stages of the egg. First, each ovary was placed in a 1 ml dish where the epithelium of the lumen was cut allowing for eggs to fall free. Next, a magnetic stirrer was used to rotate a small metal bar inside the dish containing the opened ovary. Stirring was continued until all eggs were separated from the ovarian tissue, at which point the epithelium and vascular tissue of the ovary were removed, leaving only the eggs in the dish. The eggs were then shaken and manipulated with probes until a monolayer was formed in the bottom of the dish. A photo of the eggs was taken using a digital camera mounted to an Olympus® SZH10 Research Stereoscope. These images were then uploaded into the software program ImageJ, where egg number and egg diameters were assessed using the *Cell Counter* and *Measurement* applications, respectively. These results were used to calculate the fecundity and average egg size for each female sampled (Mains et al. 2008). Additionally, the aforementioned camera and scope were used to determine what developmental stages of eggs were present in each ovary. Egg staging was done using anatomical descriptions provided by Babin et al. (2007). After these procedures were completed, the contents of each dish were placed in 5 ml vials containing 2% formalin for preservation.

Age Determination

The age of each female was determined by enumerating annuli on otoliths extracted via dissection. Sagittal otoliths from each fish were removed, dried, and then mounted to a microscope slide using Flo-Texx® liquid cover slip. Next, the otoliths were sanded using 600 grit sandpaper, then polished using 2000 grit sandpaper, creating a lateral cross-section view. A single drop of mineral oil was then added to the surface of the otolith and allowed to soak in for 10 minutes to accentuate the annuli. Fish showing signs of sexual maturation and containing only a single annulus were considered age 1; fish with 2 or 3 annuli were classified as age 2 and age 3 respectively (summarized by Campana, 2001). No young-of-the-year (YOY) were found to be sexually mature and no fish with 4 or more annuli (i.e., age 4) were recorded.

Lipid Analysis

Somatic Lipid

To determine the amount of neutral lipid stored in the somatic tissue of each fish, the percent lipid (weight of lipid / wet weight of whole body) was assessed. To do this, the fish were removed from the freezer and added to a mortar with equal weights of sodium sulfate and ground with a pestle until homogenous. Next, the homogenized mixture was transferred to a 250 ml round bottom flask to which 10 ml of solvent (petroleum-ether and ethyl acetate mixed at a 9:1 ratio) was added for every 1 gram of fish tissue. The flasks were then rotated at 125 rpm on an orbital shaker for one hour to maximize exposure of the tissue to the solvent. Next, the contents of the flask were filtered through a fritted disk funnel, allowing for the solvent-lipid solution to be separated from the ground tissue. The solution was then added to a 15 ml pre-weighed aluminum dish and placed on a heating block until all of the solvent evaporated. The aluminum

dish was then reweighed to the nearest 0.0001 gram and the difference from the original weight of the tin was determined to be the somatic lipid weight. Percent somatic lipid was then calculated as follows:

$$\textbf{\textit{Somatic Lipid}}\ (\%) = \frac{\textbf{\textit{Somatic Lipid Weight}}\ (\boldsymbol{g})}{\textbf{\textit{Whole Fish Wet Weight}}\ (\boldsymbol{g})} \times \mathbf{100}$$

Ovarian Lipid

To assess ovarian lipid content, lipid was extracted from one ovary from each fish using a direct methylation procedure (Meier et al. 2006; Parrish et al. 2015) to produce a 100 µl lipid-solvent solution (See Appendix B for detailed procedures). Analysis of each sample was performed by injecting 1.5µl subsamples into a HP 5890 gas chromatograph (GC) and peaks were identified by comparing retention times with known fatty acid standards, with C23:0 and BHT used as internal identification and quantification standards (Meier et al. 2006; Snyder and Murray, 2009). The cumulative area of the fatty acid methyl ester (FAME) peaks was representative of the total amount of lipid present in each ovary, once the peaks representing the solvent, internal standard, and BHT were excluded.

Ovarian lipid weight was then used to calculate the percent ovarian lipid:

$$\textbf{\textit{Ovarian lipid}}\ (\%) = \frac{\textbf{\textit{Ovarian lipid weight}}\ (\boldsymbol{g})}{\textbf{\textit{Gonad weight}}\ (\boldsymbol{g})} \times \mathbf{100}$$

Finally the ovarian lipid weight and somatic lipid weight were used to calculate the relative proportion of the total lipid that was invested in the ovaries (i.e., the relative ovarian lipid investment, ROLI):

$$\boldsymbol{ROLI} = \frac{\boldsymbol{Ovarian\ lipid\ weight\ (g)}}{\boldsymbol{Somatic\ lipid\ weight\ (g) + Ovarian\ lipid\ weight\ (g)}} \times \mathbf{100}$$

Data Analysis

All heteroscedastic variables were Log_{10} transformed to normalize and allow for parametric analysis. All transformed data were back-transformed to present means, standard errors, and ranges. Effects of age were determined by analysis of variance (one-way ANOVA) with Tukey multiple honest significant differences (HSD) post-hoc tests to identify differences among means. Linear regression analysis was used to determine if significant seasonal changes occurred. All data were analyzed using R statistical software (R Core Team, 2015).

Results

General length relationships

The average length of female shiners used in this study (all ages pooled) was 73 mm (range 52 – 101 mm) and the average weight was 2.7 g (range 0.98 - 6.56 g)(Table 1). A length-weight relationship was calculated for the 123 fish sampled in this study, where W = wet weight (g) and L = total length (mm):

$$\boldsymbol{Log\ W = 3.046 + -5.28 \times Log\ L}$$

A strong positive correlation between total length and whole body wet weight was found (Fig. 2; see Appendix C for other body size relationships).

Values of percent somatic lipid ranged from 1.2% (in a 58 mm, age 1 fish) to 13.9% (in a 90 mm, age 3 fish), and average values were 2.9% for age 1, 5.6% for age 2, and 5.9% for age 3 fish (Table 2). Percent somatic lipid increased with greater total length in female emerald shiners (Fig. 3).

Age Effects

Other than in June, age 2 fish made up the largest proportion of monthly samples, representing over 50% (70 fish) of the total number of fish examined in this study (Table 1). Age 1 fish represented 35% (44 fish) of the total and age 3 fish represented only 7% (9 fish). Length and weight increased during each month of the study in all three age classes, and average size and weight increased with age (Table 1). In the fall, mean values for fish of a given age class were similar to those of the next older age class the following spring; for example, the average weight of age 1 fish was 1.9 g in October and the average weight of age 2 fish in June was also 1.9 g (Table 1). Regression analysis revealed that seasonal changes in length (i.e. the growth rate) differed among age classes. Age 2 fish exhibited significantly higher growth rates compared to age 1 and age 3 fish based on differences in the slopes of the regression lines ($P < 0.05$; Fig. 4).

Table 2 summarizes age-related differences in reproductive traits measured in this study. The gonadosomatic index (GSI) and ovarian lipid weight was significantly higher in age 3 fish compared to younger age classes ($P < 0.05$). Percent somatic lipid was significantly higher in age 2 and age 3 fish compared to age 1 fish, and the relative ovarian lipid investment was significantly higher in age 3 fish ($P < 0.05$; Fig. 5). Due to the fact that only 9 fish were fully gravid when collected, fecundity could not be compared between age classes (see Appendix D) and there were no significant differences among age classes in egg diameter (Table 2).

Seasonal effects

Values of Fulton's condition factor K ranged from 0.60 to 0.74 and did not differ significantly among age classes or across months (Table 3 and Fig. 6). However, percent somatic lipid increased significantly from June through October in all age classes ($P < 0.05$; Fig. 6). Percent somatic lipid was also significantly higher in older age classes compared to age 1 individuals in all months except June, and age 3 fish had the highest percent lipid values in August and October ($P < 0.05$; Table 4 and Fig. 6).

Age 3 fish exhibited a different seasonal pattern in ovary weight compared to the other two age groups. Significantly greater ovary weights in June were followed by a distinct summer decrease in ovary weight in age 3 fish, whereas ovary weights for age 1 and age 2 fish remained relatively constant from June through August (Fig 7a). Thereafter, ovary weights of age 1 fish remained unchanged but they gradually increased in age 2 and age 3 fish, resulting in significantly different means among age classes in October (Fig. 7a). Fish in all three age classes exhibited a similar seasonal pattern of increasing somatic lipid weights, each achieving maxima in October. Older fish always had significantly more somatic lipid than younger fish, and the differences between each age class grew as the season progressed (Fig. 7b).

For fish of all age classes, ovarian lipid weights were greatest in June and were followed by mid-season decreases; thereafter, there was a gradual increase again in the fall (Table 5). Linear regression analysis indicated that the overall decrease in ovarian lipid was significant for each age class ($P < 0.05$). Although ovarian lipid weights never differed significantly between age 1 and age 2 fish, they were significantly higher in age 3 fish in June and October compared to younger age classes ($P < 0.05$, Table 5).

Fish of all age classes exhibited a similar relative ovarian lipid investment pattern, with maxima in June, a summer decrease, and a slight increase in the fall (Table 6 and Fig. 8). Linear regression analysis indicated that the overall decrease in relative ovarian lipid investment was significant for each age class ($P < 0.05$). Excluding June, when relative ovarian lipid investment was significantly greater in age 3 fish, no significant differences were seen between age classes (Table 6 and Fig. 8).

Age 1 and age 2 fish did not show any obvious seasonal changes in GSI and never differed significantly from one another (Fig. 8). For age 3 fish, GSI was significantly higher in June compared to younger age classes; after July, no statistically significant differences were seen among age classes (Fig. 8).

Discussion

Life history theory predicts that the growth, maintenance, and reproductive characteristics of organisms will be shaped by biotic and abiotic factors in their environment to maximize their reproductive success. This is illustrated by the diversity of life history strategies in fishes that have allowed them to thrive under a wide range of environmental conditions. Growth, reproductive investment, and mortality are often correlated in fishes (Gunderson, 1997). However, the exact nature of the relationship among these three factors appears to vary with body size, sex, age, population density, and also with environmental factors such as temperature, food availability and predation pressure (Kamler, 2012; Roff, 1991).

The results of this study indicate that although emerald shiners in the upper Niagara River begin reproducing at an early age (typically at age 1, see further discussion below), they exhibit an overall life history strategy that prioritizes growth early in life and has large increases in reproductive effort near the end of the lifespan. Low reproductive investment early in life most

likely maximizes somatic growth and development in two-year old emerald shiners, which display higher growth rates than either age 1 or age 3 fish. For age 3 emerald shiners, the decline in growth rates is coupled with significant increases in reproductive effort as measured by the various indices used in this study (i.e., GSI, ovarian lipid, relative ovarian lipid investment, and fecundity).

Previous studies have shown that if small body size is linked tightly to mortality risk early in life, then it is beneficial for organisms to develop a life history strategy that prioritizes growth rates over reproductive effort early in life to allow for sharp increases in reproductive effort later in life, when a larger body size has been obtained and therefore risk of mortality is reduced (Reznick, 1985; Riessen, 1999). There is some evidence of small body size being correlated with high predation risk for emerald shiners in the upper Niagara River (Cochran, 2016), and this trend appears to be common among other small bodied fishes (Scharf et al. 2000; Sih, 1994). Additionally, although size-selective overwinter mortality has not been studied in this species, a pattern of higher winter mortality in smaller individuals has been seen in a wide range of taxa (Hurst, 2007).

It is common for temperate fishes to exhibit seasonal fluctuations in growth rates and physiological condition due to annual reproductive cycles as well as variation in environmental factors such as food availability and water temperature. Lipid dynamics of female emerald shiners in the upper Niagara River appear to be shaped by both variation in environmental conditions and reproductive demands. Seasonal patterns in somatic lipid stores were similar for all age classes. Percent somatic lipid was lowest in spring and increased until reaching maxima in fall. Although no fish were collected during winter months, it is assumed that fish of a given age class collected in fall are representative of fish of the next older age class in the previous fall.

In other words, since age 2 fish in my study ended the growing season with 9% body lipid and age 3 fish started the growing season with 3% body lipid, it is likely that emerald shiners lost approximately 6% of their somatic lipid during their third winter. This general pattern is common among temperate fishes and indicates that somatic lipid stores are used to fuel energy needs during winter months when food availability is low (Hurst, 2007).

My results indicate that in all age classes, somatic lipid content was similar in June (ranging from 2.2 - 3.5 %) and increased during the growing season to reach their highest levels in October. However, the final somatic lipid content achieved before winter, as well as the rate of accumulation of lipid, varied among age classes. Mean somatic lipid levels at the end of the growing season in October were 5.6 % in age 1 shiners, 9.6% in age 2 shiners, and 12.3 % in age 3 shiners, and the rate of lipid accumulation was lowest in the age 1 fish, slightly higher in age 2 fish, and highest in age 3 fish. Previous studies have found that energy accumulation rates in fishes are often positively correlated with total length and gape size, which may result in increases in feeding rate and greater success in consuming a wider variety of food items (Scharf et al. 2000; Kamler, 2012). Emerald shiners have been shown to exhibit feeding differences associated with differences in length and gape size (Atkinson et al. 2015; Fuchs, 1967; Hartman et al. 1992; Hayer et al. 2014; Schaap, 1989), and this trend appears to be common among other small bodied cyprinids (Rabito Jr., and Heins, 1985; Schultz, 1999). It is therefore likely that the differences in lipid accumulation rates in this study are due, at least in part, to differences in body size and gape size among age classes.

When expressed on a per gram basis, it is common for energy density in fishes to increase with length until the age at first reproduction is reached, and then energy density often remains relatively unchanged after sexual maturation. In their review of the literature, Martin et

al. (2015) found that 80% of the 55 species of fish studied exhibited no significant increases in energy density with changes in length after sexual maturation. Adult female emerald shiners from this study did not follow this trend: my results indicate that percent somatic lipid increased significantly with length across all age classes, even after sexual maturity (Martin et al. 2015). High overwinter mortality linked to insufficient stored energy could increase the importance of lipid stores in temperate fishes like the emerald shiner (Hurst, 2007), resulting in higher survival rates in fish that continue to increase somatic lipid even after the onset of reproduction. Indeed, overwinter mortality appears to be inversely related to energy stores for many fishes inhabiting the Great lakes and their tributaries (Madenjian et al. 2000; Post and Evans, 1989).

Fulton's condition factor K is the most commonly used indicator of condition or overall health in fishes. Fulton's K is based on the assumption that greater weight at a given length is the result of higher energy or nutrient reserves and therefore, higher values of Fulton's K indicate better health or robustness (Froese, 2006). Despite its frequent use in fisheries studies, several criticisms of the underlying assumptions implicit in Fulton's K have arisen (Cone, 1989; Froese, 2006). Previous studies have found that as fat stores are depleted, water tends to passively diffuse into the emptying fat cells, resulting in a strong inverse relationship between lipid composition and whole body-water content in some fish species (Shearer, 1994; Sutton et al. 2000). It is, therefore, possible that body mass lost from the depletion of lipid stores might be replaced by concomitant increases in whole body water weight, resulting in relatively unchanged values of Fulton's K. This phenomenon could explain why emerald shiners caught in spring, when percent somatic lipid was lowest for all age classes, did not have significantly different values of Fulton's K compared to shiners collected in late summer and early fall, when percent somatic lipid was greatest. However, this cannot account for why values of Fulton's K did not

reflect the significant differences in percent somatic lipid composition that occurred between different age classes in my study. Cone (1989) points out that a potential issue with using Fulton's K as a condition index is that it assumes weight changes with length isometrically; if that assumption is violated, then values of Fulton's K cannot be compared across groups that show different length-weight relationships. Even though the emerald shiners in this study appear to be growing isometrically as a group (see further discussion below), it is possible that the different growth rates among age classes resulted in values of Fulton's K that do not accurately reflect differences in somatic lipid. Moreover, it is somewhat surprising that values of Fulton's K did not differ between spawning and post-spawning periods in my study, since gonad weight makes up a larger component of body weight early in the growing season. However, relatively constant values of Fulton's K across seasons and age classes appears to be common in adult emerald shiners (Hayer et al. 2014; Schaap, 1989). Given these previous findings and the results of my own study, it is recommend that Fulton's K alone not be used as a measure of overall health and robustness in emerald shiners since this index may not correlate well with energy density or lipid content.

In temperate regions, optimal conditions for reproduction and growth of early life history stages may only be available for a limited amount of time each year. It may, therefore, be necessary for fish to spawn early in spring to provide their offspring sufficient time to grow and sequester enough nutrients to survive their first winter (Hurst, 2007; Post and Evans, 1989). For fishes in temperate habitats, photoperiod and temperature appear to be the most common factors regulating reproduction (Diana, 1995). For example, Leckvarcik (2001) found that for the ironcolored shiner (*Notropis chalybaues*), the onset of gonadal development occurred within a short time frame in each of the three years of the study, indicating reliance on photoperiod.

However, in their study, the onset of spawning varied more from year to year and was dependent upon a minimum water temperature. Due to low productivity and food availability during the winter and early spring months, temperate fishes that spawn early in the spring are likely to depend on lipid reserves from the previous growing season to provide energy for reproduction. Hunter and Leong (1981) found that over two thirds of lipid accumulated by female northern anchovy (*Engraulis mordax*) during late spring and summer months, when their prey were most abundant, was allocated to reproduction the following spring. My results indicate that ovarian lipid investment was highest at the start of the spawning season in June, and this correlated with peak values of ovarian weight and the gonadosomatic index (GSI). Somatic lipid stores remained low during the spawning season and only began to increase after the peak spawning period had ended. Since the emerald shiners were in spawning condition in the spring, it is likely that some somatic lipid was used during the winter months to begin vitellogenesis and maturation of eggs. Once spawning began in the spring, current food intake could have been used to support continued development of eggs. After the spawning season ended, food intake in excess of maintenance costs may have served to replenish somatic lipid stores prior to the next winter. This pattern of lipid dynamics, characterized by winter depletion of energy stores to support maintenance and egg development, followed by early spring intake to further support egg development and restoration of somatic lipid later in the summer and early fall, is common among cyprinids in temperate habitats (Lochmann et al. 2007; Thompson et al. 1991).

In spring 2015, emerald shiners in the upper Niagara River exhibited a protracted spawning season that began in late May and continued into mid-August. The first gravid female was collected on June 1 and the last on August 18; all females collected later in the season had ovaries that were spent, indicating reproduction had ceased. Additionally, the presence of eggs at

multiple maturity stages (i.e., stages 3-4) in ovaries of most females during the spawning season indicates that the shiners exhibited asynchronous egg development, a process where egg maturation and vitellogensis of a single brood occur in staggered phases within the ovary (Blazer, 2002). Both the timing of reproduction and the asynchronous egg development I observed in this study have previously been reported in emerald shiners, and appear to be common among temperate members of the genus *Notropis* (Rabito Jr., and Heins, 1985; Schultz, 1999).

Based on examination of otoliths, four age classes of emerald shiners (young of the year or YOY, age 1, age 2, and age 3) were present in the upper Niagara River. Only 9 of the 123 female shiners examined in this study were classified as fully gravid, meaning that their ovaries contained uniformly ripe eggs at the most advanced stage of development (i.e., stage 4 yolk-bearing eggs following the terminology of Schreck and Moyle, 1990). Of the nine gravid individuals, two were age 1, indicating that emerald shiners in the upper Niagara River can reach sexual maturity after their first winter. These findings are similar to previous assessments of emerald shiner age structure and life histories (Flittner, 1964; Fuchs, 1967; Schaap, 1989). Most females examined in this study had ovaries with eggs at various stages of development, which is common in fishes displaying asynchronous egg development (as discussed above). Because of this trait, the indicators of reproductive effort used in this study (i.e., GSI, ovarian lipid weight, relative ovarian lipid investment, fecundity, and average egg diameter) are likely to underestimate the cost of reproduction since immature eggs would tend to produce lower values for most of these indicators than would be expected for fully gravid females. Indeed, the largest values for many of these indicators came from the nine fully gravid females collected early in the spawning season (see Appendix D).

When log-transformed length and weight measurements are used to estimate growth parameters, the exponent *b* from the regression analysis provides information on the type of growth exhibited by a particular species and how much weight is added to the fish per unit increase in length. In my analysis, *b* was estimated be 3.046, which indicates that emerald shiners in the upper Niagara River are exhibiting isometric growth. This growth rate is similar to that of emerald shiner populations in Lake Erie (b = 3.114) and Dauphin Lake, Manitoba (b = 3.114), and is lower than emerald shiners in Lake Simcoe, Ontario, Canada (b = 4.142) (Atkinson et al. 2015; Campbell and MacCrimmon, 1970; Flittner, 1964; Schaap, 1989). The lower growth rate of emerald shiners in the upper Niagara River compared to other populations could be due to differences in genetics, diet, or temperature. However, Atkinson et al. (2015) found that emerald shiners living in a tributary exhibited reduced growth rates compared to those living in Lake Erie, hence, the lower growth rate in emerald shiners from the upper Niagara River may also be due in part to the higher energetic costs of living in a lotic system with strong currents compared to populations living in lentic systems (Facey and Grossman, 1990).

In summary, life history studies attempt to understand the ways in which organisms balance the energetic demands of growth, maintenance, and reproduction as they age and experience different environmental conditions. In this study, I investigated how growth, reproductive effort, and lipid dynamics vary ontogenetically and seasonally for emerald shiners in the upper Niagara River. Growth rates were highest in age 2 shiners and lower in age 1 and age 3 individuals. Evidence of reproduction was found beginning at age 1, and reproductive investment as measured by ovarian lipid content was lowest in age 1 and 2 individuals and greatest in age 3 fish. Although previous studies have looked at seasonal and age-related changes in growth and reproduction in this species, this is the first study to investigate the role that lipids

play in these changes. Since emerald shiners are considered key native planktivores and an important prey item for piscivorous fishes, a better understanding of the life history characteristics of this species may lead to more informed management decisions throughout the Great Lakes.

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Table 1. Monthly length (mm) and weights (g) for all female fish collected. Values are means (± SE) and number of fish collected each month (N) is provided. No age 3 fish were found in September, and only one age 3 fish was found in July, thus, no standard error could be calculated.

	Age 1			Age 2			Age 3		
	Length (mm)	**Weight (g)**	**N**	**Length (mm)**	**Weight (g)**	**N**	**Length (mm)**	**Weight (g)**	**N**
June	57.9 ± 0.82	1.3 ± 0.05	16	65.9 ± 1.14	1.9 ± 0.10	8	87.3 ± 0.80	4.4 ± 0.25	4
July	61.2 ± 1.11	1.5 ± 0.09	11	72.8 ± 0.87	2.5 ± 0.09	18	88.0	4.3	1
August	65.2 ± 1.68	1.7 ± 0.15	6	78.3 ± 0.98	3.1 ± 0.13	24	94.0 ± 7.00	5.7 ± 0.67	2
September	69.5 ± 1.50	2.0 ± 0.14	4	84.0 ± 1.33	4.0 ± 0.20	12	-	-	0
October	69.5 ± 1.12	1.9 ± 0.10	6	88.1 ± 1.16	4.5 ± 0.30	9	94.0 ± 0.00	5.9 ± 0.44	2
Overall Mean	62.2 ± 0.82	1.6 ± 0.06	44	77.8 ± 0.89	3.1 ± 0.12	70	90.3 ± 1.68	5.0 ± 0.33	9

Table 2. Mean values (± SE) of gonadosomatic index (GSI), percent somatic lipid, ovarian lipid weight, relative ovarian lipid investment, fecundity, and egg diameter for each age class. Different letters signify statistically significant differences between age classes at $P < 0.05$ from Tukey HSD.

	Age 1	**Age 2**	**Age 3**
GSI	0.9 ± 0.06 [a]	1.2 ± 0.13 [a]	4.4 ± 1.54 [b]
% Somatic Lipid	2.9 ± 0.23 [a]	5.6 ± 0.32 [b]	5.9 ± 1.42 [b]
Ovarian lipid weight (µg)	64.3 ± 14.95 [a]	182.1 ± 35.70 [a]	1,800.6 ± 510.52 [b]
Relative ovarian lipid investment (%)	0.2 ± 0.04 [a]	0.2 ± 0.06 [a]	1.0 ± 0.41 [b]
Fecundity (# eggs)	1,025.0 ± 25.00 [a]	1,788.7 ± 349.03 [a]	3,193.0 ± 157.62 [a]
Egg diameter (mm)	0.45 ± 0.031 [a]	0.56 ± 0.066 [a]	0.64 ± 0.042 [a]

Table 3. Monthly measures of Fulton's condition factor (K) for fish of each age class. Values are means (± SE). Sample sizes (N) are given in Table 1. No age 3 fish were found in September, and only one age 3 fish was found in July so no standard error could be calculated. There were no significant differences in K across months or age classes.

	Fulton's Condition Factor (K)		
	Age 1	**Age 2**	**Age 3**
June	0.67 ± 0.010 [a]	0.63 ± 0.015 [a]	0.66 ± 0.022 [a]
July	0.65 ± 0.011 [a]	0.64 ± 0.010 [a]	0.610 [a]
August	0.61 ± 0.014 [a]	0.64 ± 0.012 [a]	0.69 ± 0.073 [a]
September	0.60 ± 0.010 [a]	0.66 ± 0.019 [a]	-
October	0.61 ± 0.024 [a]	0.66 ± 0.032 [a]	0.74 ± 0.052 [a]

Table 4. Monthly measurements of somatic lipid (%) measured for each age class. Values are means (± SE). Sample sizes (N) are given in Table 1. No age 3 fish were found in September and only one age 3 fish was found in July so no standard error could be calculated. Different letters signify statistically significant differences between age classes at $P < 0.05$ from Tukey HSD.

	% Somatic Lipid		
	Age 1	**Age 2**	**Age 3**
June	2.3 ± 0.21 [a]	3.2 ± 0.47 [a]	3.4 ± 0.90 [a]
July	2.3 ± 0.31 [a]	3.4 ± 0.27 [b]	3.6 [b]
August	2.6 ± 0.57 [a]	5.2 ± 0.43 [b]	8.8 ± 2.16 [c]
September	3.7 ± 0.53 [a]	6.9 ± 0.68 [b]	-
October	5.6 ± 0.45 [a]	9.6 ± 0.75 [b]	12.3 ± 0.43 [c]

Table 5. Monthly measurements of ovarian lipid weight (µg) measured for each age class. Values are means (±SE). Sample sizes (N) are given in Table 1. No age 3 fish were found in September so no standard error could be calculated. The ovarian lipid sample for the only age 3 fish collected in July was lost. Different letters signify statistically significant differences between age classes at $P < 0.05$ from Tukey HSD.

	Ovarian Lipid Weight (µg)		
	Age 1	**Age 2**	**Age 3**
June	120.2 ± 34.38 [a]	341.2 ± 203.38 [a]	2,614.3 ± 529.0 [b]
July	57.3 ± 28.56 [a]	186.8 ± 108.12 [a]	-
August	25.1 ± 13.29 [a]	126.1 ± 27.03 [a]	243.3 ± 179.27 [a]
September	35.0 ± 12.66 [a]	115.5 ± 29.74 [a]	-
October	38.1 ± 10.45 [a]	239.5 ± 69.66 [a]	1,014.2 ± 904.2 [b]

Table 6. Monthly measurements of relative ovarian lipid investments measured for each age class. Values are means (± SE). Sample sizes (N) are given in Table 1. No age 3 fish were collected in September so standard error could not be calculated. The ovarian lipid sample for the only age 3 fish collected in July was lost. Different letters signify statistically significant differences between age classes at $P< 0.05$ from Tukey HSD.

	Relative Ovarian Lipid Investment		
	Age 1	**Age 2**	**Age 3**
June	0.30 ± 0.087 [a]	0.80 ± 0.450 [a]	1.99 ± 0.431 [b]
July	0.14 ± 0.079 [a]	0.19 ± 0.092 [a]	-
August	0.11 ± 0.047 [a]	0.07 ± 0.014 [a]	0.06 ± 0.044 [a]
September	0.03 ± 0.009 [a]	0.04 ± 0.008 [a]	-
October	0.04 ± 0.014 [a]	0.06 ± 0.017 [a]	0.13 ± 0.104 [a]

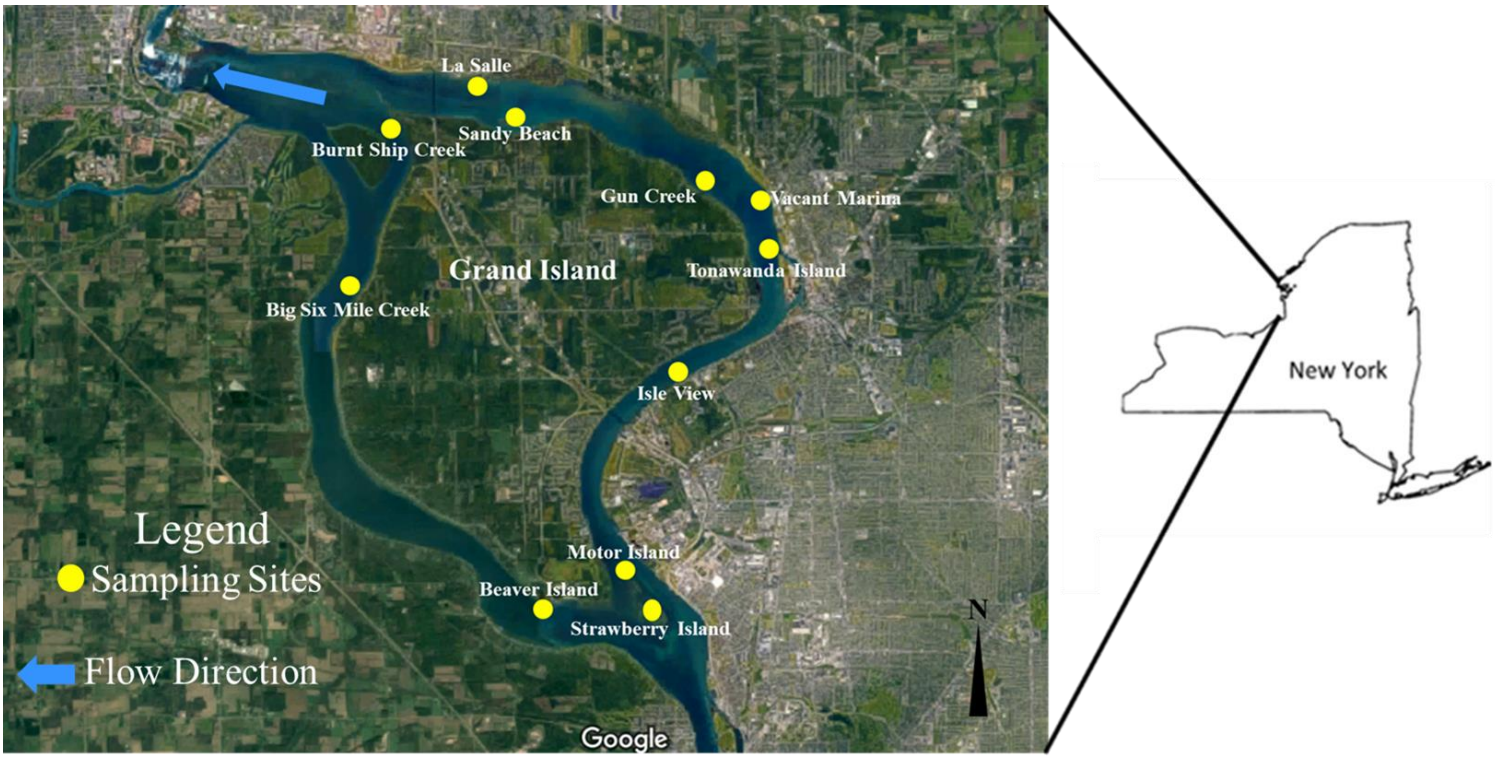

Figure 1. A map of the upper Niagara River and its location in New York State. Yellow circles mark the location of the 11 sample sites used in this study. The blue arrow indicates direction of flow of the river.

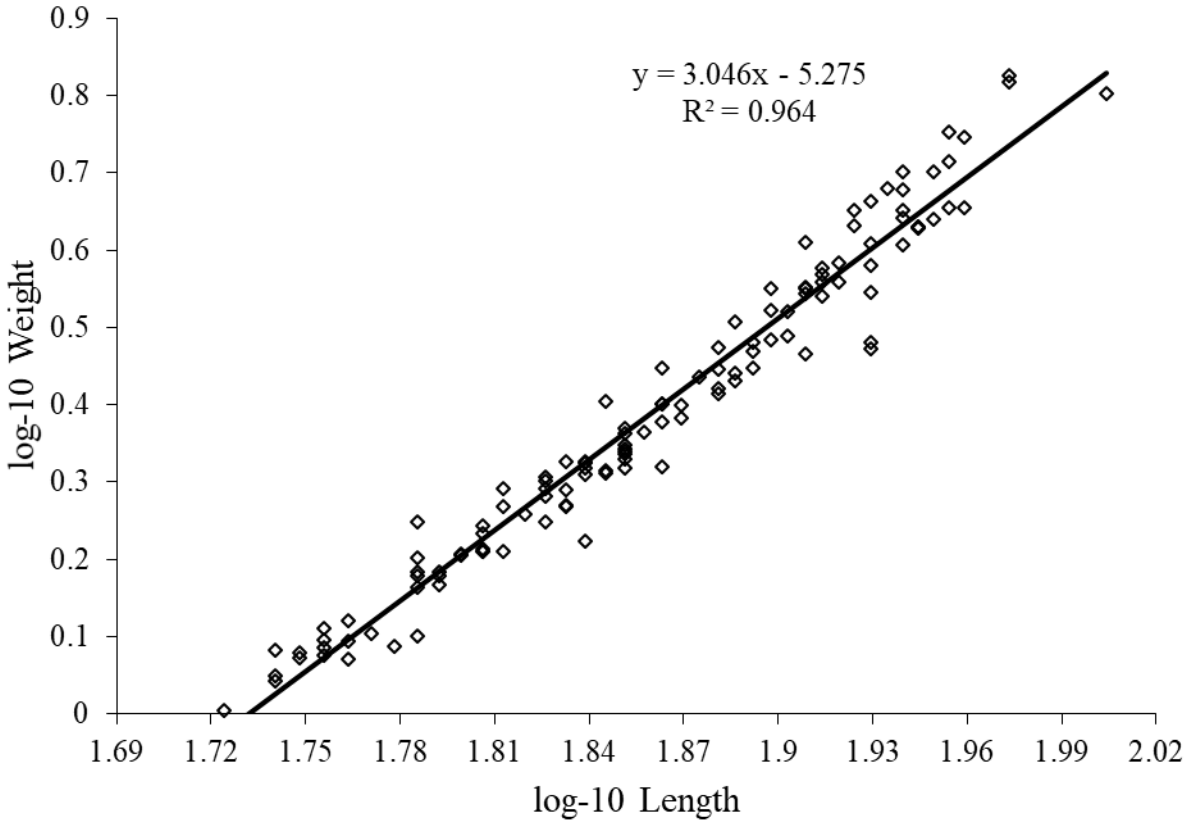

Figure 2. The length-weight relationship for female emerald shiners collected for this study (N = 123). For the regression analysis, total length was measured in mm and whole body wet weight in g

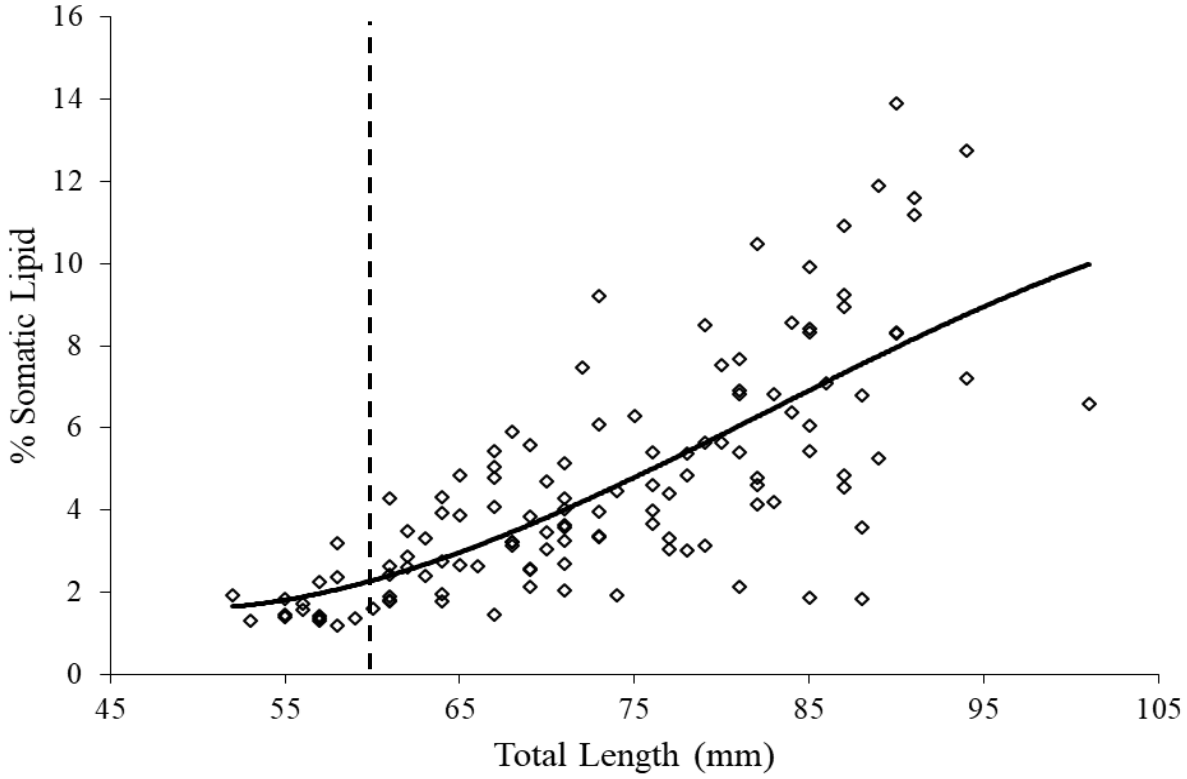

Figure 3. The relationship between percent somatic lipid and total length for female emerald shiners. The vertical dashed line represents the average size at maturity for female emerald shiners.

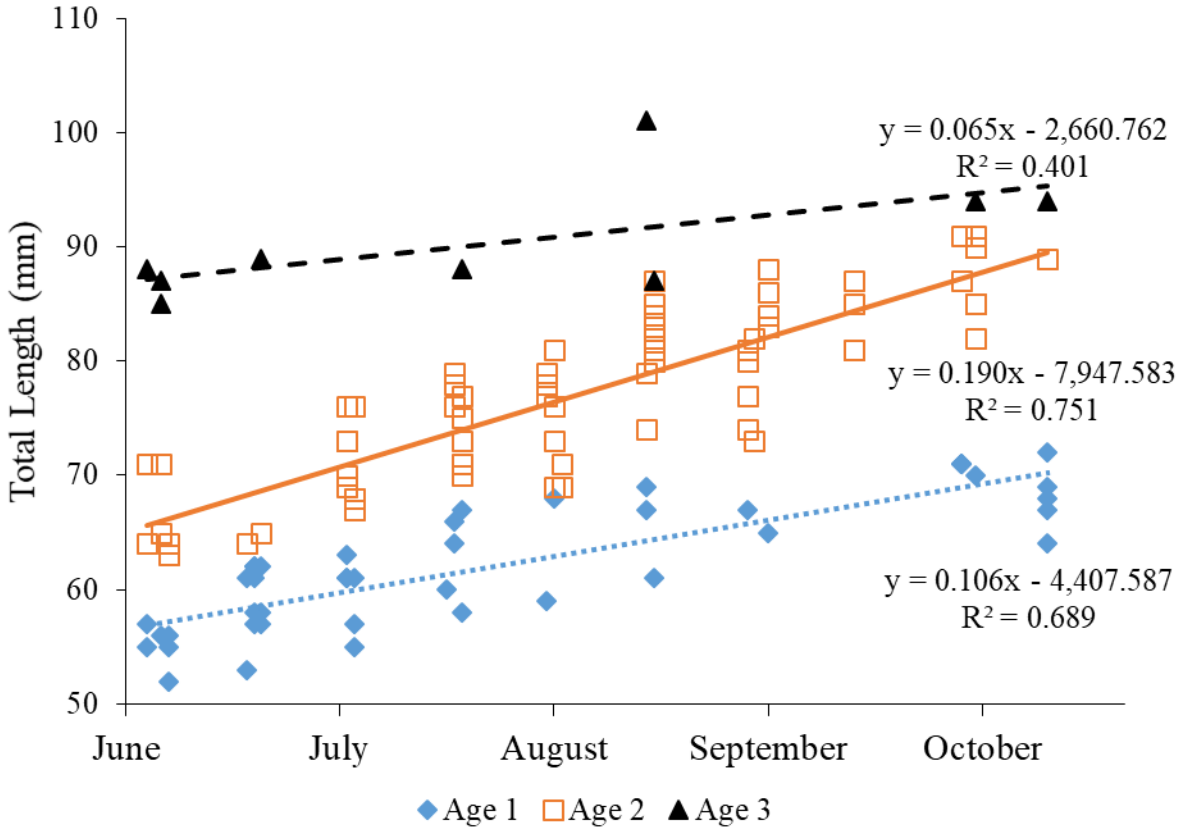

Figure 4. Regression analyses of seasonal changes in total length (mm) of female emerald shiners of each age class.

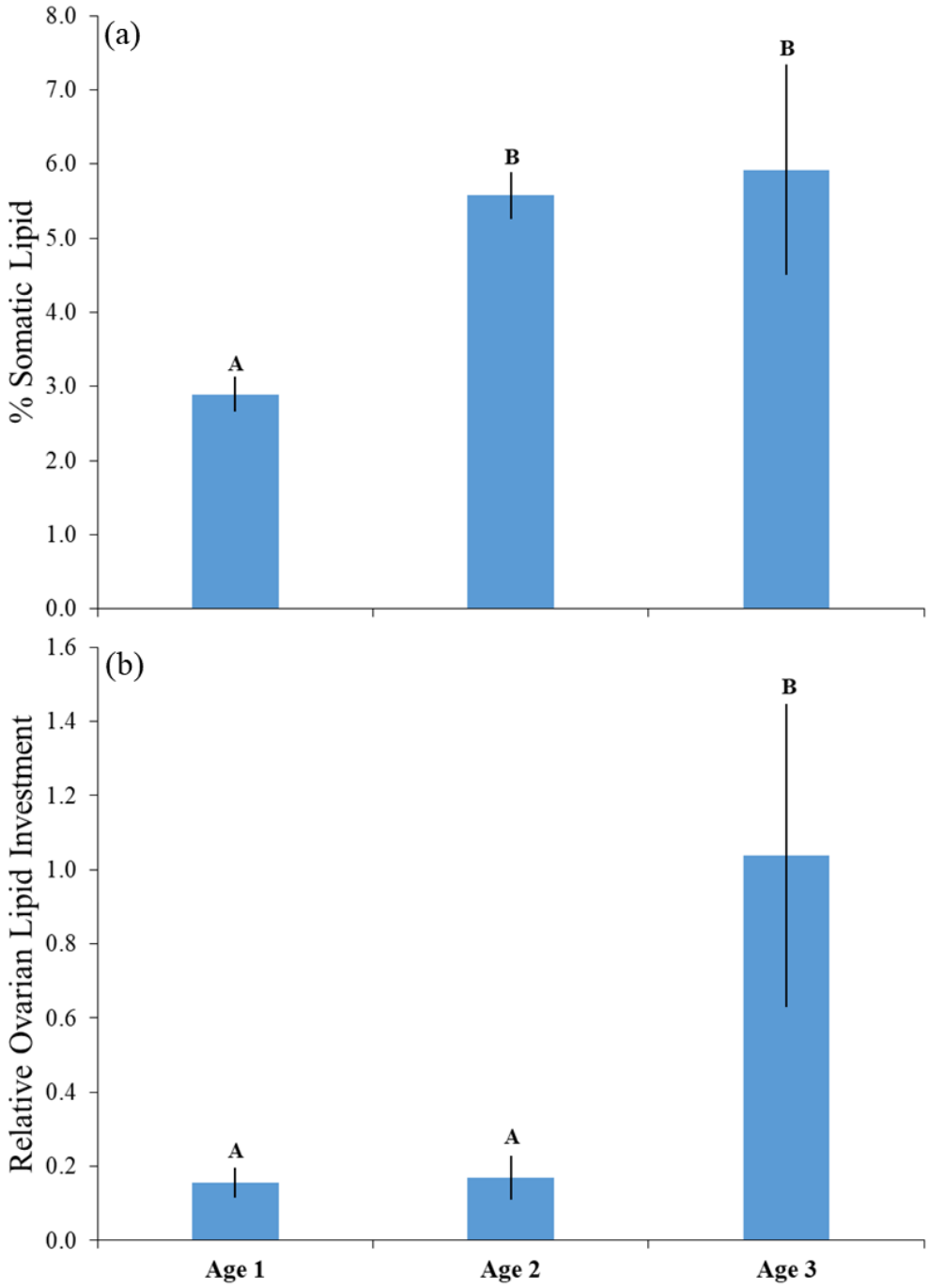

Figure 5. Percent somatic lipid (a) and relative ovarian lipid investment (b) for three age classes of female emerald shiners. Values represent means (± SE). Different letters indicate statistically significant differences between age classes at $P < 0.05$ from Tukey HSD.

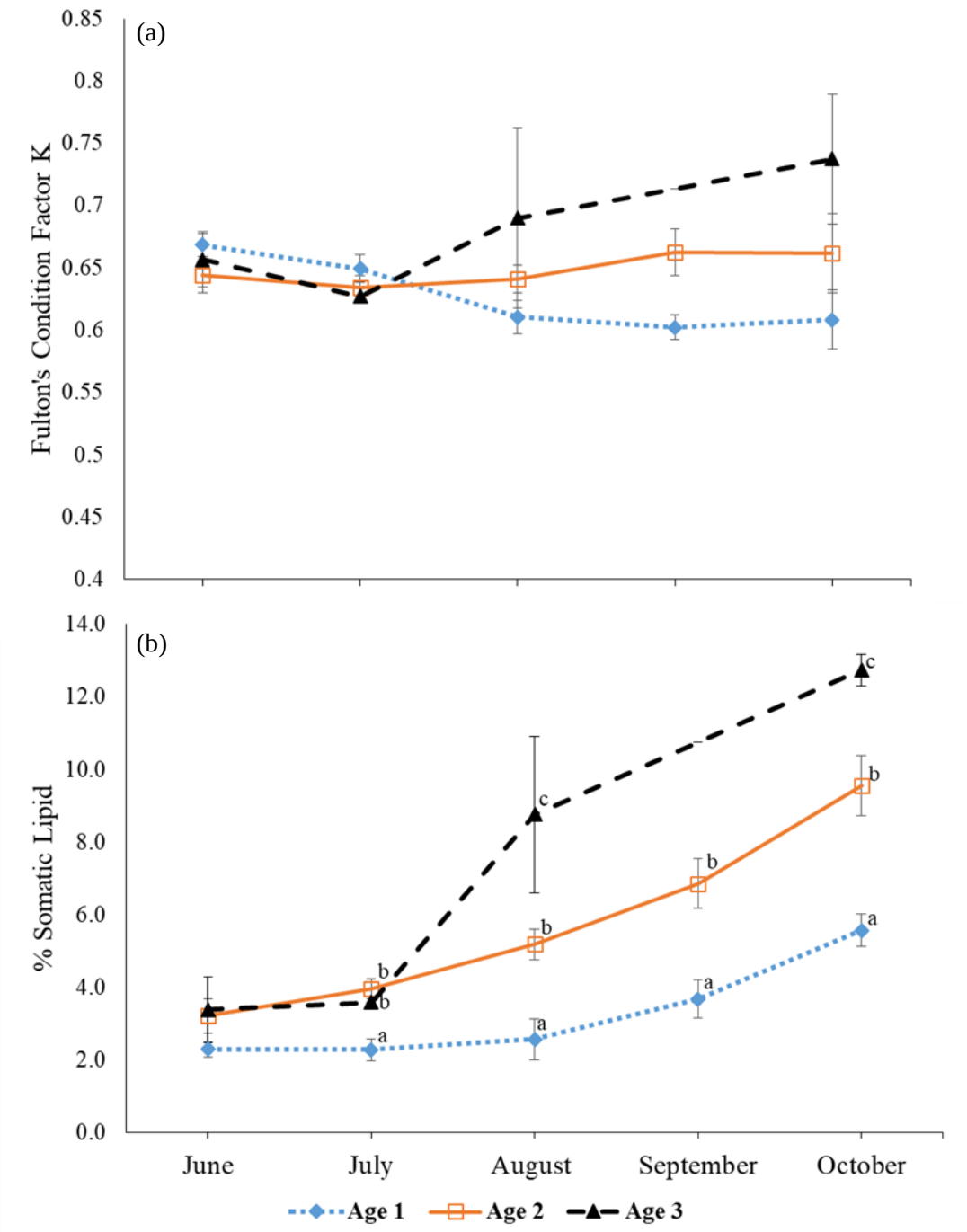

Figure 6. Seasonal changes in mean (± SE) condition factor K (a) and percent somatic lipid (b) for age 1, age 2, and age 3 female emerald shiners. Different letters indicate statistically significant differences between age classes at $P < 0.05$ from Tukey HSD.

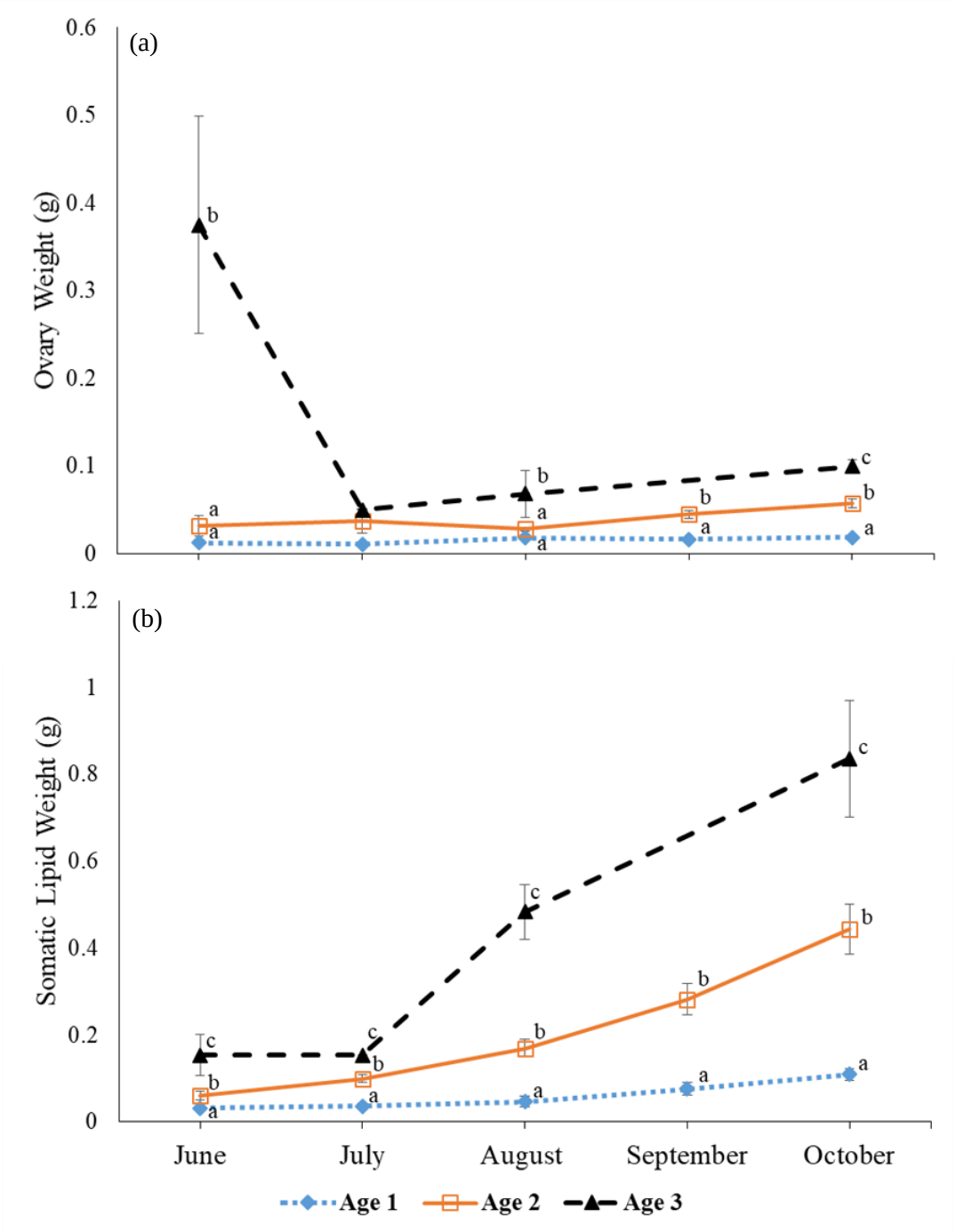

Figure 7. Seasonal changes in mean (± SE) ovary weight (a) and somatic lipid weight (b) for age 1, age 2, and age 3 female emerald shiners. Different letters indicate statistically significant differences between age classes at $P < 0.05$ from Tukey HSD.

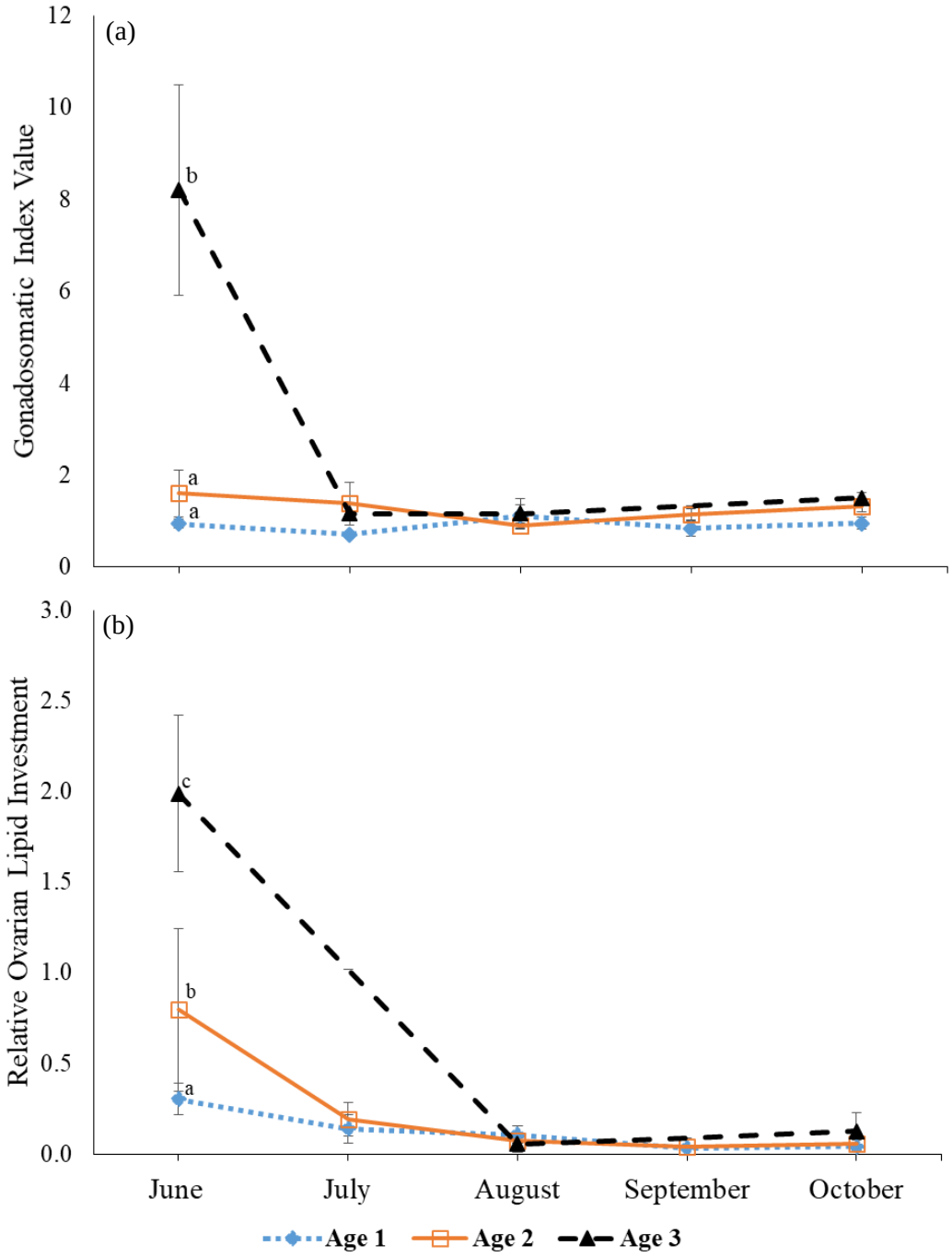

Figure 8. Seasonal changes in mean (± SE) gonadosomatic index value (a) and relative ovarian lipid investment (b) for age 1, age 2, and age 3 female emerald shiners. Different letters indicate statistically significant differences between age classes at $P < 0.05$ from Tukey HSD.

Appendix A. Gonad Removal and Sex Determination

The dissection process began with the fish being fastened to a dissecting board with two 3” pins. The fish was laid in the supine position with one pin pushed in the bottom jaw, out the top of the head and the second pin was pushed through the caudal peduncle. Both pins were pushed firmly into the dissecting board holding the fish in place. Once fastened in place, a shallow incision was made on the ventral side of the fish starting at the anus and ending at the posterior-most section of the jaw. A second incision was then made perpendicular to the first incision, just posterior to the opercula and was made as deep as the spinal cord. These cuts were made superficially to allow for epidermis and muscle tissue to be cut while avoiding damage to the internal organs. Once these incisions were made the body cavity was held open using four more 3” pins. The pins were placed between the cavity wall and the organs and pushed through the dorsal tissue into the dissecting board. With the body cavity held open, the entire digestive tract was removed from the anus to the esophagus. The organs were removed by first severing the digestive tract at the anus using scissors and then at the anterior section just below the opercula. The posterior most portion of the digestive tract was grasped with forceps and lifted out slowly while connective tissues between the digestive organs and the surrounding organs were gently severed using a dissection probe. With the digestive system removed, the swim bladder and gonads were exposed allowing for the gonads to be removed. First using a dissection probe to separate the connective tissue between them and the swim bladder, the gonads were then lifted out of the body cavity using a pair of forceps. The fish were sexed via visual determination and if uncertain, a dissection scope was utilized.

Appendix B. Procedures for Lipid and Fatty Acid Analysis

Direct Methylation Procedure

Fish tissue samples and 3 ml of methylating solution were placed in a 20 ml vial and ground with a Brinkmann Instruments Polytron® Homogenizer. The contents of the 20 ml vial were then transferred to round bottom test tubes with Teflon-lined screw caps and placed in a Thermolyne type 16500 dri-bath to be heated to ~80°C for 2 hours. The test tubes were then removed from the heat source and left to cool before 1.5 ml of ultra-pure water, 1.5 ml hexane, and 0.3 ml dichloromethane were added. The tubes were then thoroughly mixed before being centrifuged at 2000 rpm for 5 min to allow for phase separation. Next, the top layer, as well as a small amount of the middle layer, was removed to ensure that all FAMEs were extracted and were added to a clean test tube. The mixture in the original test tube was then mixed with another 1.8 ml of hexane and dichloromethane (4:1) and centrifuged as before to ensure that all FAMEs were properly separated. Again, the top layer and some of the middle layer were removed and added to the test tube containing the first extraction. Next, using a Pasteur pipet, the bottom layer of the second test tube was removed so that only FAMEs and solvent were left. The solvent was then evaporated under a stream of nitrogen leaving only the FAMEs in the tube. Finally, 100µl of hexane containing a known amount of internal injection standard (23:0 FAME) was used to re-suspend the FAMEs. Analysis of each sample was performed by injecting 1µl subsamples into a gas chromatograph programmed to measure co-migratory retention times with the internal standard mentioned above.

Ovarian Lipid Weight

Due to the fact that the ovaries of emerald shiners are so small, a gas chromatograph was used to assess their lipid content. When using this method, it is difficult to guarantee that the size of each injection is identical. In order to control for this source of variability, the area under the peak created by the BHT for all samples was averaged and used as a constant by which each sample could be adjusted. First, the area under each peak created by the lipid content of the injection (i.e., all peaks excluding the BHT peak and internal injection standard) was calculated giving the total lipid area. Next, the total lipid area for each sample was adjusted by the deviation of its observed BHT peak from the average BHT peak size, in order to correct for the variation in injection amounts. The resulting value was the Adjusted Lipid Area:

$$\textbf{Adjusted Lipid Area} = \sum_{\boldsymbol{ni}} \boldsymbol{pa} \times \left(\frac{\mathbf{1}}{\boldsymbol{\Delta BHTni}}\right)$$

Where *ni* = fish, *pa* = total lipid area and ΔBHT = the quotient of the average area under the BHT peak divided by observed area under the BHT peak for that sample. A regression equation representing the line of best fit calculated for the adjusted lipid area of 4 injections of known lipid weights (Appendix 3) was calculated;

$$\boldsymbol{y = 4,982,371x + 10,810; R^2 = 0.997}$$

The equation was then rearranged to solve for *x* (lipid weight) and the adjusted lipid area for each ovary was input as the y variable to calculate ovarian lipid weight in grams (see Fig. B1).

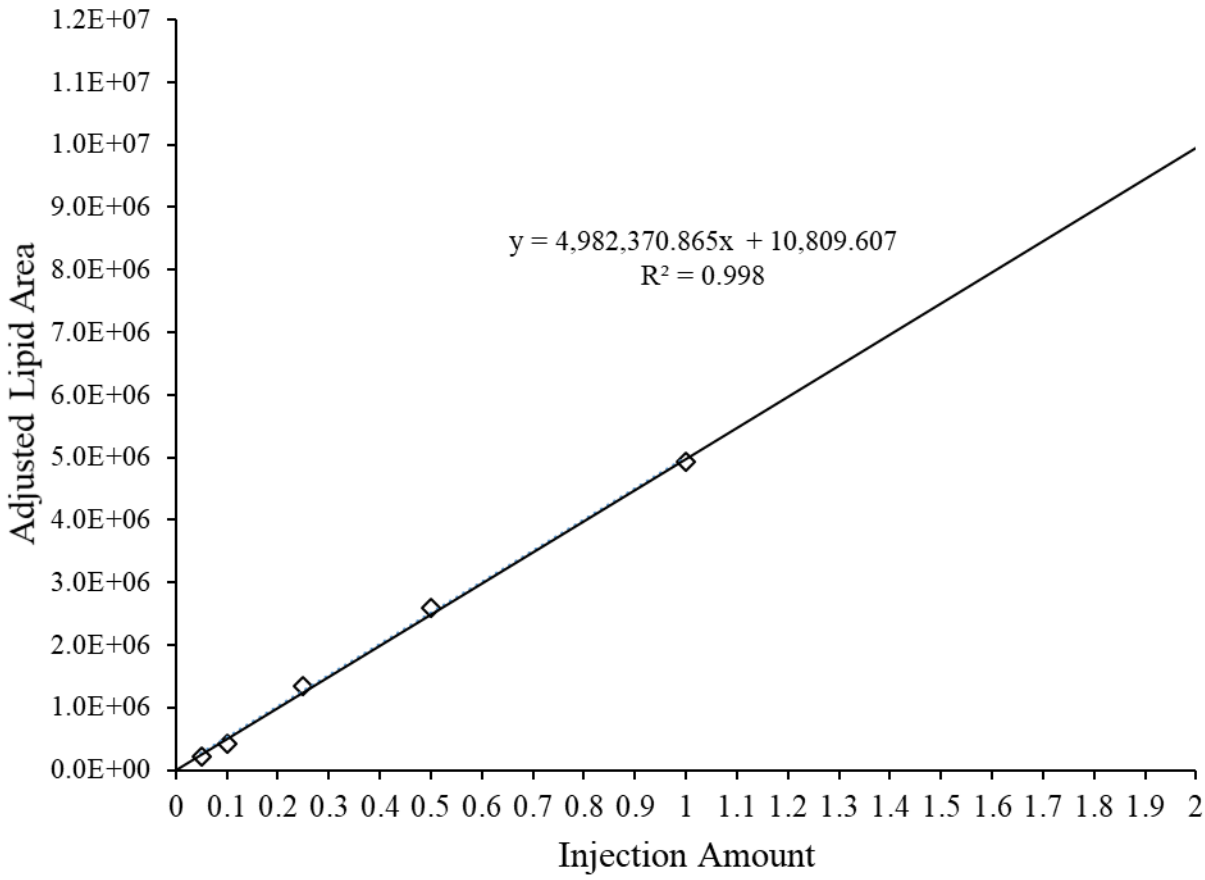

Figure B1. A regression equation representing the line of best fit calculated for the adjusted lipid area of four injections of known lipid weight used to covert the adjusted lipid area from the gas chromatograph to lipid weight in grams.

Appendix C. Whole Body Weight Regressions

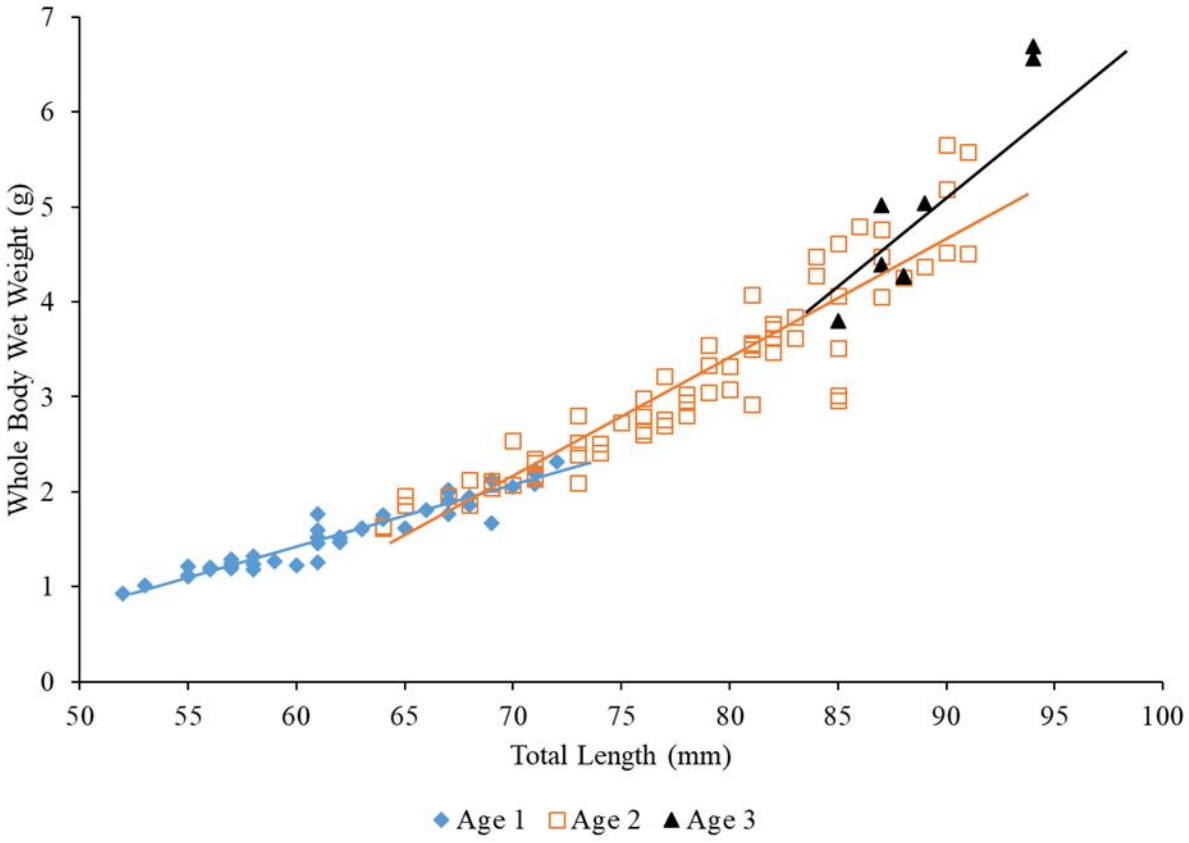

Figure C1. Length-weight relationship for the three age classes of female emerald shiners collected for this study (n = 123). For the regression analysis, total length was measured in mm and whole body wet weight in grams. Significantly different ($p < 0.05$) slopes are indicated by an *.

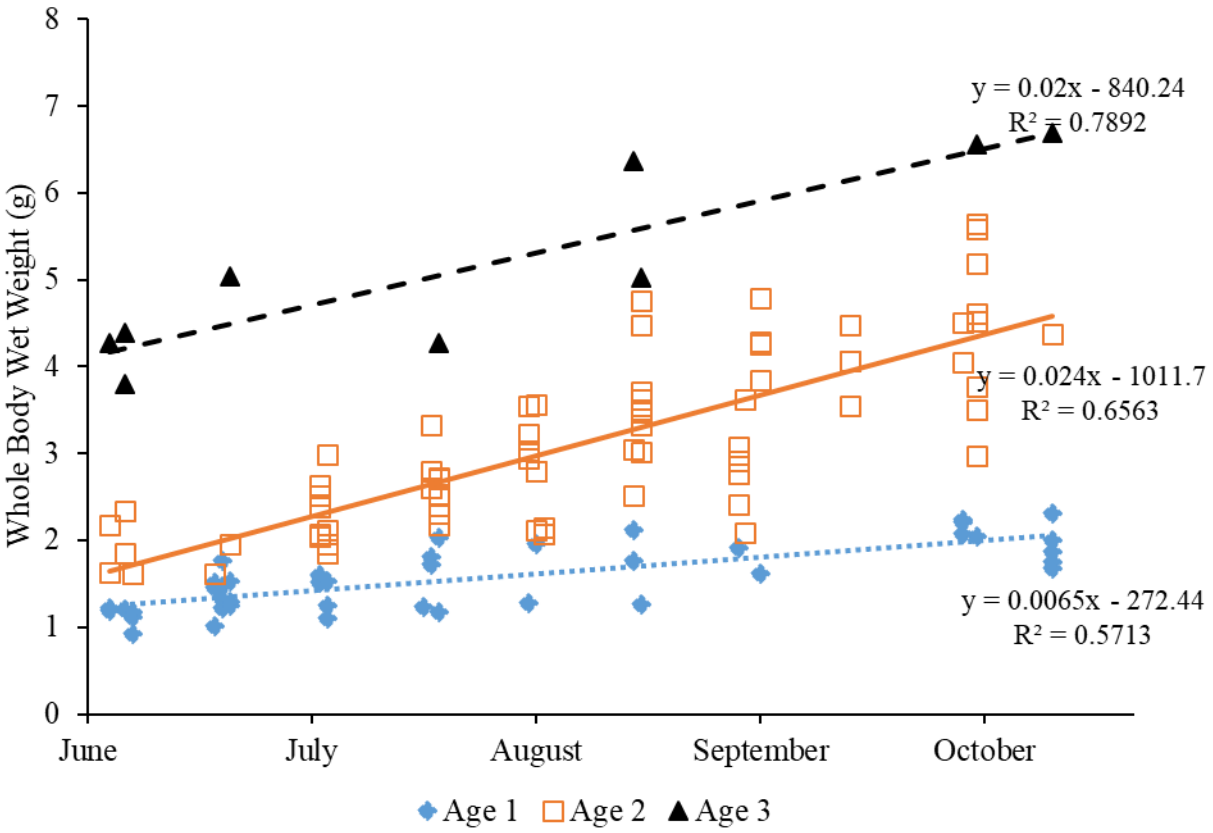

Figure C2. Regression analysis of changes in whole body wet weight (g) of female emerald shiners from bi-weekly samples collected from the upper Niagara River. Age 2 shiners exhibited the largest rate of increase in weight during the sampling season.

Appendix D. Fecundity Data

Table D1. The whole body wet weight (weight), fecundity, age, and number of eggs per gram of whole body wet weight for the fish that were gravid at the time they were collected (n = 9). The box below the table contains the mean (± SE) values of the number of eggs per gram of whole body wet weight for each age class.

Weight	Fecundity	Eggs/gram	Age
5.0	3500	695.0	3
4.4	3294	750.5	3
4.3	3224	755.0	3
3.8	2754	724.4	3
3.0	2464	826.0	2
2.8	1604	573.3	2
2.2	1298	597.6	2
1.6	1050	651.0	1
1.3	1000	793.7	1

Eggs per gram body weight	Age 1	Age 2	Age 3
	722.3 (± 101)	665.7 (± 139)	731.2 (± 27)

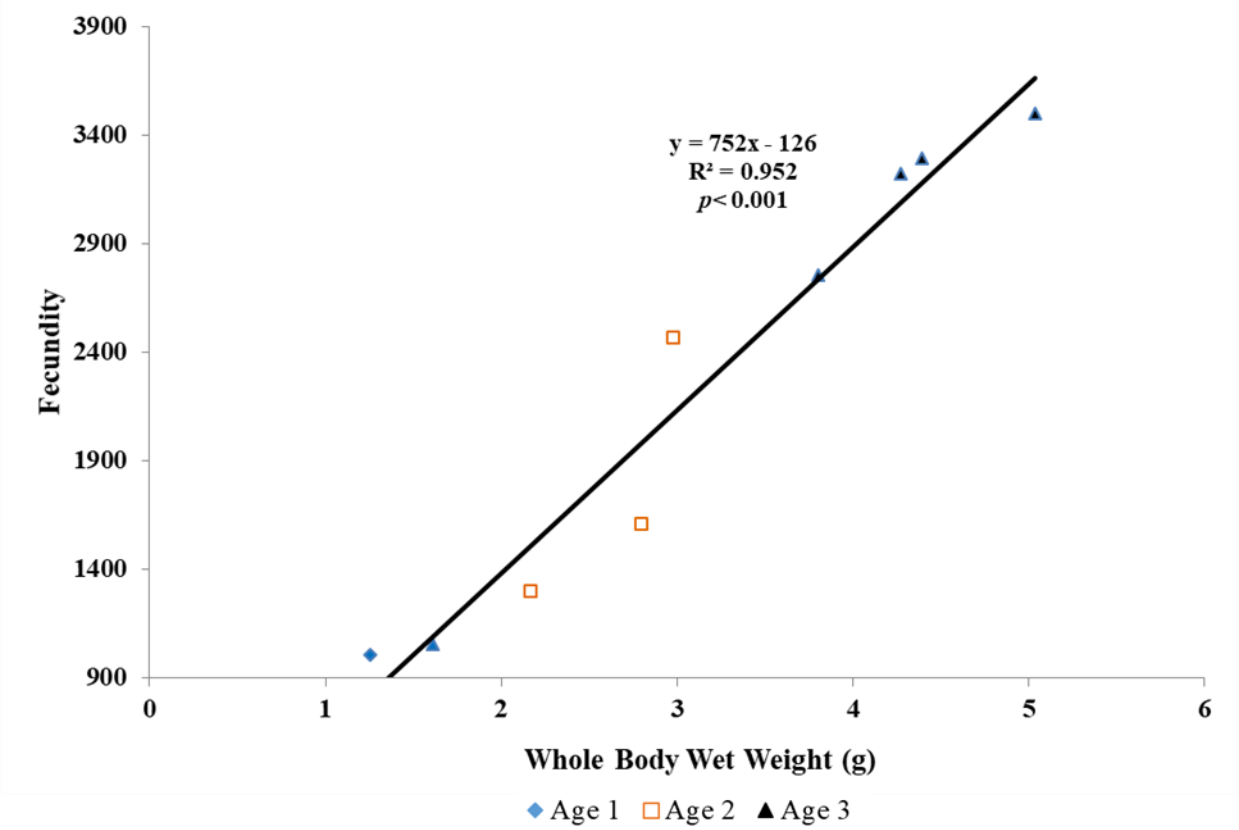

Figure D1. Regression analysis of fecundity and whole body wet weight for gravid emerald shiners (n= 9).