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Lake Ontario Alewife, Alosa pseudoharengus, maturation and reproduction dynamics

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Lake Ontario Alewife, *Alosa pseudoharengus*, maturation and reproduction dynamics

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Abstract

Since their introduction in Lake Ontario, alewife (*Alosa pseudoharengus*) have dominated the forage fish community, making them the primary food source for the lake's economically valuable sport fish populations. Therefore, alewife population dynamics can impact fishery success and management. Recently observed declines in alewife abundance and year class strength variability further increase the need to better understand alewife reproduction. The objectives of this study were to quantify maturation and reproductive dynamics of Lake Ontario alewife by 1) determining if alewife display determinate or indeterminate fecundity, 2) determining if age 2 alewife could be considered part of the spawning stock, and 3) assessing reproductive potential across alewife ages 2 to 6. We collected alewife from various locations in Lake Ontario from October 2017 to October 2018 and measured gonadosomatic index, condition factor, gonad development, spawning potential, batch fecundity, and embryo survival data. Evidence of a prolonged spawning season and the presence of multiple batches of advanced oocytes in the ovaries of alewife suggest this species display indeterminate fecundity (i.e., can spawn multiple batches of eggs in a single spawning season). Spawning potential (observed spawning and or the presence of mature gonads) was observed in 63.9% of age 2 females and 90.4% of age 2 males captured in June and July, indicating age 2 alewife should be considered part of the spawning stock. This was confirmed by the successful survival of embryos of age 2 parents. When comparing embryo survival data among all ages, older females displayed higher embryo survival, and our beta regression model suggested female age best explained observed. In addition, alewife older than age 2 appeared to have a higher proportion of indeterminate spawners, further suggesting older alewife have increased reproductive output vs younger fish. However, the lack of variation

in relative batch fecundity among ages suggest other variables, such as size may better explain this variability.

1. Introduction

1.1. Alewife status and ecology

Alewife (*Alosa pseudoharengus*) is a species of herring belonging to the family Clupeidae and is identified by its large eyes, silver colored body, and "saw-tooth" belly. As a pelagic planktivorous species native to eastern North American waters of the Atlantic Ocean (from Nova Scotia to North Carolina), it has both anadromous and landlocked (freshwater resident) populations. Alewife was first discovered in Lake Ontario in 1873, likely after entering via the Hudson – Mohawk River drainage, the New York Finger Lakes, and the Erie Canal (Smith *et al.* 1970). The opening of the Welland Canal in 1829 provided a route for their movement to the other Great Lakes. In the late 1940's and 1950's, overfishing and the introduction of the parasitic sea lamprey (*Petromyzon marinus*), had significantly reduced the abundance of predatory lake trout (*Salvelinus namaycush*) in the Great Lakes. By 1960, due to the lack of predatory pressure, alewife became widespread in the Great Lakes basin (O'Gorman and Stewart 1999).

In Lakes Michigan, Huron, and Ontario, alewife had become so abundant massive die offs occurred which fueled the public to demand a solution to alewife overpopulation (O'Gorman and Stewart 1999). Fisheries managers successfully introduced hatchery raised Pacific salmon and created a multi-million-dollar recreational fishery in the region that in turn had the added benefit of controlling alewife through predatory pressure (Bence and Smith 1999, O'Gorman and Stewart 1999, Connelly and Brown 2009). To this day, alewife remain an important food source for these predatory salmonines. Stable isotope analysis conducted in Lake Ontario by Colborne et al. (2016) confirmed the significance of alewife in the diet of lake trout. Interestingly, McCommish and Miller (1975) found that lake trout 58.3 cm or greater in total length tended to consume large alewife (126-182 mm in total length) exclusively. In addition, through the use of fatty-acid

signatures, Happel *et al.* (2017) found that alewife were preyed upon by Chinook salmon (*Oncorhynchus tshawytscha*), coho salmon (*Oncorhynchus kisutch*), brown trout (*Salmo trutta*), lake trout, rockbass (*Ambloplites rupestris*), and smallmouth (*Micropterus dolomieu*) bass. Chinook and coho salmon diets were composed nearly exclusively of alewife, while brown trout, lake trout, rockbass, and smallmouth bass displayed a mixed diet of alewife and round goby (*Neogobius melanostomus*).

Alewife are the dominant species in the Lake Ontario fish community. As an invertebrate consuming prey fish, they are naturally more abundant than piscivores. The 2016 survey conducted by the United States Geological Survey - Lake Ontario Biological Station (USGS-LOBS) and the New York State Department of Environmental Conservation (NYSDEC) found that alewife made up 89% of the total fish catch and 93% of the total pelagic prey fish caught in bottom trawl survey of American waters of Lake Ontario (Weidel *et al.* 2016). The survey conducted in 2018 also indicated that alewife continued to dominate lake biodiversity and were 80% of the total catch (Weidel *et al.* 2018). In addition to their abundance, alewife act as a lipid rich food source (Madenjian *et al.* 2000, Futia *et al*. 2019). Of Lake Ontario prey fish sampled in 2015 and 2016, overall lipid content of alewife (11.0 ± 3.7%) was significantly greater than that of rainbow smelt (4.7 ± 1.3%) and round goby (4.0 ± 1.8%) (Futia *et al.* 2019). Madenjian *et al.* (2000) observed similar results in Lake Michigan. As lipid content in fish has been positively associated with fitness, a higher lipid content will result in larger individuals with increased fitness (e.g., fecundity, condition factor) (Henderson and Nepszy 1994, Hixon *et al*. 2014). A lipid rich diet should translate to a lipid rich predator with higher fitness (Madenjian *et al.* 2000). It is also worth noting alewife is a valuable prey source beyond the Great Lakes region. In Claytor Lake, Virginia, alewife are consumed by predators like walleye (*Stizostedion vitreum*), white bass

(*Morone chrysops*), and striped bass (*Morone saxatilis*) (Kohler and Ney 2011). They are also an important food source to anadromous fish like migrating striped bass in North Carolina that have been shown to rely almost solely of blueback herring (*Alosa aestivalis*) and alewife (Trent and Hassler 1966).

Although alewife populations are valuable food sources in the Great Lakes and beyond, this species can have negative impacts on the systems they inhabit. Alewife have an elevated level of thiaminase, an enzyme shown to decrease thiamine (vitamin B_1) in certain species that prey on them (Tillitt *et al.* 2005). Thus, alewife have been linked to thiamine deficiency complex (TDC), and consequently, early mortality syndrome (EMS). EMS and its negative impacts on recruitment and fish populations are well documented for coho salmon, lake trout, steelhead trout, and Atlantic salmon (*Salmo salar*) (Fitzsimons *et al.* 1999, Ketola *et al.* 2000, Madenjian *et al.* 2008, Fitzsimons *et al.* 2010, Riley *et al.* 2011, O'Gormon *et al.* 2013, Futia and Rinchard 2019).

Alewife can also negatively impact systems they inhabit as predators. Alewife are size-selective planktivores, meaning they feed on the largest available zooplankton (Mills *et al.* 1992, Madenjian *et al.* 2008). The presence of alewife has been connected to the reduction in size and abundance of larger species of zooplankton like *Daphnia* spp., *Diaptomus minutus* (Brooks and Dodson 1965, and Warshaw 1972). In addition, due to their ability to filter feed, alewife are able to continue feeding on smaller plankton, giving alewife a competitive edge over native planktivores (Crowder and Binkowksi 1983). Therefore, the introduction of alewife has been attributed in part to the decline of native Great Lakes planktivorous salmonids, such as whitefish (*Coregonus clupeaformis*) (Madenjian 2008). In addition, Crowder (1983) speculated that the bloater (*Coregonus hoyi*) in Lake Michigan evolved fewer and shorter gill rakers and shifted to benthic habitat and diet as a result of competition with alewife. The negative impact of alewife on

bloater is further supported by the observed recovery of bloater after the decline of alewife in Lake Ontario between 1971 and 1998 (Owens *et al*. 2003). Although zooplankton are the primary food source of alewife of all ages, adult alewife are known to consume larval fish and eggs of species such as walleye, lake trout, and other planktivores (Brooking *et al.* 1998, Madenjian *et al.* 2008, O'Gorman *et al.* 2013). Madenjian (2008) and O'Gormon (2013) concluded that predation of larvae and eggs by alewife likely contributed to the decline of yellow perch (*Perca flavescens*), deepwater sculpin (*Myoxocephalus thompsonii*), burbot (*Lota lota*), Atlantic salmon , lake trout, and emerald shiner (*Notropis atherinoides*) in the Great Lakes basin.

1.2. Alewife Population Dynamics

Despite their successful naturalization throughout the Eastern United States alewife populations are not always stable. Reproductive success of northern fish often relies heavily on water temperatures during a given phase of early life history (O'Gorman *et al.* 2004). This is particularly true of alewife in the Great Lakes. Mass mortalities of alewife have been observed in the region because alewife are stressed when water temperatures dip below 3°C, which is common in the Great Lakes (O'Gorman *et al.* 2004, Madenjian *et al.* 2005, Hook *et al*. 2007). When age-0 alewife have short growing seasons, the negative impacts of these cold winters can result in high mortality (O'Gorman *et al.* 2004, Madenjian *et al.* 2005, Hook *et al.* 2007). Results of these pressures are currently being observed in Lake Ontario leaving a "gap" in a certain year class. In 2015, the age 1 alewife had extremely low abundance (after an exceptionally long and cold winter), resulting in few age 2 fish in 2016, age 3 fish in 2017, and so on (Figure 1, Weidel *et al.* 2019). In addition, as we examine the preliminary 2019 abundance, the lack of age 5 and 6 fish abundance suggests predation pressure on large, old alewife, increased (Figure 1; Weidel *et al.* 2019). The loss of the 2015-year 1 class in combination with increased predation pressure on large, old

alewife, resulted in an overall decline in the abundance of large, old alewife in 2019, making the young (ages 2 and 3), small alewife a large component of the spawning stock (Wiedel *et al.* 2019). Therefore, it is imperative to understand if age 2 alewife can reproduce and better understand alewife reproduction in Lake Ontario.

1.3. Alewife Reproduction: Understanding Maturation and Fecundity

The seasonal distribution of alewife (Figure 2) suggests that water temperature is an important factor in alewife reproduction and recruitment (O'Gorman *et al*. 2013, Weber *et al.* 2015, B. Weidel, USGS personal communication). Alewife will overwinter offshore to thermal regulate (O'Gorman and Stewart 1999). As water temperatures increase, landlocked alewife move from the deep, offshore benthic habitat pelagic zone to near shore waters to spawn (Figure 2) (O'Gorman *et al*. 2013, Weber *et al.* 2015, B. Weidel, USGS personal communication). Like their anadromous counterparts, landlocked alewife spawn in late-spring and summer and are broadcast spawners (Bronte *et al*. 1991). Broadcast spawners will release their eggs into the open water and provide little to no parental care; this strategy is typical of other clupeid and many other marine species (Blaxter and Hunter 1982, Bronte *et al*. 1991). Their eggs are adhesive for roughly 24h and will sink unless buoyed by currents; eggs are incubated in water near 24°C for 3-4 days before hatching (Dimaggio *et al.* 2014, Weber *et al.* 2015). After hatching, alewife spend anywhere from 1 to 3 months in their nursery areas before moving back into deeper water as water temperature's decrease (Davis and Schultz 2009, Weber *et al.* 2015). Some observed triggers for these migrations include heavy rainfall, high water levels, and sharp drops in water temperature (Mullen et al. 1986).

In order for females to mature and produce eggs, female alewife undergo oogenesis - the development of oocytes within the ovary - like other teleost species (Wallace and Selman 1981,

Ganias *et al*. 2015). In oogenesis, multiple oogonial stem cells within the ovary undergo meiosis to become primary oocytes or previtellogenic oocytes. These oocytes contain cytoplasm and a centrally located nucleus or germinal vesicle (Figure 3). As these oocytes increase in size as does the nucleus and multiple nucleoli appear; the aggregated nucleoli become surrounded with cytoplasmic organelles known as yolk nuclei or cortical alveoli. The appearance of these cortical alveoli indicates the oocyte has reached the endogenous vitellogenic stage (Figure 3). The oocytes remain in this stage as these cortical alveoli migrate to the periphery of the cell in preparation for receiving vitellogenin. Vitellogenin is a protein-based substance synthesized in the mother's liver that provides the necessary building blocks for egg yolk. Once vitellogenin begins to enter the oocytes, those oocytes are referred to as exogenous vitellogenic oocytes (Figure 3). The accumulation of vitellogenin causes a drastic increase in size of the oocytes. Vitellogenesis ends when the oocytes reach their full size and the oocyte begins final maturation. During this process, the nucleus resumes meiosis and migrates to the periphery of the oocyte. Once the migration of the nucleus (germinal vesicle migration) is complete, the nucleus breaks down (germinal vesicle breakdown) and the oocyte is hydrated, further increasing in size. Oocytes that have undergone these processes are referred to as final maturation oocytes (Figure 4). Final maturation oocytes are the largest oocytes and are considered ripe. After the oocytes are spawned a follicular envelope known as post-ovulatory follicles (POFs) are left behind (Wallace and Selman 1981, Lowerre-Barbieri *et al.* 2011, Wooten and Smith 2015). Figure 4 is a conceptual flow chart from Wooten and Smith (2015) illustrating the entire process.

Certain fish can recruit new oocytes throughout the spawning period and release eggs in more than one spawning bout. Consequently, these fish display indeterminate fecundity and are often referred to as multiple spawner fish (Rinchard and Kestemont 1996, Ganias *et al.* 2015). On

the other hand, there are fish that display determinate fecundity. This is when an entire stock of oocytes are prepared before the spawning season and are released in one spawning bout (Ganias *et al.* 2015). In the past, researchers believed alewife displayed determinate fecundity; however, recent research on anadromous alewife suggest they display intermediate fecundity (Norden 1967, Ganias *et al.* 2015). Ganias *et al.* (2015) found oocytes at different stages of maturity in anadromous alewife. In addition, they used a numeric model of oocyte growth to indicate batches of oocytes recruited at the beginning of the spawning season had enough time to develop and be spawned in the same season. The uncertainty in whether landlocked alewife in the Great Lakes have multiple spawner potential could help explain some of the variability in fecundity estimates seen in the literature and better understand their spawning behavior (Table 1) (Nigro and Ney 1982, Bronte *et al*. 1991). Therefore, it is important to determine if landlocked alewife from Lake Ontario present indeterminate fecundity.

As we mentioned before, it is also important to determine the age at which an individual first spawns, i.e., if age 2 alewife can reproduce. A study by Nigro and Ney (1982) found that southern alewife in Claytor Lake, VA, spawned as early as age 1 (160 mm). These female alewife were similar in total length to older alewife in northern lakes (ages 2 and 3), suggesting total length may be an important factor to age at maturity (Table 1). In Lake Superior, females were observed spawning as early as age 2 (140 mm). Although the proportion of spawning female increased with age, an entire cohort (age group) was not observed spawning until age 5 (Bronte *et al.* 1991). In the same study, males were spawning at age 1; however, the highest frequency of individual male spawning per age group was observed at age 3 (150 mm), and the proportion of spawning males declined at older ages (Bronte *et al.* 1991). Other research suggests that age at first spawning of alewife occurs between ages 2 and 3 (from 120 to 225 mm) (Norden 1967, Mullen *et al.* 1986,

Palkovacs *et al.* 2008). These lengths at maturity are similar to what is observed in Lake Ontario (Figure 5) (B. Weidel, USGS personal communication). Interestingly, Lake Ontario alewife at a given age appear to be larger – in body weight (g) - than they were in the past (Figure 6) (B. Weidel, USGS personal communication). Therefore, alewife may be able to spawn sooner (with respect to age) than they have in the past.

The effect of age on the reproductive potential of Lake Ontario alewife also needs to be examined. This is because assuming that many small, young, female fish have the same reproductive output as fewer larger, older females (when biomass is equal) can be considered a pitfall of fisheries management (Hixon et *al.* 2014). In a variety of species, Big Old Fat Fecund Female Fish (BOFFFFs) have displayed, greater relative fecundity (the number of eggs per gram of body weight), variation in the number of oocyte batches in multiple spawning fish, and greater offspring quality (size and or survival), than smaller younger fish (Hixon *et al.* 2014). Relative fecundity is important because unlike absolute fecundity (total number of ripe eggs in a female), relative fecundity corrects for body weight. Interestingly, relative fecundity and egg size has been shown to increase with age or size in Atlantic herring (*Clupea harengus*), and Pacific herring (*Clupea pallasi*), two species of the same family as alewife (Hixon *et al.* 2014). Therefore, determining reproductive potential among ages, i.e., if big old fat fecund female alewife have the same value as other BOFFFFs, is important in understanding alewife reproductive potential.

2. Objectives and Hypotheses

The objectives of this study were to provide information on the maturation and reproductive dynamics of Lake Ontario alewife by 1) determining if alewife display determinate or indeterminate fecundity, 2) determining if age 2 alewife could be considered part of the spawning stock, and 3) comparing and assessing reproductive potential across alewife ages 2 to 6.

To reach these objectives, gonadosomatic index and condition factor, gonad development, spawning potential, batch fecundity, and embryo survival data were collected and analyzed.

My hypotheses are as follows:

1. H_o: Alewife do not reproduce at age 2.

   H_a: Alewife do reproduce at age 2.

2. H_o: Females spawn all of their eggs in a single spawning event.

   H_a: Females can spawn multiple batches of eggs during their spawning season.

3. H_o: Alewife reproductive success is constant among age after maturity.

   H_a: Alewife reproductive success differs among age groups after maturity.

3. Materials and Methods

3.1. Alewife Collection

Alewife were collected throughout American waters of Lake Ontario (Figure 7) over a one-year period from October 2018 to October 2019 (October, April, May, June, July, and August) using bottom trawl, seining, and electrofishing methods (Table 2). Considering alewife is a pelagic species and only comes nearshore to spawn, nearshore samplings were conducted in May, June, July, and August by myself, and the members of Department of Environmental Science and Ecology at SUNY Brockport. In June and July, alewife were collected nearshore via electroshocking and seine nets with 0.5-1" mesh. The electroshocking boat was used in Bald Eagle Creek Marina, Kendall, NY, while seine nets were deployed in Hamlin Beach State Park in Hamlin, NY, and Ontario Beach Park in Rochester, NY. In all nearshore sampling scenarios, sampling occurred between 9:30 p.m. and 1:00 a.m. in order to target spawning individuals. Offshore bottom trawl sampling events were conducted at various depths by the USGS-LOBS and NYSDEC when alewife were in deeper water in October, April, and July. These trawls were

conducted in Rochester, Point Peninsula, Fairhaven, Oswego, and Southwick. I was fortunate enough to join the USGS-LOBS for bottom trawl sampling in October 2017 and 2018.

As length is often used as a preliminary indicator of age in fish (Chen and Paloheimo 1994), alewife of varying lengths were targeted in an attempt to collect data on individuals of different ages. In April, we were able to guide sampling efforts by using a length at age key provided by the USGS-LOBS (Table 3). In all other months, individuals of varying lengths were sampled.

After collection, alewife regardless of collection method were transported to Dr. Rinchard's lab at SUNY Brockport for processing. Fish from offshore bottom trawls were transported frozen or on ice, while alewife from nearshore sites were transported alive in a large cooler with supplemental aeration via bilge pump (SEAFLO, Xiamen, Fujian, China).

3.2. Fish Processing

Upon arrival in the lab, fish were weighed (g) and measured (total length, mm) using a light top loading balance scale (Mettler Toledo, Columbus, OH) and standard meter ruler (Swanson, Frankfort, IL), respectively. Gonads were excised and weighed using a precision balance scale (Mettler Toledo) and sex was recorded. For some individuals, if gonad development was not apparent, sex was later determined histologically (Figure 8). During the spawning season, if females were releasing eggs, all eggs were extracted by applying pressure to the abdomen; egg weight (g) was also taken using a light top loading balance scale (Mettler Toledo) and added to gonad weight to get the total gonad weight. If males were releasing milt, only minute amounts of milt were extracted by applying pressure to the abdomen and, therefore, no milt weight was recorded.

3.3. Gonadosomatic Index and Condition Factor

Gonadosomatic Index (GSI) was calculated to assess gonad development in relation to body weight for all individuals using the formula: $GSI\ (\%) = \frac{\text{gonad weight x 100}}{\text{body weight}}$ with gonad and body weight expressed in g. Condition factor was calculated to examine relative body condition of alewife using the formula: $K = \frac{\text{body weight x 100}}{\text{total length}^3}$ with body weight expressed in g and length in cm.

3.4. Histology Preparation

One gonad (per individual) was fixed in a 20-ml disposable scintillation vial (Thermo Fisher Scientific Inc., Waltham, MA) containing Bouin's solution (75 ml saturated aqueous solution of picric acid, 25 ml formalin, and 5 ml of glacial acetic acid). Bouin's solution is a common fixative for tissue preparation which also acts as a staining mordant. After 48 h, samples were placed in 70% ethanol. The 70% ethanol was replaced once a week for two to three weeks to remove excess Bouin's fixative from the samples. Next, the samples were placed in a tissue cassette (Thermo Fisher Scientific Inc.) and submerged in successive baths of increasing concentrations of ethanol and xylene, and three baths of paraffin wax to embed the sample; which allowed for the preservation of tissue over time (Table 4). The increasing concentration of ethanol was used to dehydrate the tissue over a gradient to avoid excessive shrinkage. The xylene acted as a clearing solvent to allow the paraffin wax to impregnate the tissue. The three paraffin baths were kept liquid in a laboratory oven (Thermo Fisher Scientific Inc.) at approximately 80°C. After embedding, samples were removed from the cassette, centered in a metal tray – with the labelled cassette back placed on top – and filled with liquid paraffin using a histo-embedder (Leica Biosystems Inc., Buffalo Grove, IL). Trays containing the sample and paraffin were then placed on an adjacent cold plate of the histo-embedder at -10°C for 15-45 min to solidify. After the

paraffin solidified, the solid blocks of paraffin containing the sample were removed from the metal tray.

Paraffin blocks containing the samples were chilled on ice - which allowed thinner sections to be obtained by providing support for harder elements within the tissue specimen - then sectioned with a microtome (Leica Biosystems Inc.). Select male testis were cut in order to identify sex and determine if age 2 males produced spermatozoa. Testis were cut at 6 µm. All ovaries were cut between 6-20 µm (larger oocytes required higher section thickness) to determine gonad development. Using tweezers, ribbons of sections were picked up and placed on the surface of the water in a water bath (Boekel Scientific, Feasterville, PA) at 50-60°C so they could flatten out. A 25x75x1 mm frosted slides (Thermo Fisher Scientific Inc.) were used to pick the sections out of the water. Slides were then set upright to dry in slide racks (Thermo Fisher Scientific Inc.). Slides were stained once dry or within 72 h of sectioning. Before proceeding with the staining protocol, the slides were deparaffinized and rehydrated - incomplete removal of paraffin can cause poor staining of the section. The slide racks were places in three-consecutive xylene, ethanol, and water baths. Once done, slides racks were moved into hematoxylin and eosin stain baths (Humason 1979). Hematoxylin stained the nucleus blue-purple and eosin stained the cytoplasm and vitellogenin red-pink. For hematoxylin, tap water was used post-staining to allow the stain to develop followed by a brief acid-ethanol dip to prevent over-staining. To make the stain permanent the tissue was then dehydrated with ethanol and cleared with xylene (Table 5). When complete, cover glass slips (Thermo Fisher Scientific Inc.) with a drop of Permount Mounting Medium (Thermo Fisher Scientific Inc.) were laid on top the stained specimen. This product set a thin, adhesive layer that effectively cemented the cover glass to the slide. In addition, it formed an airtight barrier that preserved the staining quality and maintains the optical qualities of the

specimen. After drying overnight, slides were ready to be examined using a compound microscope at 40 to 100x magnification (Motic, Kowloon Bay, Kowloon).

3.5. Histology Analysis

Each ovary was classified according to the most advanced stage of oocytes present based on the criteria adapted from Rinchard and Kestemont (1996) and Wallace and Selman (1981) (Table 6). Ovarian development was examined by a histomorphometric analysis modified from Rinchard and Kestemont (1996). Two parameters were examined: (1) the distribution of oocyte size, assessed by measuring 20 diameters of each oocyte stage present in the ovary and (2) the relative proportion (%) of each stage, i.e., by counting 100 to 200 oocytes per ovary and then dividing the percentage of a given stage by the corresponding mean diameter. Only spherical oocytes which had been sectioned through the nucleus were measured. To measure the oocytes, five to fifteen pictures of different areas within each ovary – with caution to ensure each picture represented different oocytes – were taken with a digital microscope imager (Celestron Inc., Torrance, CA) and its respective software. Then, the diameter of 20 oocytes of each oocyte stage present in the ovary were measured (µm) using ImageJ image processing program. The frequency of oocyte measurements in 50 µm intervals was calculated for each oocyte stage. To count oocytes, slides were projected onto white paper fixed to flat surface using a micro projector (Bausch & Lomb, Rochester, NY) under "high" magnification. Oocytes (regardless of stage) were identified and the number of oocytes in each stage recorded.

3.6. Spawning Potential

Spawning potential for the given spawning season was assessed for individuals captured in June and July. Female fish were considered able to spawn if (1) eggs were released when pressure was applied to the abdomen upon capture and/or (2) class 3 (or more advanced) ovaries

were present upon histological examination. Male fish were considered able to spawn if (1) milt was released when pressure was applied to the abdomen, or (2) spermatozoa was present in testes during histological examination. Spawning potential of alewife was calculated as:

$$spawning\ potential\ (\%) = \frac{(number\ of\ fish\ displaying\ spawning\ potential \times 100)}{total\ number\ of\ fishes\ samples\ in\ June\ and\ July}.$$

3.7. Batch Fecundity

Absolute batch and relative batch fecundity of alewife were calculated using the gravimetric method – the relation between ovary weight and oocyte density in the ovary (Muchlisin 2014). Absolute batch fecundity illustrated the numbers of eggs that would be spawned in a single spawning bout while relative batch fecundity illustrated the number of eggs spawned in a single spawning bout per gram of bodyweight. Depending on the individual, 1) loose eggs or 2) a sub-sample of the remaining ovary (the ovary not used in histology analysis) was weighed and placed in Gilson's fluid (100 ml 60% ethanol; ,880 ml water,15 ml 80% nitric acid, 18 ml glacial acetic acid, and 20 g mercuric chloride) to preserve the eggs and degrade ovarian tissue (Klibansky and Juanes 2007). This allowed the eggs to be released from the tissue, so they could be manipulated and counted manually under a dissecting microscope (Leica Biosystems Inc.). For fish that spawned, all oocytes sampled were counted; while only the largest oocytes (considered the batch that would be spawned) were counted in ovarian samples. Absolute batch fecundity was calculated as: $absolute\ batch\ fecundity = \frac{oocyte\ count \times total\ weight\ of\ the\ gonad}{subsample\ of\ gonad\ weight\ (eggs\ or\ ovary)}$; relative batch fecundity was calculated as: $relative\ batch\ fecundity\ = \frac{absolute\ batch\ fecundity}{weight\ (g) of\ the\ fish}$.

3.8. Artificial Reproduction and Embryo Survival

To evaluate the viability of alewife eggs, artificial reproduction was conducted, and percent embryo survival was calculated. Eggs were stripped from all females releasing eggs and divided

into sub-samples based on the number of available males. Again, total length was used as a precursor for age for both male and female fish and age was confirmed later using otoliths (Table 3). Each combination of eggs and milt was considered a cross (Figure 9). For each cross, sperm was added to the eggs and mixed with de-chlorinated municipal water from the lab to activate sperm and allow fertilization to take place. After one minute, the eggs were rinsed to remove excess sperm and debris. Eggs were then immediately moved into corresponding labeled baskets, comprised of PVC piping and a mesh bottom, then placed in a 4-tray vertical incubator (MariSource, Fife, WA). De-chlorinated municipal water was run on a flow through system while eggs incubated. Eggs were incubated for 48-72h so embryos could develop to the pigmented eyed stage, which is characterized by the appearance of pigments in the eyes of the embryo (Figure 10). After incubation, eggs were moved into modified 60 x 15 mm petri dishes and examined under a dissecting scope at 10-40x magnification. Petri dishes were modified with PVC piping and rubber cement so that all eggs were contained within the field of magnification to ensure all eggs were accounted for. Pictures of the modified petri dishes (containing all embryo) were taken using the digital microscope imager and its respective software. Total eggs and embryos at pigmented eyed stage were counted from the pictures taken; $\text{embryo survival rate (\%)} = \frac{\text{\# alive embryos x 100}}{\text{\# total embryos}}$.

3.9. Age

The sagittae otoliths were removed from all fish for aging. Once removed, otoliths were placed into 1.5 ml microcentrifuge tubes (Thermo Fisher Scientific Inc.) and sent to the USGS-LOBS. Once there, the microcentrifuge tubes containing the otoliths were opened and placed in their lab oven (Thermo Fisher Scientific Inc.) overnight at roughly 60°C, to dry the samples. When dried, the microcentrifuge tubes were closed so no otoliths were lost. Custom made multi-well silicone mounting trays with 50 depressions were prepared by labeling every 5 depressions with

the corresponding individual ID. Then, under a dissecting scope (Olympus Corp., Shinjuku, Tokyo, Japan) at 10-40x magnification with reflected light, otoliths were moved using forceps and/or fine tipped probes into their corresponding depression in the mounting tray. Otoliths were manipulated so the sulcus, or grooved/concaved side, was facing down then each otolith was moved to the edge of the depression. Once in position, a small drop of Cytoseal-60 (Thermo Fisher Scientific Inc.) was added to the middle of the depression and otoliths were moved in contact with the Cytoseal-60 (Thermo Fisher Scientific Inc.) to hold them in place. This was done with each pair of otoliths until multiple trays were completed. Next, otoliths were cleaned (cautiously, without scratching or otherwise damaging the otolith) using forceps and fine tipped probes. This made the annuli – growth rings – easier to see when aging. Once all otoliths were cleaned, enough Cytoseal-60 (Thermo Fisher Scientific Inc.) was added to completely cover the depression, the trays were set to dry overnight.

The following day, otoliths were interpreted for age. Otoliths were examined with the Olympus dissecting scope with reflected light under 10 to 40x magnification (with the option of a 1, 1.25, or 1.6x multiplier). Annuli were counted from the focus – center of otolith – to the edge. In alewife collected during the spring, the edge of the otolith was counted as an annulus because spring is the start of the growing season; therefore, we presumed that the new annulus had just begun. Interpreted age was recorded as the number of annuli counted. In order to control quality, at least two individuals from the USGS-LOBS interpreted each pair of otoliths and final otolith age was agreed upon by all individuals involved in the interpretation.

3.10. Statistical Analyses

Statistical analyses on GSI, condition factor, and absolute batch and relative batch fecundity data were performed using IBM SPSS Statistics 25. Before the analyses, the assumptions

for parametric tests were evaluated. Normality of data was assessed using a combination of histograms and QQ-plots (to examine normal distribution), and the Shapiro-Wilks and Kolmogorov-Smirnov statistical tests. Homogeneity of the variance were tested using Levene's test. In this study, the Mann-Whitney U test and the Kruskal-Wallis test was used to compare means when data failed to meet the assumptions of parametric test. For all non-parametric analyses, the data failed to meet the assumption of a normal distribution. When assumptions of the parametric tests were met, an independent t-test or one-way ANOVA with post-hoc tests was used to compare two or more means, respectively.

For male alewife, a one-way ANOVA and Tamhane post-hoc test (equal variances not assumed) were used to test the effect of age on GSI and examine pairwise comparisons. A Kruskal-Wallis and Mann-Whitney U test were used to test GSI data of female alewife. For male alewife, ages 1 (n = 0), 4 (n = 1), and 7 (n = 2) were not included in this part of the analysis due to their low sample sizes. For female alewife, ages 4 (n = 1) and 7 (n = 1) were removed from the analysis due to their low sample sizes. When analyzing GSI data from October 2017 and October 2018, an independent t-test was used to compare average GSI of all fish (male and female) between months. The Kruskal-Wallis and Mann-Whitney U tests were also used to examine condition factor throughout the year by comparing average condition factor of male and female alewife among months.

Absolute batch and relative batch fecundity data were analyzed by comparing ages 2, 3, and 6 using a one-way ANOVA and Kruskal-Wallis respectively. There was one age 7 individual that was removed from both analyses due to the sample size.

Embryo survival data was analyzed in R Core Team (2013). A beta regression model was used to determine if the observed variability in embryo survival was explained by female and or

male age. The beta regression was chosen considering 1) the proportion data did not have constant numerator and denominators, and 2) the data was not normally distributed and was suggested appropriate based on the describe distribution function "descdist" in the fitdistrplus package (Delignette-Muller and Dutang 2015). After removing zero values (n = 11), the "fitdistrplus" package in R confirmed the data followed a beta distribution and therefore the beta regression was appropriate. The beta regression model set included models with explanatory factors: female age, male age, female and male age, and interaction between the two, and a null model. Akaike information criterion (AIC) was used to determine differences in the model fits.

4. Results

The GSI of both male and female alewife remained low, at or below 2%, from October 2017 to April 2017 and then increased in June and July (Table 7; Figure 11). Male GSI increased gradually from June to July, while female GSI increased rapidly in July. During July, both male and female GSI reached their peak. However, females had significantly higher GSI than males (8.5 ± 2.1% vs. 4.1 ± 1.5%, Mann-Whitney, U = 351.5, n = 133, P = 0.000) (Figure 11). After the spawning season, in October 2018, GSI of males and females dropped to below 2%. Average GSI of all fish in October 2018 was not significantly different than October 2017 (0.9 ± 0.04 vs. 0.9 ± 0.1%, independent t-test, t = -0.218, df = 108, P = 0.828).

The influence of age on GSI was examined in males and females collected in the same location throughout July. Age was a significant factor in GSI of male alewife in July (ANOVA, F = 4.502, n = 44, df = 3, P = 0.008). However, the Tamhane post-hoc test did not show any significant differences in pairwise comparisons (Table 8; Figure 12). Age did not significantly affect female GSI in July (Kruskal-Wallis, H = 6.9, n = 79, df = 3, P = 0.074). Age 5 females had

the highest GSI (8.65 ± 1.5%), while age 6 females had the lowest GSI (6.22 ± 2.3%) (Table 9; Figure 13).

The average condition factor of male and female alewife throughout this study was 0.7 ± 0.03 and 0.7 ± 0.01, respectively. Male condition factor ranged from 0.8 ± 0.03 in October 17 to 0.6 ± 0.01 in July 2018 and varied significantly throughout the sampling period (Kruskal-Wallis, H= 31.4, n = 168, df = 4, P = 0.000) (Table 10). Female condition factor ranged from 0.8 ± 0.01 in October 2017 to 0.6 ± 0.01 in June 2018 and was significantly different among all months except between April and July (Kruskal-Wallis, H = 71.7, n = 255, df = 4, P = 0.000) (Table 11).

4.1. Gonad Development in Females

4.1.1. General

Changes observed in female GSI corresponded with the development of their ovaries. Females with previtellogenic and endogenous vitellogenesis ovaries displayed GSI at or below 2%, while females with advanced ovaries (exogenous vitellogenesis, final maturation, and intermediate multiple spawner) displayed higher GSI (Table 12). The percent frequency of ovarian classes in females (regardless of age) throughout this study is illustrated in Figure 14.

In October 2017, alewife presented either previtellogenic (75%) or endogenous vitellogenesis ovaries (25%). Females with previtellogenic ovaries contained only previtellogenic oocytes averaging 72.96 ± 15.19 µm, while females with endogenous vitellogenesis ovaries contained both previtellogenic and endogenous vitellogenic oocytes. Previtellogenic oocytes were identified by the centrally located nucleus (or germinal vesicle) and averaged 71.23 ± 4.45 µm. Endogenous vitellogenic oocytes were larger than previtellogenic oocytes and identified by the presence of cortical alveoli (or yolk nuclei) and averaged 143.54 ± 4.07 µm (Table 12).

The ovaries of the females collected in April 2018 were still at either the previtellogenic stage (38.3%) or at the endogenous vitellogenesis stage (61.7%). The increase of females with endogenous vitellogenesis ovaries coincided with GSI increase and a surge in the size of their endogenous vitellogenic oocytes. Average diameter of previtellogenic oocytes ranged from 87.50 ± 22.80 µm (previtellogenic ovaries) to 90.32 ± 13.76 µm (endogenous vitellogenesis ovaries). The size of the endogenous vitellogenic oocytes in the endogenous vitellogenesis ovaries reached 213.84 ± 29.59 µm, the largest size for this stage of oocytes observed in this study (Table 12).

In June 2017, females presented either previtellogenic (41.2%), endogenous vitellogenesis (35.3%), or exogenous vitellogenesis (23.5%) ovaries. Fish with exogenous vitellogenesis ovaries presented three batches of oocytes. Within exogenous vitellogenesis ovaries only a small proportion (3.95 ± 1.38%) of oocytes moved into the exogenous vitellogenic stage, while most oocytes in the ovary were either in the previtellogenic (83.75 ± 5.55%) or endogenous vitellogenic stage (12.30 ± 4.26%). Exogenous vitellogenic oocytes observed in the exogenous vitellogenesis ovaries were characterized by the accumulation of vitellogenin and a dramatic increase in size (350.65 ± 36.75 µm) (Table 12). The presence of multiple batches of oocytes within exogenous vitellogenesis ovaries showed females were developing a batch of oocytes to be spawned (alongside less advanced oocytes). This coincided with an increase in monthly GSI. In the same month, average size of previtellogenic oocytes among ovarian stages ranged from 79.94 ± 6.99 µm (previtellogenic ovaries) to 83.64 ± 12.75 µm (endogenous vitellogenesis ovaries) and endogenous vitellogenic oocytes ranged from 203.55 ± 14.69 µm (exogenous vitellogenesis ovaries) to 204.34 ± 60.94 µm (endogenous vitellogenesis ovaries).

Females were at different stages of maturity in July. Alewife displayed endogenous vitellogenesis (2.7%), exogenous vitellogenesis (66.7%), final maturation (6.7%), and

intermediate multiple spawner (24%) ovaries. In all ovaries examined, exogenous vitellogenic oocytes and final maturation oocytes were never observed in the same ovary at the same time. Final maturation ovaries indicated fish were spawning in July. In addition, the presence of intermediate multiple spawner ovaries indicated that some fish likely spawned a batch of oocytes in June, and at least one more batch was being recruited for another spawning event. Multiple batches of oocytes were observed in all advanced ovaries (exogenous vitellogenesis, final maturation, and intermediate multiple spawner ovaries). Like the previous month, only a small portion of oocytes matured inti; the advanced stages. In exogenous vitellogenesis ovaries, 7.82 ± 3.50% of oocytes present were in the exogenous vitellogenic stage while the rest were in the endogenous vitellogenic (12.36 ± 4.99%) and previtellogenic stages (79.82 ± 7.39%). Final maturation ovaries contained 6.34 ± 2.13% of final maturation oocytes alongside endogenous vitellogenic (10.44 ± 2.38%) and previtellogenic (83.10 ± 2.80%) oocytes. After the accumulation of reserve vitellogenin during exogenous vitellogenesis, oocytes averaged 489.83 ± 27.61 µm when entering final maturation. Final maturation was classified by germinal vesicle migration and breakdown, and a slight increase in size due to hydration. Lastly, intermediate spawner ovaries contained POFs as well as 1.85 ± 1.45% of exogenous vitellogenic oocytes alongside endogenous (10.60 ± 5.31%) and previtellogenic (87.66 ± 5.24%) oocytes (Table 12).

Like October 2017, October 2018 ovaries were either previtellogenic (70.4%) or endogenous vitellogenesis (29.6%). Average diameter of previtellogenic oocytes between ovarian classes ranged from 73.53 ± 9.17 µm (endogenous vitellogenesis ovaries) to 78.98 ± 9.98 µm (previtellogenic ovaries) µm and endogenous vitellogenic oocytes averaged 168.04 ± 18.24 µm (endogenous vitellogenesis ovaries).

4.1.2. Gonad Development and Age

All age groups displayed multiple batches of oocytes in advanced ovaries (exogenous vitellogenesis, final maturation, and or intermediate multiple spawner) throughout the year (Figure 15). Age 1 fish displayed previtellogenic, endogenous vitellogenesis, exogenous vitellogenesis ovaries. The exogenous vitellogenesis ovaries were observed in individuals with an average total length (TL) of 133.00 ± 2.83 mm, which were 22.2% (n = 2) of all age 1 individuals sampled (n = 9) (Table 13).

Previtellogenic, endogenous vitellogenesis, exogenous vitellogenesis, final maturation, and intermediate multiple spawner ovaries were observed in age 2 fish. Average TL of age 2 individuals based on ovarian class ranged from 138.00 ± 13.90 to 161.00 ± 11.31 mm. Exogenous vitellogenesis, final maturation, and intermediate multiple spawner ovaries were observed in 48% n = 32), 3% (n = 2), and 7% (n = 5) of all Age 2 individuals (n = 67), respectively. The average total length of these individuals increased with ovarian development: 145.06 ± 11.43 mm (exogenous vitellogenesis), 149.00 ± 15.56 mm (final maturation), and 161.00 ± 11.31 mm (intermediate multiple spawner) (Table 13, Figure 16).

Again, previtellogenic, endogenous vitellogenesis, exogenous vitellogenesis, final maturation, and intermediate multiple spawner ovaries were observed in age 3 fish. Average TL of age 3 individuals based on ovarian class ranged from 160.71 ± 7.83 to 174.00 mm. Exogenous vitellogenesis, final maturation, and intermediate multiple spawner ovaries were observed in 33% (n = 10), 3% (n = 1), and 3% (n = 1) of all age 3 individuals (n = 30) respectively. Again, the average total length of these individuals increased with ovarian development: 164.60 ± 10.50 (exogenous vitellogenesis), 168.00 (final maturation), and 174.00 mm (intermediate multiple spawner) (Table 13, Figure 16).

No advanced ovaries (exogenous vitellogenesis, final maturation, or intermediate multiple spawner) were observed in age 4 individuals (n = 6). In 67% of age 4 individuals, alewife averaged 184.75 ± 7.50 mm and displayed previtellogenic ovaries, while 33% that averaged 183.00 ± 7.07 mm displayed endogenous vitellogenesis ovaries.

Age 5 individuals displayed previtellogenic, endogenous vitellogenesis, exogenous vitellogenesis, and intermediate multiple spawner ovaries. Average TL of age 4 individuals based on ovarian class ranged from 176.60 ± 6.58 to 182.20 ± 5.26 mm. Exogenous vitellogenesis and intermediate multiple spawner ovaries were observed in 22% (n = 4) and 22% (n = 4) of all age 4 individuals (n = 18) respectively. The average total lengths of these individuals were 176.75 ± 8.85 mm (exogenous vitellogenesis) and 180.00 ± 8.04 mm (intermediate multiple spawner) (Table 13, Figure 16).

Like age 2 and 3 fish, age 6 fish displayed previtellogenic, endogenous vitellogenesis, exogenous vitellogenesis, final maturation, and intermediate multiple spawner ovaries. Average TL of age 6 individuals based on ovarian class ranged from 181.45 ± 8.04 to 195.00 mm. Exogenous vitellogenesis, final maturation, and intermediate multiple spawner ovaries were observed in 17% (n = 6), 6% (n = 2), and 19% (n = 7) of all age 6 individuals (n = 36) respectively. The average total length of these individuals did not have a clear trend with ovarian development: 188.50 ± 9.95 mm (exogenous vitellogenesis), 178.00 ± 8.49 mm (final maturation), and 187.00 ± 7 mm (intermediate multiple spawner) (Table 13, Figure 16).

Lastly, a single age 7 individual with a TL of 195.00 mm displayed an endogenous vitellogenesis ovary.

Interestingly, age 2 alewife did not display endogenous vitellogenesis ovaries until April 2019 and exogenous vitellogenesis ovaries until July 2019. Age 3 and older individuals displayed

these ovaries in October 2017 and June 2018, respectively. In addition, in each month, older individuals displayed a higher frequency of the most advanced ovarian stage present. Most importantly, 12.5 % of age 2 individuals (Figure 16) displayed intermediate multiple spawner ovaries in July, while 38.7% of older individuals displayed intermediate spawner ovaries (Figure 17).

4.2. Spawning Potential

Of all fish examined for spawning potential - through field observations of individuals spawning and histological analyses of gonad development - in June and July 2018, 77.4% of males and 58.3% of females displayed spawning potential (Table 14). Both age 2 males (90.4%) and females (63.9%) displayed the highest frequency of spawning potential (excluding the single age 7 female that displayed spawning potential); interestingly, no spawning potential was observed in age 1 males while 50% of age 1 females displayed spawning potential (Tables 15 and 16). More specifically, field observations indicated males were spermiating as early as June 2018. 76% of all males captured in June (n = 129) were spermiating while 88% of all males captured in July (n = 68) were spermiating. Only one female was observed ovulating in June 2018 while all other ovulating females (n = 13) were captured in July 2018.

4.3. Batch Fecundity and Embryo Survival

Fecundity data were collected from females ages 2 to 7. Absolute batch fecundity of alewife sampled ranged from 3,764 to 10,112 eggs and averaged 7,231 ± 497 eggs among all ages. Age 2 fish displayed the lowest average absolute batch fecundity (6,100 ± 2,146), while the age 7 individual displayed the highest (9,082 eggs) (Table 17, Figure 18). There was no significant difference in absolute batch fecundity among age groups 2, 3, and 6 (ANOVA, $F = 0.810$, $n = 17$, $df = 1$, $P = 0.381$).

Relative batch fecundity (number of eggs/g of fish) ranged from 161 to 487 eggs/g and averaged 254 eggs/g among all ages. The age 7 individuals displayed the lowest relative batch fecundity (186 eggs/g), while age 2 fish displayed the highest average relative batch fecundity (271 ± 103 eggs/g) (Table 17, Figure 18). There was no significant difference in relative batch fecundity among age groups 2, 3, and 6 (Kruskal-Wallis, H = 0.345, n = 17, df = 2, P = 0.842).

Among crosses of different aged male and female fish, the age 6 female-age 2 male cross had the highest embryo survival rate (26 ± 22%), while the age 5 female- age 6 males had the embryo survival rate (0%) (Table 18). When examining embryo survival only based on female age, age 6 individuals displayed the highest average embryo survival rate (23 ± 20%) and age 5 individuals displayed the lowest (3 ± 5%) (Table 19). Of the explanatory factors (female age, male age, female and male age, and interaction between the two, and a null model) examined in our model, the only modeling scenario with an AIC lower than our null was the model including only female age (Table 20). The R^2 value of the female age model was 0.19766 ± 0.08663.

5. Discussion

5.1. General Gonad Development and Multiple Spawner Potential

GSI of alewife was low throughout most of the year but peaked, along with gonad development, just before nearshore water temperatures in Lake Ontario – measured in Rochester, NY – reached their peak in August. This suggest temperature influenced gonad development (Figures 11 and 20). The increase in GSI and field observations of male and female alewife spermiating and ovulating indicated the spawning season occurred in June and July. In addition, histological analyses revealed advanced ovaries in these months. More specifically, the presence of the intermediate multiple spawner ovaries in July suggested those individuals could have spawned in June. The link between gonad development and water temperature has been observed

in other alewife populations and Atlantic herring – another Clupeidae and close relative to the alewife (Nigro and Ney 1982, Ma *et al.* 1998, Ganias *et al.* 2015).

While the gonad development of both male and female alewife appears to be related to water temperature, sex specific GSI trends differed. Female alewife displayed a higher GSI than males through most of the year; which was expected (Figure 11). Teleost females often have a higher tissue and energy demand for gonad development (Wallace and Selman 1981, Parker *et al.* 2017). For example, significantly higher energy content per unit mass of gonad was identified in female black eye goby (13% higher per mass unit) (*Rhinogobiops nicholsii*) and Atlantic Salmon (47% higher per unit mass) (Parker *et al*. 2017). In addition, female alewife appeared to be able to remain in non-advanced ovarian stages such as endogenous vitellogenesis for a long period of time and develop into advanced ovaries rapidly. This coincided with the rapid increase and peak in GSI observed in July (Table 12, Figures 10, 13, and 14 A-E). Rapid maturation in females has been observed in other alewife populations as well (Ganias *et al.* 2015). Although it was earlier than July (April 25–May 17, 2012), a rapid increase of female GSI was also observed in an anadromous population (Newmarket, New Hampshire); where GSI varied from 4.19 to 16.22% over a 5-week period (Sullivan *et al.* 2019). The rapid increase in maturation was not observed in males. However, in June 2018, males displayed a higher GSI and proportion of spawning potential between sexes suggesting males were ready to spawn before females (within the season) (Figure 11). Male fish maturing before female fish within the season has also been observed in Atlantic Herring (Ma *et al.* 1998).

In addition to providing insight on trends in gonad development, these data shed light on the multiple spawner potential of alewife in Lake Ontario. The high and variable GSI of both male and female fish through four separate sampling events in July and the presence of spermiating

males in all 4 sampling events and spawning females in 3 sampling events illustrated a prolonged spawning season. This was further supported by the presence of intermediate spawner ovaries in July 2018. These findings, in conjunction with multiple batches of oocytes developing in advanced ovaries show that alewife display indeterminate fecundity (i.e., have multiple spawner potential) (Wallace and Selman 1981, Rinchard and Kestemont 1996, Ganias *et al*. 2015). These findings were similar to the Connecticut population that was deemed to have multiple spawner potential in that individuals from the Connecticut population also displayed a prolonged spawning season (uprunners collected as early April 6 2006, and as late as May 30th) and advanced oocyte growth was observed in ovaries containing POFs during the spawning season (Ganias *et al*. 2015).

5.2. Age at First Spawning

Based on observations of spermiation in the field, embryo survival data, and histological analyses, it appeared male fish spawned at age 2 (Tables 16 and 18). With that said, only 2 age 1 males were captured in June and July of 2018, so this is likely not a good representation of their spawning potential. The presence of exogenous vitellogenesis ovaries suggested female alewife displayed spawning potential as early as age 1. However, no age 1 females were observed spawning in the field or involved in the embryo survival or batch fecundity portion of this study. On the other hand, the presence of all stages of ovarian development, an increase in GSI during the spawning season, fecundity estimates, and successful embryo survival, of age 2 alewife indicated age 2 alewife were part of the spawning stock (Table 13, Figures 14A, 15, 17, and 19). Similar results were observed in landlocked alewife of the Great Lakes and beyond; alewife in Cayuga Lake, New York, Lake Michigan, Michigan, and Claytor Lake, Virginia were observed to spawn as early as age 2 (Table 1) (Rothschild *et al.* 1966, Norden *et al.* 1967, Nigro and Ney 1982). It is also worth noting age 1 (133.00 ± 2.83 mm) and age 2 (147.44 ± 12.63 mm) alewife

that displayed spawning potential in this study were greater in total length than age 2 (120 mm) and age 3 (127 mm) alewife that were observed spawning in Cayuga Lake, New York. This further supports the previously mentioned theory that there may be a size threshold related to first spawning instead of an age threshold.

5.3. Age Effect on Reproductive Potential

Some of our results suggest that age is an important factor in reproductive potential of Lake Ontario, while others do not. In male fish, age was identified as a significant factor in GSI and age 2 individuals displayed the highest GSI of the sampled ages (Table 8). In addition, the frequency of spawning potential was greatest in age 2 males (90.4%) (Table 16). Thus, it appeared younger males may invest more in gonad development and spawn at a higher frequency relative to older alewife. With that said, statistical analysis could not identify significant difference in pairwise comparisons of male GSI. This was likely due to the sample size of age 3 (n = 6) and 4 (n = 9) fish reducing the power of our tests. The small sample size of older fish means we should also use caution when interpreting spawning potential data.

For female fish, a similar trend was observed in spawning potential where age 2 fish displayed the highest frequency of spawning potential (63.9%) (excluding the single age 7 individual). It is worth noting the differences in spawning potential frequency among females ages 2, 5, and 6 was less than 5% (Table 15). With that said, the spawning potential frequency data may have been misleading. These data only illustrate June and July. Additionally, we may have missed some older alewife during our sampling efforts in June and July. In some other teleosts - like the plaice (*Pleuronectes platessa*) – larger older females will spawn in different locations than smaller younger fish (Hixon *et al.* 2014). Therefore, the ovarian frequency data – which examines the whole year – appears more robust. Interestingly, these data suggest a reproductive edge to older

alewife. A higher frequency of older alewife were likely spawning based on the fact that alewife older than age 2 have a higher frequency of more advanced stages of development throughout the year (Figures 15 and 16). Additionally, the higher frequency of intermediate multiple spawner ovaries in older alewife (38.7%) compared to that of age 2 alewife (12.5%) suggest 1) not all alewife display indeterminate fecundity and 2) of individuals that do, older alewife do so at a higher frequency (Figures 15 and 16). These results coincide with the literature that within multiple spawner fish like drum (*Aplodinotus grunniens*), anchovy (*Engraulidae*), striped bass, and other teleosts, larger older females may produce more batches of eggs over a longer period each season (Hixon *et al.* 2014). The model used to assess embryo survival further suggest alewife have an additional reproductive edge over younger alewife (Table 20). Also, when looking at female age alone, embryo survival was highest in crosses with age 6 females (23 ± 20%) (Table 19). The combination of these results would suggest that older alewife offer higher rates of embryo survival and a higher frequency of older individuals are multiple spawner fish when compared to younger individuals. However, due to the low associated R^2 value, our model does not explain the variability in embryo survival very well. A higher R^2 may have been achieved with a larger sample size, less sporadic embryo survival data, and or additional variables. The sporadic embryo survival results may have resulted from handling stress in the laboratory. It was also challenging to find an egg basket that would provide adequate oxygenation without the eggs being evacuated from the basket. Examining other variables (e.g., male and female size, male and female stress, tank effect, and date) could have identified a factor with more explanatory power. Based on the literature, size would likely be an important factor. Sometimes, age appears to be a significant factor in the reproductive potential of teleost's because reproductive potential generally increases with female age simply as a function of body size (Hixon *et al.* 2014). Ultimately, our results suggest older fish

produce progeny with higher embryo survival. It is also worth noting, no effect of age on female reproductive potential was observed in GSI, or fecundity data. Although there were no significant differences in fecundity data, batch fecundity increased with age (Table 17, Figure 19). However, once we corrected for size by calculating relative fecundity, young alewife displayed the highest relative fecundity and the variation among groups was greatly reduced (Table 17, Figure 19). Again, this suggests there may be other factors, like size, that are more important than age.

5.4. Batch and Absolute Fecundity

Considering Lake Ontario alewife are multiple spawner fish, their batch fecundity does not represent all eggs that could be spawned in a given season (i.e., absolute fecundity). Using findings from the research performed by Ganias *et al.* (2015), we can estimate (with some assumptions) the absolute fecundity of Lake Ontario alewife. Ganias *et al.* (2015) suggested alewife can spawn at least 3 batches of oocytes in a season. Therefore, assuming landlocked alewife are able to spawn the same number of batches as anadromous alewife, and batch fecundity is constant throughout the season, absolute fecundity of Lake Ontario alewife would range from 11,290 to 30,335. Up to 3 batches of oocytes were observed in Lake Ontario alewife at a time, so it is plausible all 3 batches could be spawned. However, one must still use caution interpreting this estimate as batch fecundity is not always constant throughout the season. In some teleost species, like the Atlantic Silverside (*Menidia menidia*), batch fecundity varies significantly throughout the spawning season in that batch fecundity in the beginning of the season is lowest and peak batch fecundity is reached in the middle of the season (Conover 1984). Regardless, as previously discussed, the multiple spawner potential of alewife has a profound effect on absolute fecundity and therefore likely explains some of the extreme variability observed in Table 1.

5.5. Conclusion

This study was the first in recent history that examined the reproductive and maturation dynamics of Lake Ontario alewife. We learned, with some caveats, that Lake Ontario alewife 1) can reproduce at age 2, 2) can spawn multiple times in a single spawning season, and 3) may have variability in reproductive success. It appeared relative fecundity did not change among ages but embryo survival and the proportion of multiple spawner individuals increased with age. With that said, based on the literature, this variation may not be based on age alone. Examining variations in multiple spawner potential, batch fecundity, and embryo survival, among both female size and age would help us better understand which factor is more important in the reproductive potential of Lake Ontario alewife.

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Tables

Table 1. Characteristics of sexually mature female alewife in inland water adapted from Nigro and Ney (1982) and supplemented with data from Bronte (1991). Median (Claytor Lake) or mean (Cayuga Lake, Lake Superior, and Lake Michigan) values reported with ranges in parentheses.

Location	Age Class	Total length (mm)	Total Eggs/Fish	Gonad Weight (g)	Eggs/g ovary
Claytor Lake, Virginia			17,300	4.1	4,220
(Nigro & Ney 1982)	1	160	(13,200 - 24,000)	(3.6 - 5.8)	(3,900 - 4,420)
			35,400	7.9	4,480
	2	216	(27,300 - 49,200)	(6.7 - 8.4)	(4,180 - 4,710)
			39,100	8.9	4,400
	3	225	(31,700 - 49,000)	(7.6 - 10.1)	(4,010 - 4,540)
Seneca Lake, New York			-		
(Odell *et al.* 1934)	3	145	(10,000 - 12,000)		
Cayuga Lake, New York			8,800		
(Rothschild *et al.* 1966)	2	120	(7,800 - 9,000)		
			8,000		
	3	127	(5,800 - 10,000)		
Lake Michigan					
(Norden *et al.* 1967)	2	160	11,147		3,380
	3	176	16,100	4.4	3,670
	4	192	22,400	6.6	3,400
Lake Superior					
(Bronte *et al.* 1991)	2-5	187	63,559 ± 1,624		

Table 2. Number of total alewife collected at each location throughout this study.

Location	Oct '17	Apr '17	Jul '17	Jun '17	Aug '17	Oct '18
Rochester (Offshore)	25	165				49
Bald Eagle Creek						
Marina			137	34		
Charity			15			
Rochester (Nearshore)			15	105		
Hamlin			4	20		
Oswego			32			
Point Peninsula			48			
Southwick			5			
Fairhaven						67

Table 3. Age estimation of alewife in April at given total length in millimeters estimated by USGS using otolith and length frequency data (B. Weidel, USGS personal communication).

Age	Size Class (mm)
1	50-105
2	106-145
3	146-155
3-4	156-165
4	166-175
4-5	176-180
5+	181+

Table 4. Embedding procedure used in this study.

Step	Bath	Time (h)
1	80% ethanol	1
2	90% ethanol	1
3	100% ethanol	1
4	100% ethanol	1
5	100% xylene	1
6	100% xylene	1
7	100% xylene	1
8	Paraffin wax	Overnight (12-16)
9	Paraffin wax	1
10	Paraffin wax	1

Table 5. Hematoxylin and Eosin staining procedure used in this study.

Step	Bath	Time (min.)
1	100% xylene	3
2	100% xylene	3
3	100% xylene	3
4	100% ethanol	3
5	100% ethanol	3
6	100% ethanol	3
7	95% ethanol	3
8	80% ethanol	3
9	Deionized water	5
10	Hematoxylin	3
	Rinse with deionized water	
11	Tap water	5
12	Acid ethanol	Dip 8-12x
13	Tap water	1
14	Tap water	1
15	Deionized water	2
	Blot excess water from slide holder before eosin	
16	Eosin	0.5
17	95% ethanol	5
18	95% ethanol	5
19	95% ethanol	5
20	100% ethanol	5
21	100% ethanol	5
22	100% ethanol	5
23	100% xylene	15
24	100% xylene	15
25	100% xylene	15

Table 6. Microscopic characteristics for the determination of ovarian class and maturity stages of oocytes in the ovary of teleost fish adapted from Wallace and Selman (1981) and Rinchard and Kestemont (1996).

Ovary Class	Oocyte Stages present in ovary through maturation	Description of most advanced stage
(1) Previtellogenic	Previtellogenic (1) oocytes	Oocytes with vacuole free cytoplasm
(2) Endogenous vitellogenesis	Previtellogenic (1) and endogenous vitellogenic (2) oocytes	Appearance of yolk vesicles, occupy 2 or 3 rings in the cytoplasm periphery (early endogenous vitellogenesis. In addition, oocytes can be full of yolk vesicles. Follicular and cellular layer are differentiated (late endogenous vitellogenesis)
(3) Exogenous vitellogenesis	Previtellogenic (1), endogenous vitellogenic (2), and exogenous vitellogenic (3) oocytes	Oocytes accumulate yolk globules and yolk vesicles are at the periphery of the cytoplasm
(4) Final Maturation	Previtellogenic (1), endogenous vitellogenic (2), and fully mature (4) oocytes.	Appearance of the micropyle and migration of the germinal vesicle to the micropyle
(5) Intermediate Multiple Spawner	Previtellogenic (1), endogenous vitellogenic (2) oocytes, and postovulatory follicles (POF). Exogenous vitellogenic (3) oocytes may also be present but are not required.	The pre- and postovulatory follicles hypertrophy, the yolk substance degenerates leaving behind an empty follicle

Table 7. Gonadosomatic index (GSI; mean ± standard deviation) and condition factor (K; mean ± standard deviation) of male and female alewife at different sampling events throughout the study. Bold indicates the data used in Figure 11. "Nearshore" and "Offshore" is used to describe different sampling methods used in Rochester.

Sex	Date	Location	GSI (%)	K	n
F	**10/1/2017**	**Rochester (Offshore)**	**1.0 ± 0.2**	**0.8 ± 0.0**	**16**
F	**4/23/2018**	**Rochester (Offshore)**	**2.0 ± 0.6**	**0.7 ± 0.1**	**81**
F	6/6/2018	Hamlin	1.6 ± 1.7	0.5 ± 0.1	5
F	**6/11/2018**	**Bald Eagle Creek**	**2.1 ± 1.3**	**0.6 ± 0.1**	**15**
F	6/25/2018	Rochester (Nearshore)	7.2	0.6	1
F	6/28/2018	Rochester (Nearshore)/Hamlin	3.0	0.3	1
F	7/3/2018	Point Peninsula	6.9 ± 2.5	0.7 ± 0.2	42
F	**7/9/2018**	**Bald Eagle Creek**	**6.4 ± 3.2**	**0.6 ± 0.1**	**17**
F	**7/12/2018**	**Bald Eagle Creek**	**7.4 ± 2.8**	**0.6 ± 0.1**	**26**
F	**7/16/2018**	**Bald Eagle Creek**	**7.1 ± 2.0**	**0.7 ± 0.1**	**12**
F	**7/25/2018**	**Bald Eagle Creek**	**8.5 ± 2.1**	**0.7 ± 0.1**	**30**
F	7/25/2018	Oswego	7.5	0.7	1
F	**10/3/2018**	**Fairhaven**	**0.8 ± 0.3**	**0.7 ± 0.1**	**35**
F	**10/22/2018**	**Rochester (Offshore)**	**1.0 ± 0.2**	**0.8 ± 0.1**	**23**
M	**10/1/2017**	**Rochester (Offshore)**	**0.5 ± 0.1**	**0.8 ± 0.1**	**7**
M	**4/23/2018**	**Rochester (Offshore)**	**1.2 ± 0.5**	**0.8 ± 0.6**	**67**
M	**6/11/2018**	**Bald Eagle Creek**	**3.5 ± 2.7**	**0.6 ± 0.1**	**17**
M	6/19/2018	Hamlin	6.8 ± 1.4	0.7 ± 0.1	7
M	6/25/2018	Rochester (Nearshore)	6.3 ± 1.3	0.7 ± 0.1	71
M	6/28/2018	Rochester (Nearshore)/Hamlin	5.1 ± 1.8	0.6 ± 0.1	32
M	7/2/2018	Rochester (Nearshore)	5.1 ± 1.4	0.7 ± 0.1	15
M	7/3/2018	Point Peninsula	2.0 ± 1.7	0.6 ± 0.1	5
M	**7/9/2018**	**Bald Eagle Creek**	**4.1 ± 1.5**	**0.6 ± 0.1**	**18**
M	7/9/2018	Oswego	1.4 ± 1.1	0.5 ± 0.0	2
M	**7/12/2018**	**Bald Eagle Creek**	**4.1 ± 1.2**	**0.6 ± 0.1**	**17**
M	**7/16/2018**	**Bald Eagle Creek**	**4.0 ± 0.6**	**0.7 ± 0.0**	**7**
M	7/20/2018	Charity	1.6 ± 0.8	0.6 ± 0.0	12
M	**7/25/2018**	**Bald Eagle Creek**	**2.6 ± 1.5**	**0.6 ± 0.1**	**6**
M	7/25/2018	Oswego	2.3 ± 1.2	0.7 ± 0.1	29
M	**10/3/2018**	**Fairhaven**	**0.9 ± 0.5**	**0.7 ± 0.1**	**29**

Table 8. Gonadosomatic index (GSI; mean ± standard deviation) and condition factor (K; mean ± standard deviation of male alewife at a given age in July.

Age	GSI (%)	K	n
2	4.4 ± 1.2	0.7 ± 0.04	26
3	4.0 ± 1.3	0.6 ± 0.05	6
5	2.7 ± 1.0	0.5 ± 0.05	3
6	2.9 ± 1.5	0.6 ± 0.04	9

Table 9. Gonadosomatic index (GSI; mean ± standard deviation) and condition factor (K; mean ± standard deviation) of female alewife at a given age in July.

Age	GSI (%)	K	n
2	7.9 ± 2.9	0.7 ± 0.06	38
3	7.7 ± 2.3	0.7 ± 0.09	18
5	8.7 ± 1.5	0.6 ± 0.04	5
6	6.2 ± 2.3	0.6 ± 0.05	18

Table 10. Condition factor (K, mean ± standard deviation) of male individuals displayed in Figure 11 (illustrating gonadosomatic of comparable male and female alewife throughout the year).

Month	K	n
October 2017	0.8 ± 0.03^{c}	7
April 2018	0.8 ± 0.07^{bc}	67
June 2018	0.6 ± 0.02^{ab}	17
July 2018	0.6 ± 0.01^{a}	48
October 2018	0.7 ± 0.02^{bc}	29

Table 11. Condition factor (K, mean ± standard deviation) of female individuals displayed in Figure 11 (illustrating gonadosomatic of comparable male and female alewife throughout the year).

Month	K	n
October 2017	0.8 ± 0.01^{a}	81
April 2018	0.7 ± 0.01^{b}	81
June 2018	0.6 ± 0.01^{c}	15
July 2018	0.7 ± 0.01^{b}	85
October 2018	0.7 ± 0.01^{d}	58

Table 12. Oocyte diameter (mean ± standard deviation) and proportion for a given ovary class (1 = previtellogenic, 2 = endogenous vitellogenesis, 3 = exogenous vitellogenesis, 4 = final maturation, and 5 = intermediate multiple spawner) and month. Frequency of ovary class per month is also displayed with average gonadosomatic index (GSI) for each ovary class in a given month of females used in histology analyses.

Month	Ovary Class and Proportion (%)	Previtellogenic Oocyte Diameter (µm) and Proportion (%)	Endogenous Vitellogenic Oocyte Diameter (µm) and Proportion (%)	Exogenous Vitellogenic Oocyte Diameter (µm) and Proportion (%)	Final Maturation Oocyte Diameter (µm) and Proportion (%)	GSI (%)	n
October 2017	1 (75)	72.96 ± 15.19 (100)				1.02 ± 0.25	12
	2 (25)	71.23 ± 4.45 (96.35 ± 1.41)	143.54 ± 4.07 (3.65 ± 1.41)			1.02 ± 0.14	4
April 2018	1 (38.3)	87.50 ± 22.80 (100)				1.51 ± 0.36	23
	2 (61.7)	90.32 ± 13.76 (89.48 ± 5.38)	213.84 ± 29.59 (10.52 ± 5.38)			2.13 ± 0.54	37
June 2018	1 (41.2)	71.26 ± 10.82 (100)				0.98 ± 0.47	7
	2 (35.3)	83.64 ± 12.75 (85.48 ± 1.47)	204.34 ± 60.94 (14.52 ± 1.47)			2.5 ± 0.81	6
	3 (23.5)	79.94 ± 6.99 (83.75 ± 5.55)	203.55 ± 14.69 (12.30 ± 4.26)	350.65 ± 36.75 (3.95 ± 1.38)		4.00 ± 0.50	4
July 2018	2 (2.7%)	71.68 ± 6.97 (81.60 ± 6.36)	173.10 ± 9.48 (18.40 ± 6.36)			2.19 ± 0.06	2
	3 (66.7)	70.94 ± 11.99 (79.82 ± 7.39)	225.87 ± 21.72 (12.36 ± 4.99)	432.40 ± 50.49 (7.82 ± 3.50)		7.53 ± 1.92	50
	4 (6.7)	68.00 ± 7.70 (83.10 ± 2.80)	251.23 ± 23.70 (10.44 ± 2.38%		489.83 ± 27.61 (6.34 ± 2.13)	10.28 ± 1.59	5
	5 (24.0)	77.11 ± 13.21 (87.66 ± 5.24)	266.05 ± 35.64 (10.60 ± 5.31)	387.52 ± 25.41 (1.85 ± 1.45)		5.70 ± 1.98	18
October 2018	1 (70.4)	78.98 ± 9.98 (100%)				1.02 ± 0.19	19
	2 (29.6)	73.53 ± 9.17 (94.95 ± 2.97)	168.04 ± 18.24 (5.05 ± 2.97)			1.09 ± 0.19	8

Table 13. Diameter (mean ± standard deviation) of oocytes at different classes of ovarian development (1 = previtellogenic, 2 = endogenous vitellogenesis, 3 = exogenous vitellogenesis, 4 = final maturation, and 5 = intermediate multiple spawner) and total length (mean ± standard deviation) at a given age of females used in histology analyses.

Otolith Age	Ovary Class	Previtellogenic Oocyte Diameter (µm)	Endogenous Vitellogenic Oocyte Diameter (µm)	Exogenous Vitellogenic Oocyte Diameter (µm)	Final Maturation Oocyte Diameter (µm)	TL (mm)	n
1	1	63.97 ± 5.96				126.17 ± 10.76	6
	2	76.60	166.40			130.00	1
	3	75.05 ± 5.09	213.63 ± 5.34	390.28 ± 18.70		133.00 ± 2.83	2
2	1	72.78 ± 16.66				138.00 ± 13.90	22
	2	75.92 ± 13.91	160.62 ± 39.19			140.33 ± 10.23	6
	3	70.20 ± 10.33	220.66 ± 19.94	419.42 ± 52.43		145.06 ± 11.43	32
	4	74.43 ± 2.02	268.45 ± 32.46		508.90 ± 26.66	149.00 ± 15.56	2
	5	77.12 ± 12.26	253.83 ± 41.78	369.60 ± 27.33		161.00 ± 11.31	5
3	1	94.04 ± 12.33				160.71 ± 7.83	7
	2	90.83 ± 12.67	197.88 ± 37.16			168.36 ± 11.46	11
	3	73.02 ± 15.60	233.79 ± 27.50	447.11 ± 50.48		164.60 ± 10.50	10
	4	62.65	228.15		460.00	168.00	1
	5	56.60	238.05	371.10		174.00	1
4	1	93.99 ± 10.80				184.75 ± 7.50	4
	2	77.53 ± 16.65	175.08 ± 42.11			183.00 ± 7.07	2
5	1	88.52 ± 24.81				176.60 ± 6.58	5
	2	79.00 ± 5.08	197.80 ± 49.18			182.20 ± 5.26	5
	3	61.59 ± 8.54	232.56 ± 19.78	466.11 ± 37.82		176.75 ± 8.85	4
	5	74.57 ± 7.61	259.31 ± 32.30	393.43 ± 16.87		180.00 ± 8.04	4
6	1	121.20				195.00	1
	2	90.78 ± 15.57	225.37 ± 22.34			181.45 ± 8.04	20
	3	82.31 ± 10.44	225.23 ± 26.24	414.11 ± 71.02		188.50 ± 9.95	6
	4	64.25 ± 9.69	245.55 ± 1.77		485.67 ± 26.20	178.00 ± 8.49	2
	5	78.24 ± 14.29	276.37 ± 34.39	401.88 ± 25.39		187.00 ± 7	7
7	2	61.95	160.35			195.00	1

Table 14. Frequency of spawning potential for male and female alewife captured in June and July 2018.

Sex	Spawning Potential (%)	Total n
F	58.3	144
M	77.4	186

Table 15. Frequency of female alewife sampled that were considered spawning (observed ovulation and or advanced ovaries in histological analyses) at a given age captured in June and July 2018.

Age	Average TL (mm)	Spawning Potential (%)	Total n
1	133.00 ± 2.83	50	4
2	147.44 ± 12.63	63.9	61
3	165.67 ± 9.90	42.9	28
4	NA	0	1
5	178.39 ± 8.02	61.5	13
6	189.36 ± 8.55	61.1	36
7	195	100	1

Table 16. Frequency of male alewife sampled that were considered spawning (observed spermiating) at a given age captured in June and July 2018.

Age	Average TL (mm)	Spawning Potential (%)	Total n
1	NA	0	2
2	140.20 ± 6.93	90.4	118
3	154.45 ± 13.34	80	25
4	180 ± 2.83	66.7	3
5	182.26 ± 6.12	59.4	32
6	185.33 ± 4.16	50	6

Table 17. Batch and relative batch fecundity of alewife at a given age captured in June and July 2018.

Age	Batch fecundity	Relative batch fecundity (# egg/g of fish)	n
2	6100 ± 2146	271 ± 103	10
3	8535 ± 1100	244 ± 58	5
6	8694 ± 163	223 ± 15	2
7	9082	186	1

Table 18. Frequency of embryo survival (mean ± standard deviation) of all crosses of male and female at a given age.

Female Age	Male Age	Embryo Survival (%)	n
2	2	11 ± 18	12
	3	2 ± 2	3
	6	12 ± 19	4
5	2	1 ± 1	3
	3	14	1
	4	2	1
	6	0	1
6	2	26 ± 22	13
	3	18 ± 31	3
	4	4	1
	6	22 ± 4	2

Table 19. Frequency of embryo survival (mean ± standard deviation) based on female age.

Female Age	Embryo Survival (%)	n
2	9 ± 17	19
5	3 ± 5	6
6	23 ± 20	21

Table 20. Number of model parameters (K) and Akaike Information Criterion (AIC) of beta regression embryo survival model.

Model	K	AICc
Female Age	3	-45.51
Null	2	-42.92
Female and Male Age	4	-40.62
Male Age	3	-39.08
All factors with interaction	5	-37.91

Figures

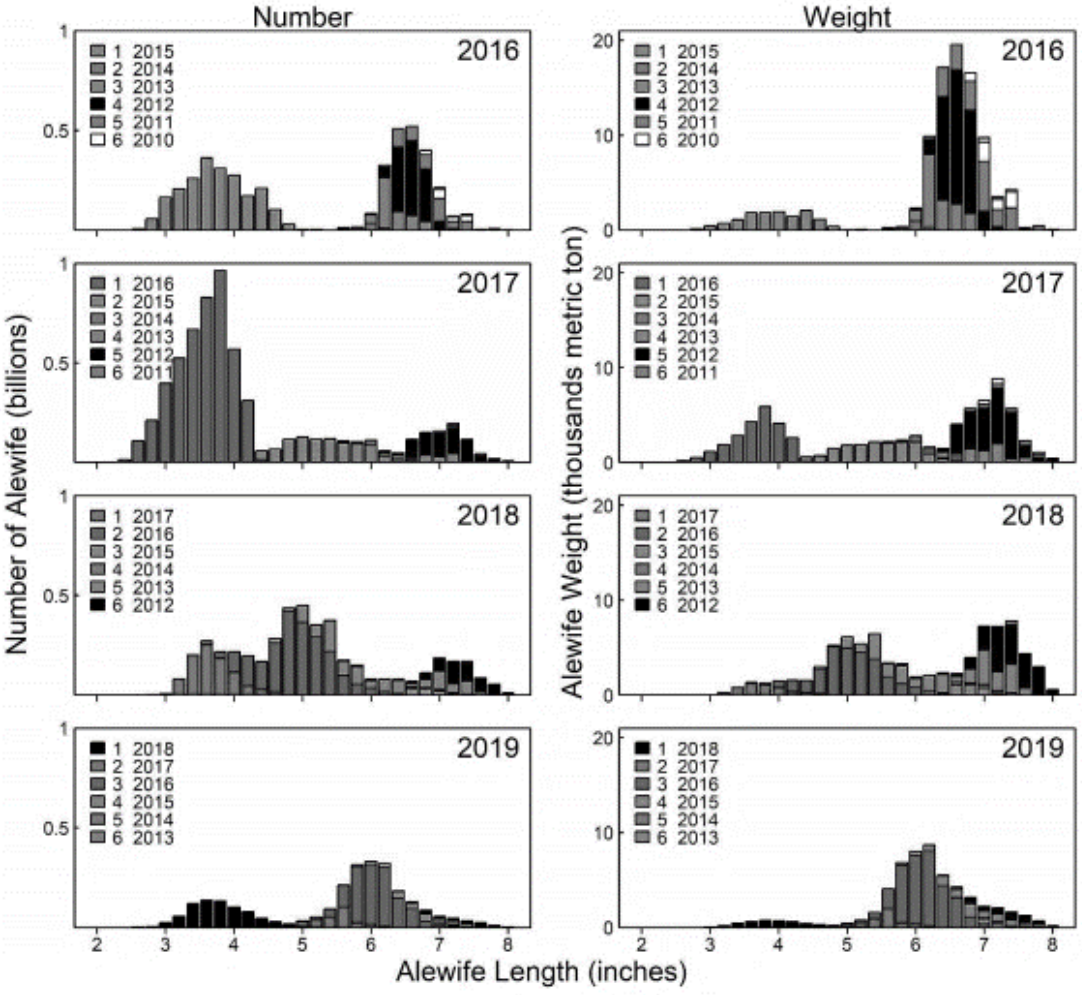


Figure 1. Lake Ontario Alewife size and age structure based on whole-lake survey results, 2016-2019. The horizontal position of a bar indicates Alewife length, while the bar height illustrates the number or weight. The year in which alewife are born (year class) is depicted by the different colors and is the same across each panel. Data was collected for the Lake Ontario pelagic prey fish assessment by the USGS and NYSDEC (Weidel *et al.* 2019).

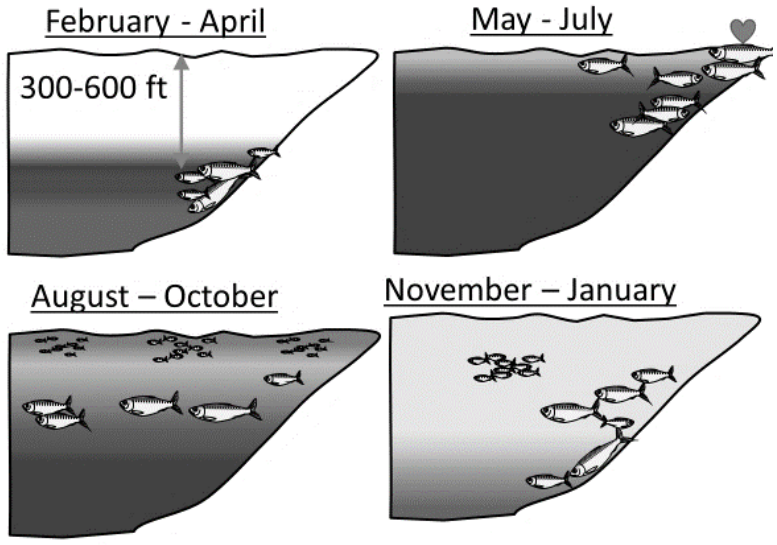


Figure 2. Illustration of alewife distribution throughout the year based on bottom trawl surveys conducted by USGS-LOBS and NYSDEC. The "heart" symbol indicates alewife spawning. Note that alewife distribution follows the thermocline and that larger alewife tend to stay deeper than smaller (younger) alewife when possible (B. Weidel, USGS personal communication).

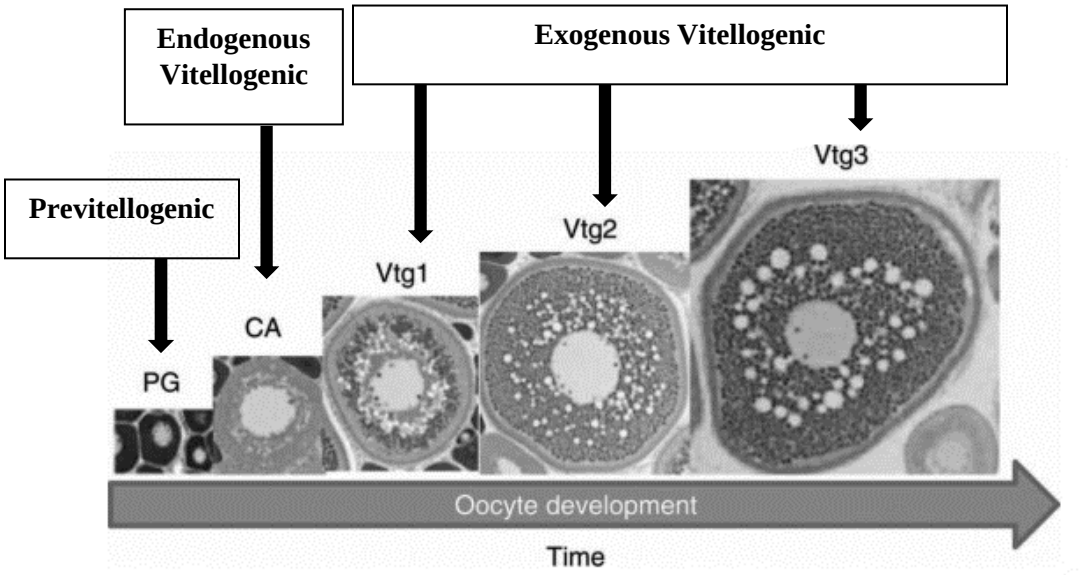


Figure 3. Progression of oocyte growth and developmental stages that are most commonly identified in fishes: primary growth (PG), cortical alveolar (CA), and yolked or vitellogenic (Vtg; Vtg1, Vtg2, and Vtg3 = primary, secondary, and tertiary vitellogenesis, respectively) adapted from Lowerre-Barbieri *et al.* (2011). In this study, PG is referred to as previtellogenic, CA is referred to as endogenous vitellogenic, and yolked or vitellogenic stages are referred to as endogenous vitellogenic oocytes. Species shown is the spotted seatrout (*Cynoscion nebulosus*).

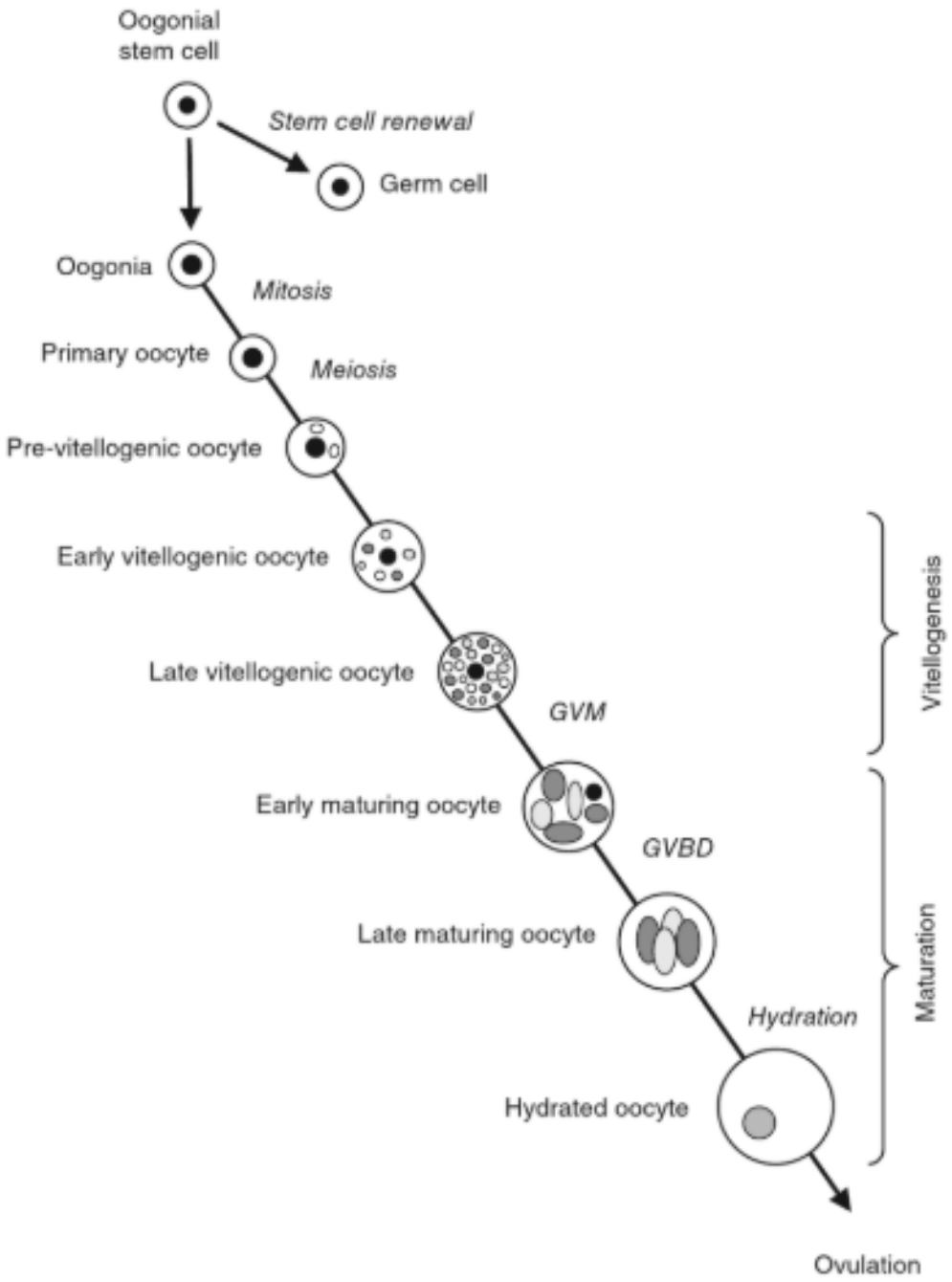


Figure 4. A summary of the process of oocyte development and maturation in female fish from Wooten and Smith (2015). GVM, germinal vesicle migration; GVBD, germinal vesicle breakdown.

Figure 5. Total length (mm) of alewife collected in Lake Ontario from the 1980s to 2015. Year class determined by length and otolith data. Data was collected for the Lake Ontario pelagic prey fish assessment by the USGS and NYSDEC (B. Weidel, USGS personal communication).

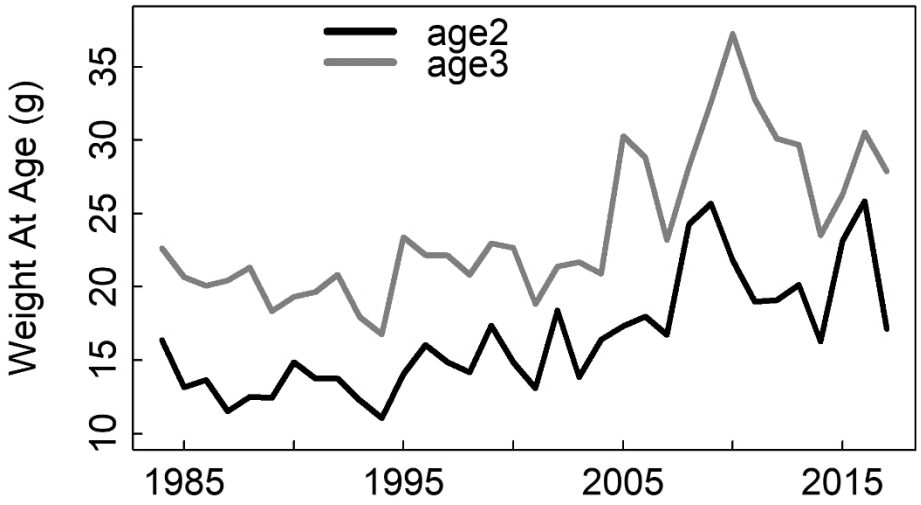


Figure 6. Weight (g) of Lake Ontario alewife from 1980's to 2017. Year class was determined by length and otolith data. Data were collected for the Lake Ontario pelagic prey fish assessment by the USGS and NYSDEC (B. Weidel, USGS personal communication).

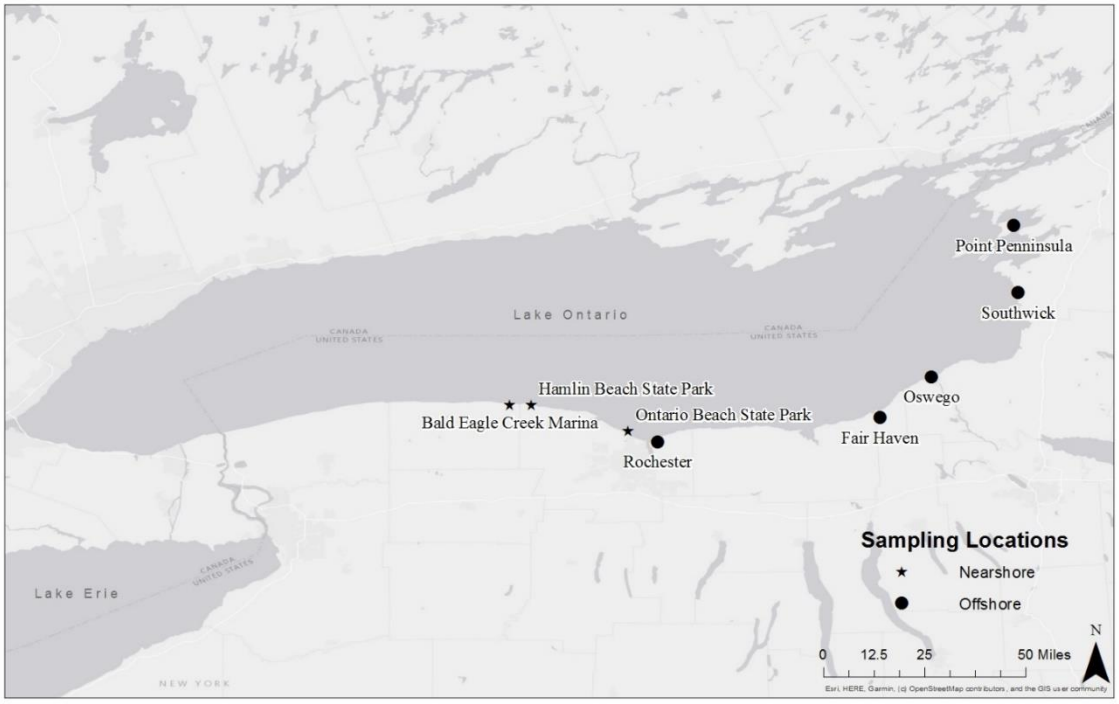


Figure 7. Sample sites located in American waters of Lake Ontario. Circles indicate off shore sites where bottom trawls at varying depths were conducted by the NYSDEC and USGS, while stars indicate nearshore sites where boat electroshocking and seining took place.

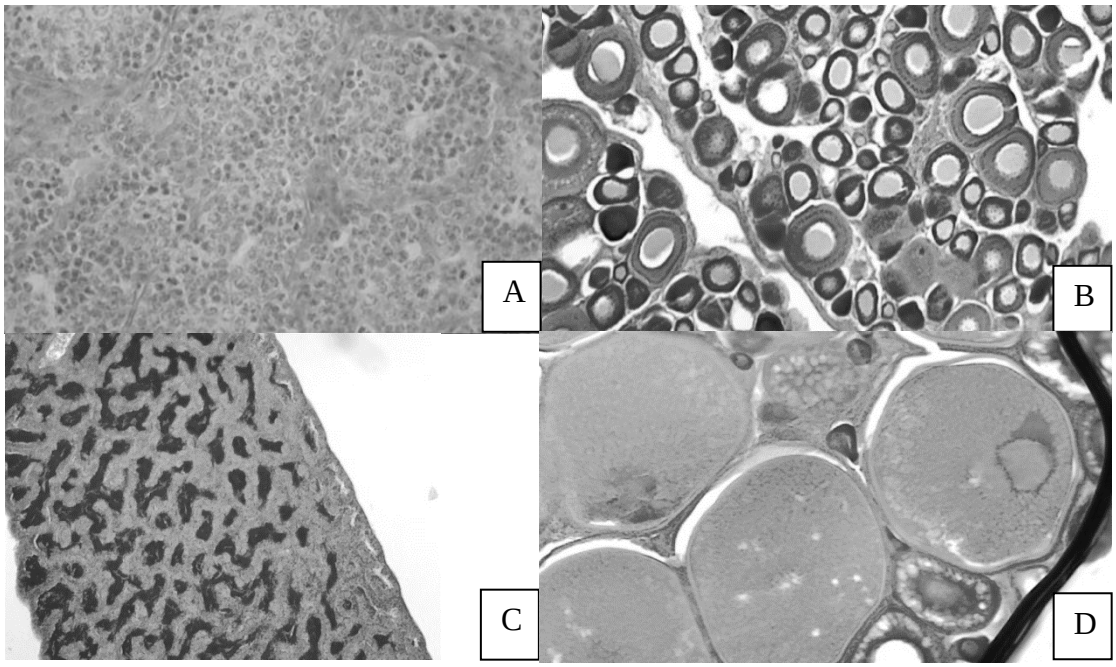


Figure 8. Cross section views of immature male testis (A) and female ovary (B) at x100 magnification and mature male testis (C) and mature female ovary (D) at x40 magnification in alewife.

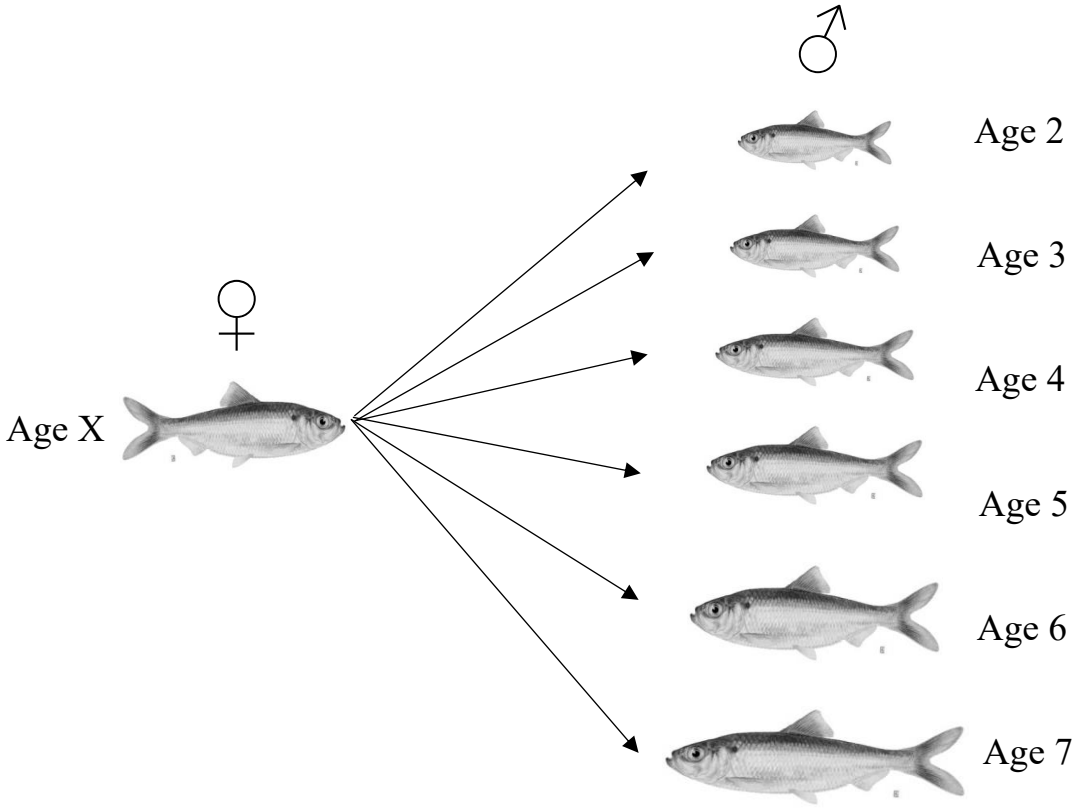


Figure 9. Method to determine crosses for artificial reproduction and embryo survival. Eggs were stripped from all individuals releasing eggs and divided into sub-samples based on the number of available males. Total length was used as a precursor for age for both male and female and age was confirmed later on using otoliths.

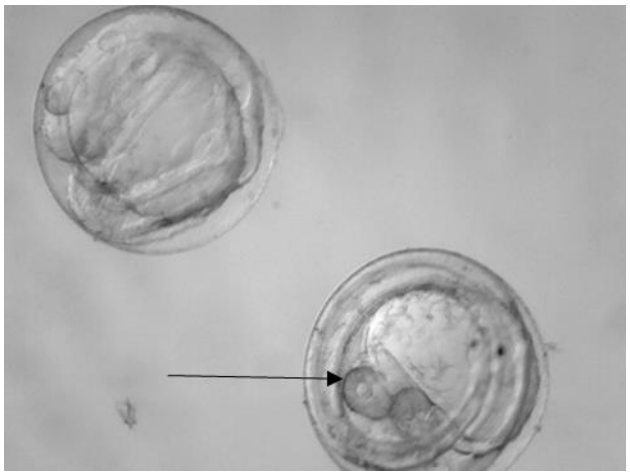


Figure 10. Alewife embryo at the pigmented eyed stage. Arrow indicates the pigmented eyes of the embryo.

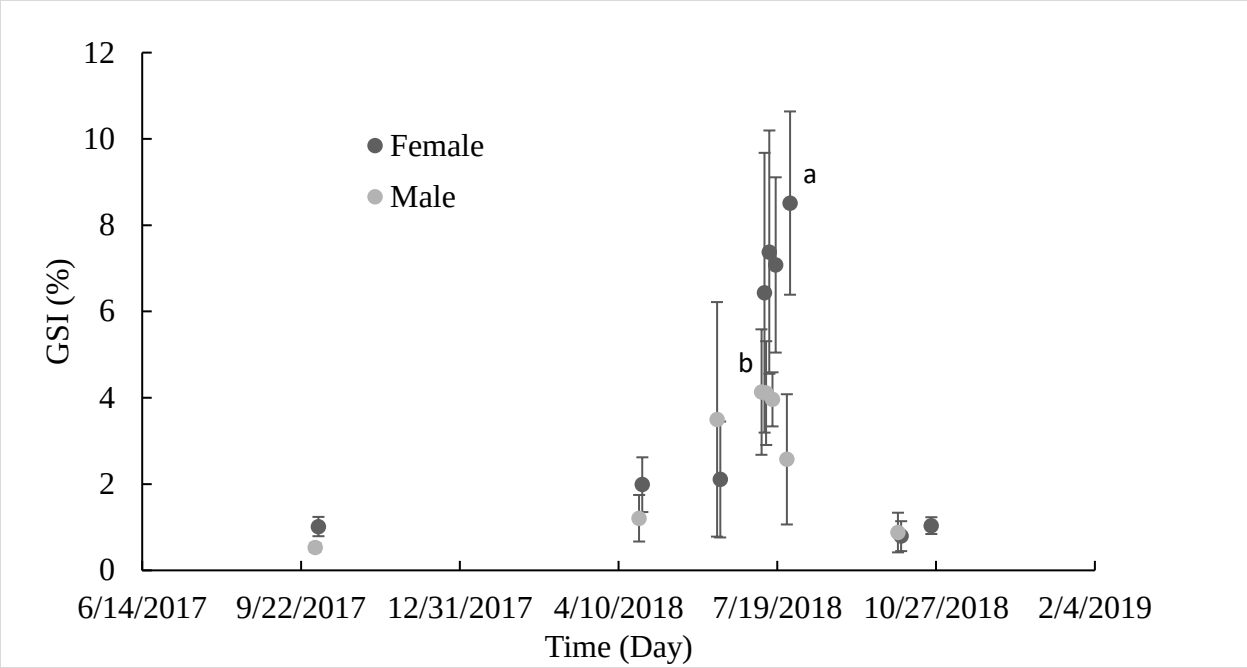


Figure 11. Gonadosomatic index (GSI; mean ± standard deviation) of male and female alewife from comparable sampling events. Comparable sampling events indicated fish were from the same location and sampling date. Superscript indicates significant difference between peak GSI of male and female alewife (Mann-Whitney U test).

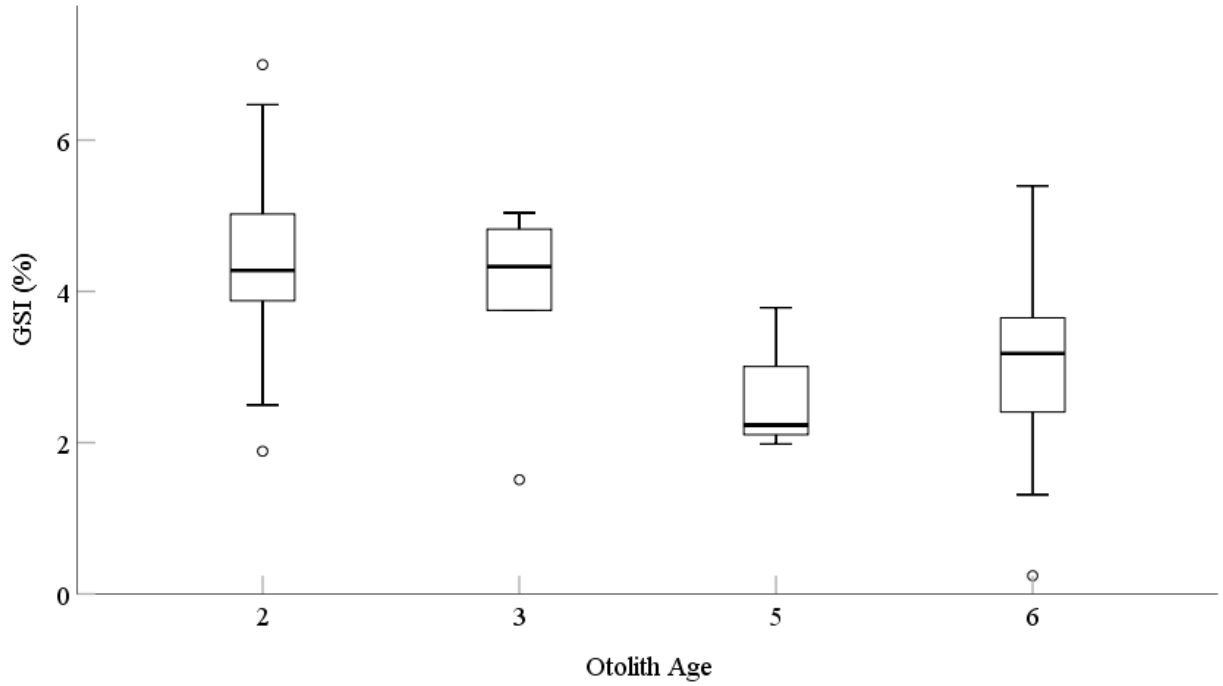


Figure 12. Gonadosomatic index (GSI; box plot) based on age of male alewife. Data in this figure illustrates fish captured in the same location (Bald Eagle Creek Marina) throughout July.

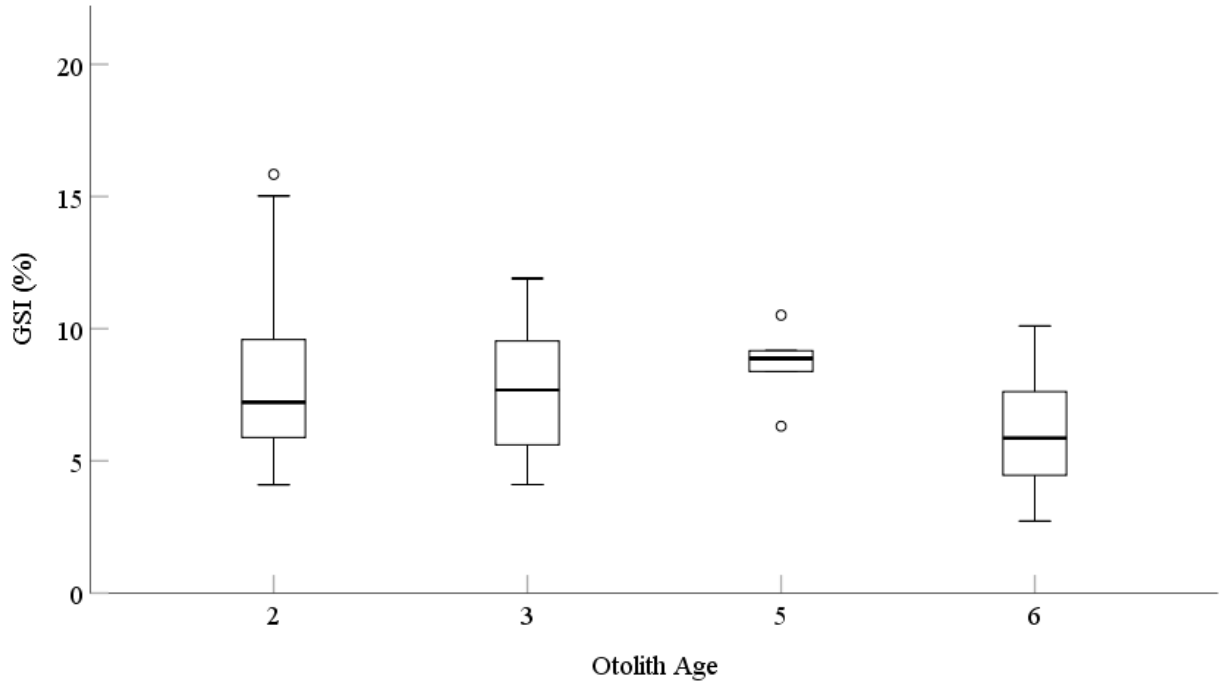


Figure 13. Gonadosomatic index (GSI; box plot) based on age of female alewife. Data in this figure illustrates fish captured in the same location (Bald Eagle Creek Marina) throughout July.

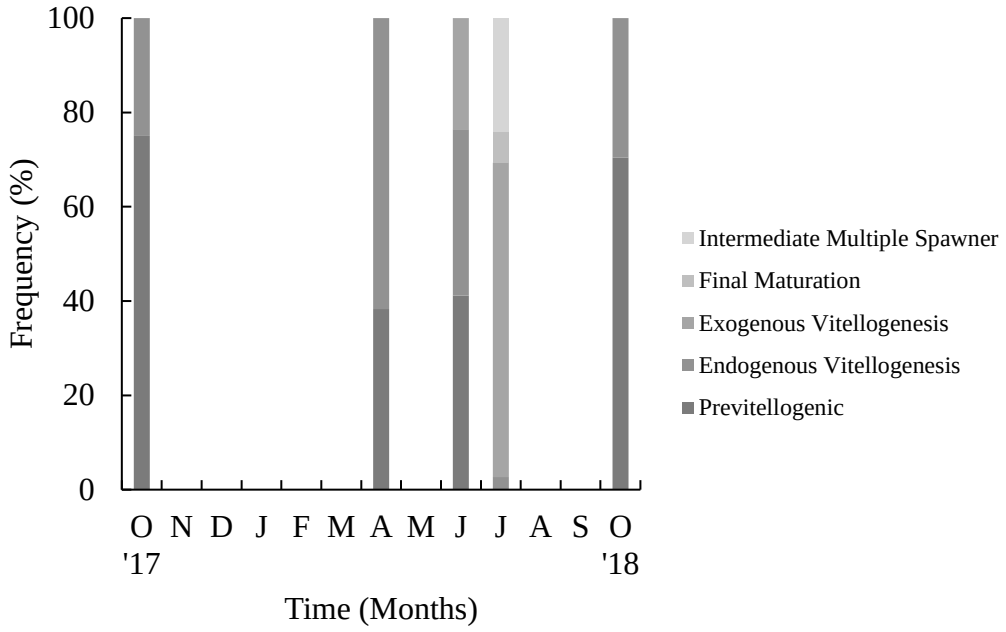
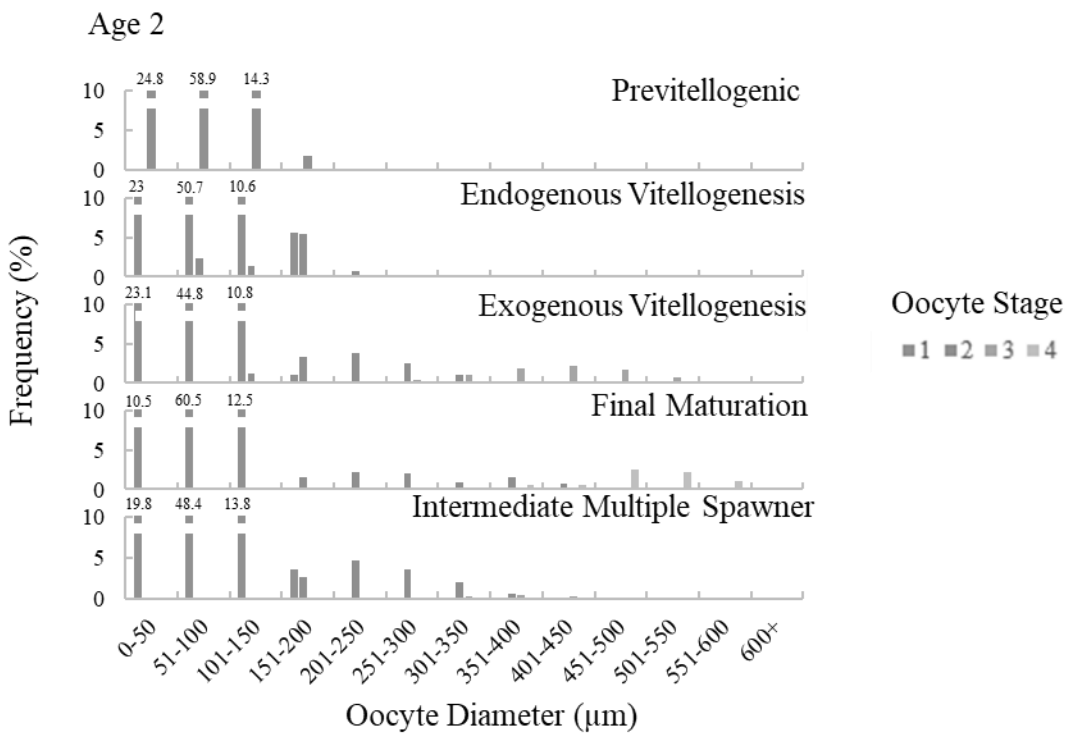
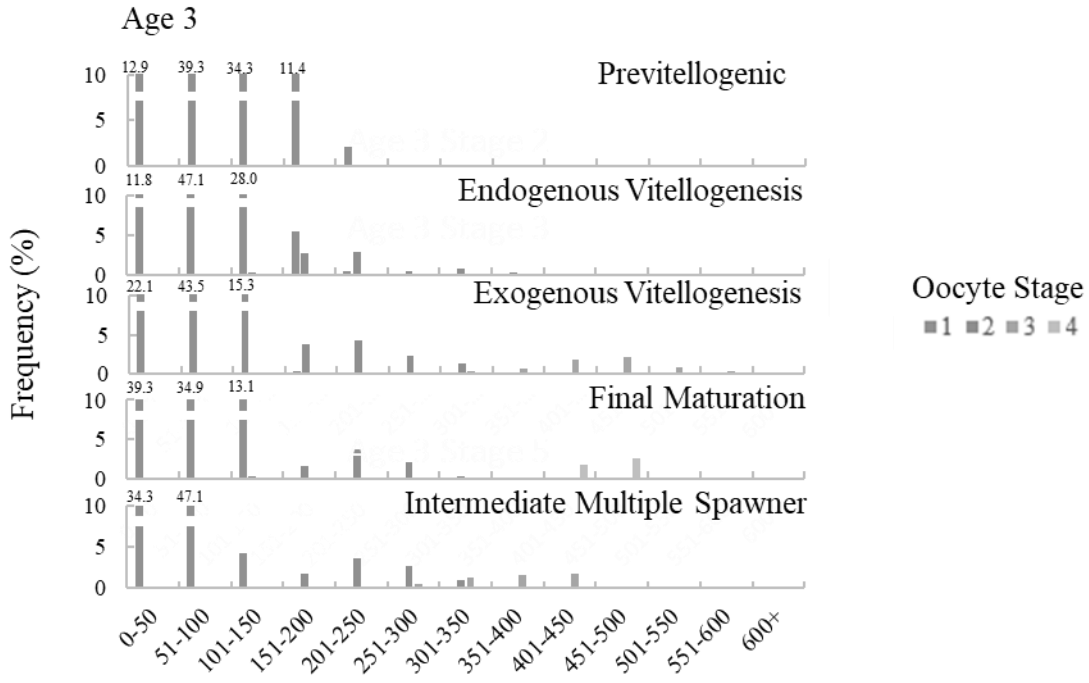


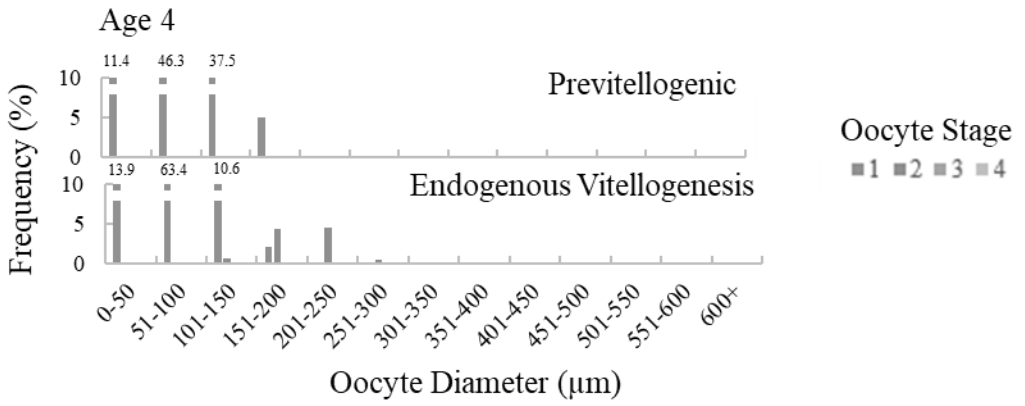
Figure 14. Change in the percent frequency of ovary classes for all individuals regardless of age in histology analyses.


Age 2
Previtellogenic
Endogenous Vitellogenesis
Exogenous Vitellogenesis
Final Maturation
Intermediate Multiple Spawner
24.8 58.9 14.3
23 50.7 10.6
23.1 44.8 10.8
10.5 60.5 12.5
19.8 48.4 13.8
Frequency (%)
0-50 51-100 101-150 151-200 201-250 251-300 301-350 351-400 401-450 451-500 501-550 551-600 600+
Oocyte Diameter (µm)
Oocyte Stage
1 2 3 4

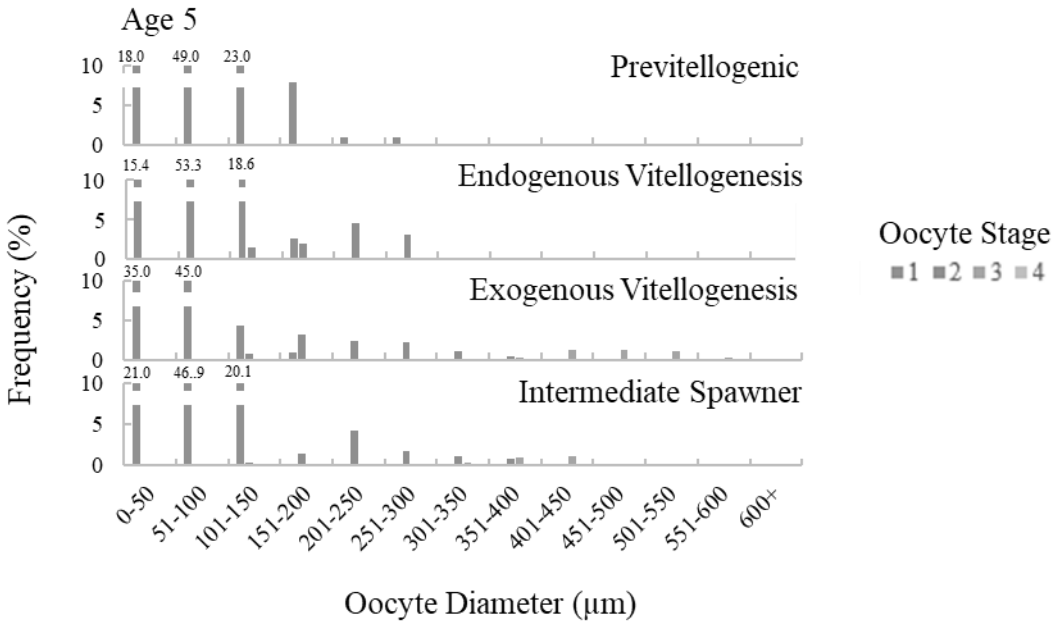
A


Age 3
Previtellogenic
Endogenous Vitellogenesis
Exogenous Vitellogenesis
Final Maturation
Intermediate Multiple Spawner
12.9 39.3 34.3 11.4
11.8 47.1 28.0
22.1 43.5 15.3
39.3 34.9 13.1
34.3 47.1
Frequency (%)
0-50 51-100 101-150 151-200 201-250 251-300 301-350 351-400 401-450 451-500 501-550 551-600 600+
Oocyte Stage
1 2 3 4

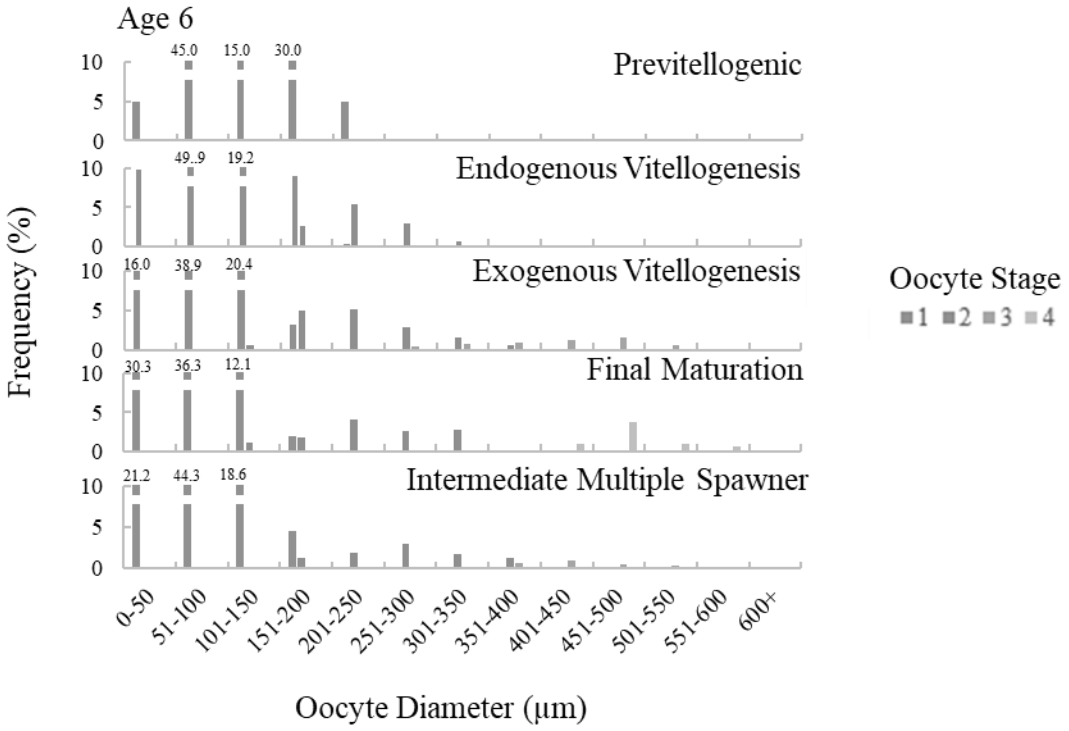
B


Age 4
Previtellogenic
Endogenous Vitellogenesis
Frequency (%)
11.4
46.3
37.5
13.9
63.4
10.6
0-50
51-100
101-150
151-200
201-250
251-300
301-350
351-400
401-450
451-500
501-550
551-600
600+
Oocyte Diameter (µm)
Oocyte Stage
1 2 3 4

C


Age 5
Previtellogenic
Endogenous Vitellogenesis
Exogenous Vitellogenesis
Intermediate Spawner
Frequency (%)
18.0
49.0
23.0
15.4
53.3
18.6
35.0
45.0
21.0
46..9
20.1
0-50
51-100
101-150
151-200
201-250
251-300
301-350
351-400
401-450
451-500
501-550
551-600
600+
Oocyte Diameter (µm)
Oocyte Stage
1 2 3 4

D



E

Figure 15. Average oocyte-size frequency distribution (µm) of each oocyte stage (1 = previtellogenic, 2 = endogenous vitellogenic, 3 = exogenous vitellogenic, and 4 = final maturation) in a given ovary class (previtellogenic, endogenous vitellogenesis, exogenous vitellogenesis, final maturation, and intermediate multiple spawner) of age A) 2, B) 3, C) 4, D) 5, and E) 6, female alewife.

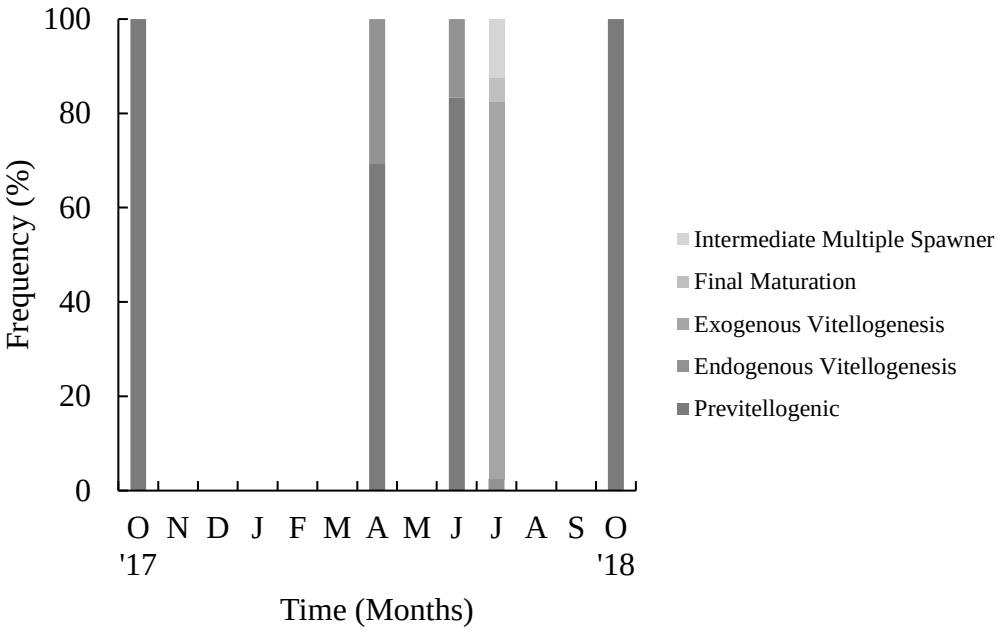


Figure 16. Change in percent frequency of ovary classes for age 2 individuals in histology analyses.

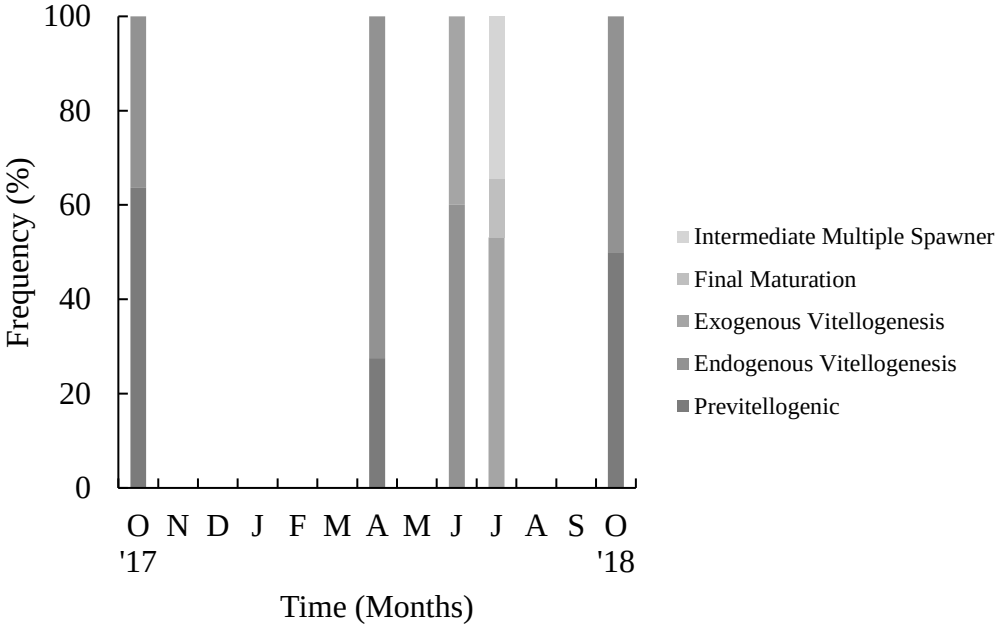


Figure 17. Change in percent frequency of ovary classes for all individuals of age 3 and older alewife in histology analyses.

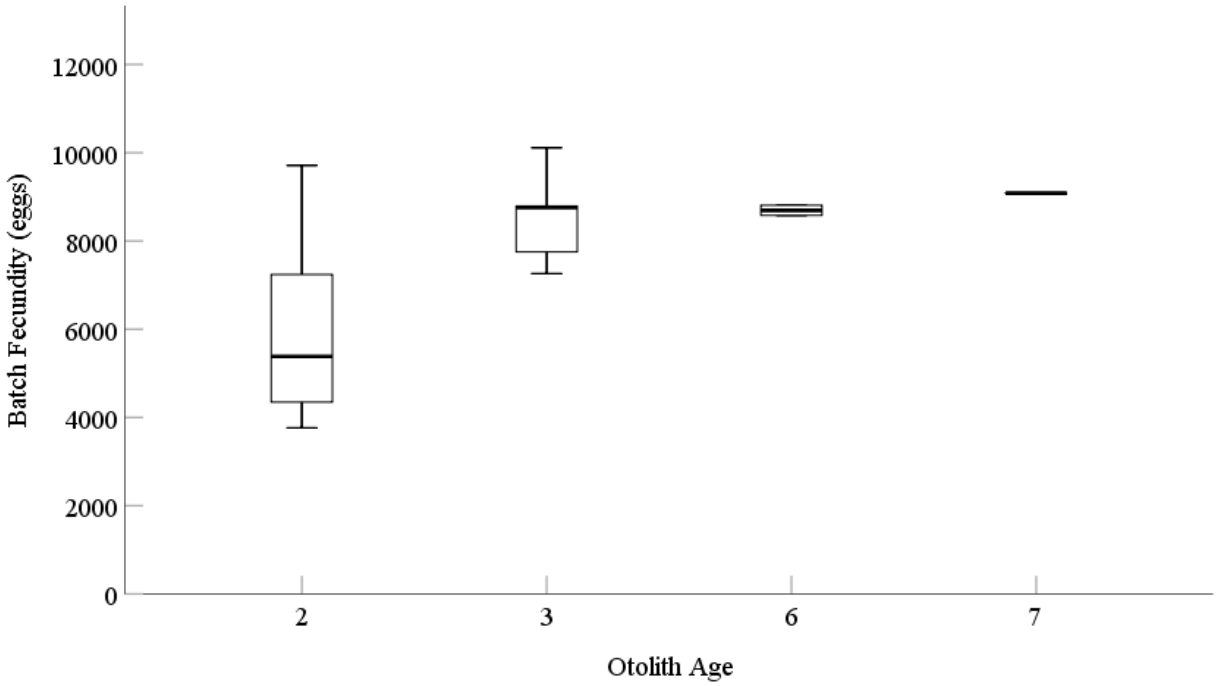


Figure 18. Batch fecundity (box plot) of female alewife captured throughout the spawning season.

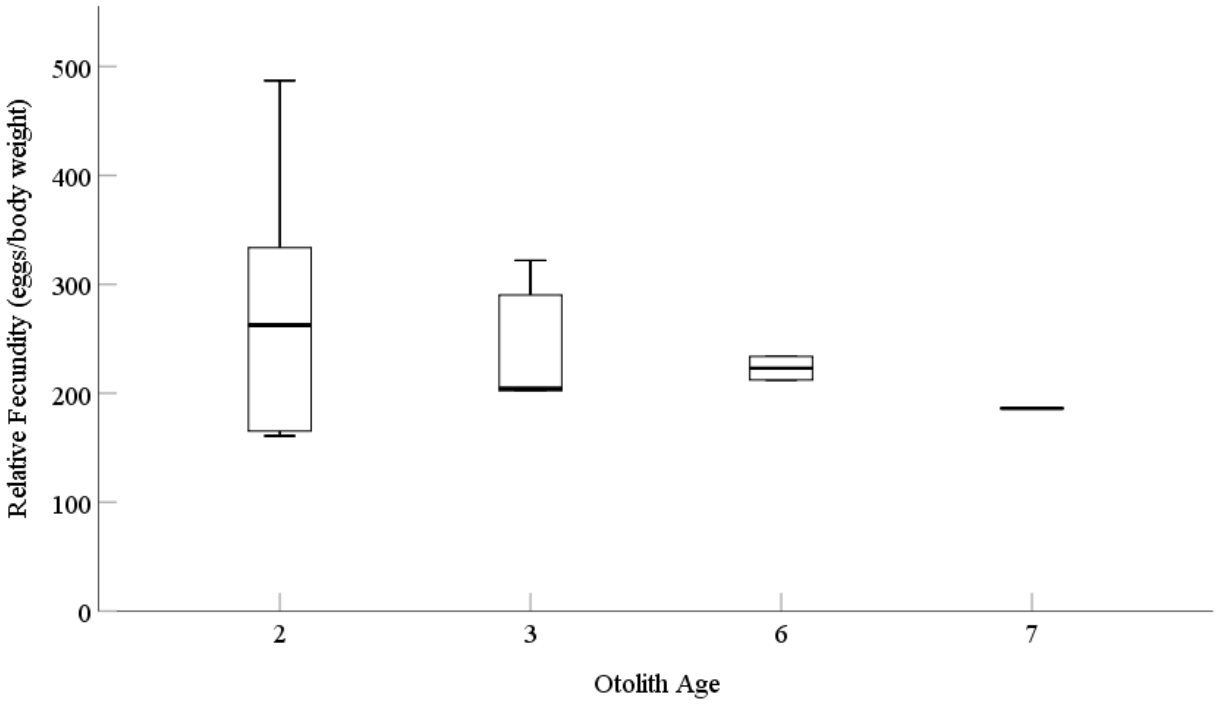


Figure 19. Relative batch fecundity (box plot) of female alewife captured throughout the spawning season.

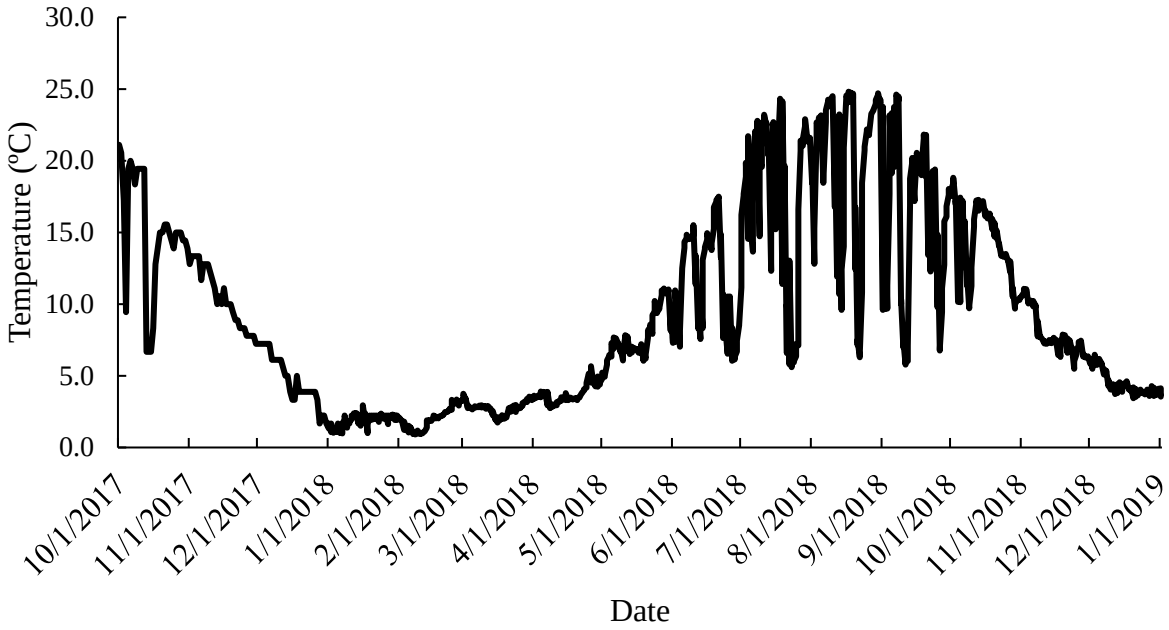


Figure 20. Water temperatures taken from October 2017 to January 2019 at the Monroe County NY Water Intake Station in Rochester, NY at a depth of 13.7 m.