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## **An investigation of juvenile alewife (*Alosa pseudoharengus*) habitat use and growth using natural markers**

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An investigation of juvenile alewife (*Alosa pseudoharengus*) habitat use and growth  
using natural markers

A Thesis

Submitted in Partial Fulfillment of the Requirements of the Degree of  
Master of Science in Biology

University of Southern Maine

Biology Department

By

Gregory Norman LaBonte

2016

THE UNIVERSITY OF SOUTHERN MAINE  
DEPARTMENT OF BIOLOGICAL SCIENCES

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using natural markers

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## ABSTRACT

This research attempts to connect patterns in growth and migration of an anadromous species. The goal of this research was to understand habitat movements and growth of juvenile alewives (*Alosa pseudoharengus*) in the Penobscot Estuary and Bay through the use of otolith microchemistry, otolith growth increments, and a laboratory stable isotope turnover study. Understanding the connection between growth and movement of juvenile alewives may lead to more accurate and sophisticated conservation and restoration methods for anadromous species. National Oceanic and Atmospheric Administration and Maine Department of Marine Resources captured ninety-one fish of the same age, weight, and length in the Bay and Estuary. I used otolith microchemistry to determine habitat use and associated growth rate. Alewife otolith increments grew fastest in freshwater (5.23  $\mu\text{m}/\text{day}$ ), at intermediate rates in the bay (4.79  $\mu\text{m}/\text{day}$ ), and slowest in the estuary (4.26  $\mu\text{m}/\text{day}$ ). Otolith microchemistry patterns revealed extensive movement between bay and estuary habitats, suggesting juveniles utilize both habitats. Restoration efforts typically focus on freshwater to estuary transitions; however, these data suggest that estuary to bay transition zones play an important role in juvenile alewife growth.

A comparison of stable isotope values for fast and slow turnover tissues is another way to infer recent habitat movement in juvenile alewife. Using juvenile alewife captured in the Penobscot estuary, I conducted a laboratory diet switch experiment to determine stable isotope turnover rates of  $\delta^{15}\text{C}$  and  $\delta^{15}\text{N}$  in muscle (slow) and liver (fast) tissue. Diet-1 was saltwater based and nutrient poor, whereas diet-2 was freshwater based and nutrient rich. Due to the poor fat content in diet-1, turnover rate was slower during the first phase of the experiment than expected. However, as expected, liver turned over 20

days faster than muscle on diet-1. Diet-2 only lasted for 20 days, and turnover results were inconclusive. These results confirmed that liver tissues turn over faster than muscle tissue. However, turnover rates slowed significantly when the fish's growth slowed.

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# INTRODUCTION

Diadromous fish are uniquely different from other types of teleosts in their use of habitats with varying salinities, specifically ocean, lakes and streams, during their life history (Gross 1987, McDowall 1987). Within diadromy there exist three main life history patterns: anadromy, catadromy, and amphidromy. Anadromous species grow at sea and reproduce in freshwater. Some anadromous species are capable of reproducing and growing entirely within freshwater habitats, although their osmoregulatory capability to switch habitats persists (Gross 1987, Jonsson 1985). For example, alewife (*Alosa pseudoharengus*) have anadromous populations as well as natural and introduced land-locked populations within their native range. These contrasting life histories lead to the question, why migrate across salinities, which is energetically costly due to physical exertion while migrating and osmoregulatory changes (Gross 1987), when a fish can persist as a resident in freshwater habitats?

Stable isotopes, particularly nitrogen and carbon, are often used to determine trophic structure within a community (Post 2002). Carbon isotopes reflect carbon sources (habitat), and nitrogen isotopes reflect trophic position and are carried through the food web. Within the past five years, researchers have begun focusing on the length of time required for stable isotopes to reach equilibrium in various tissues of the organism as food sources change (Buchheister and Latour 2010, Heady and Moore 2013, Nelson et al. 2011). Experimental validation of turnover rates are important for strong ecological inferences on resource use, trophic interactions (Chen et al. 2012, Heady and Moore 2012), and habitat use. Understanding tissue turnover rates is particularly useful for

highly migratory fish, as tissue turnover rates can provide useful insight into recent habitat use (Heady and Moore 2012).

This thesis combines (a) an investigation of variation in estuarine and marine growth and habitat use of juvenile alewife as inferred from otolith microchemistry and growth increments and (b) a laboratory study examining stable isotope turnover rates of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  in muscle and liver tissue of juvenile alewife. In this thesis, I:

- a) compare growth rates of individual juvenile alewife caught in the Penobscot estuary and bay, Maine, during October and May, 2013 and 2014 using otolith microchemistry and growth increments. Teasing apart alewife life history at the individual level will help researchers to decipher hidden complexities that may not be perceptible through large scale studies. An understanding of natural variation within a population could allow more specific approaches to conservation and management practices.
- b) present a laboratory study that involved a diet-switch experiment to investigate whether  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  turnover rates differ between juvenile alewife muscle and liver tissues. Understanding juvenile alewife stable isotope turnover rates could enable scientists to infer recent habitat use from stable isotopes.

# CHAPTER 1: EXAMINING THE RELATIONSHIP BETWEEN HABITAT USE AND GROWTH OF JUVENILE ALEWIFE (*ALOSA PSEUDOHARENGUS*) USING OTOLITH MICROCHEMISTRY AND GROWTH INCREMENTS

**Abstract:** *Diadromy is a unique feature among a small group of teleosts that migrate across habitats of varying salinities. These potentially costly migrations involve changes in growth rates, physiology, predators, and food availability, ultimately leading to the question, why migrate? In this study, I investigated habitat use and associated growth of juvenile alewife, an anadromous species, in a Northwest Atlantic estuary and bay system (Penobscot River, Maine, USA). Juvenile alewives were captured in both the estuary and bay via pelagic trawl. I used otolith microchemistry and growth increments to determine if growth rates for alewife differed between freshwater, estuarine, and bay habitats. Alewife had the fastest growth rate in fresh water and the slowest in estuarine habitat. Growth rates in the bay were marginally faster than in the estuary. These findings suggest that juvenile alewife may utilize bay habitats to facilitate growth as they age. Juvenile alewife appear to move actively to and from bay and estuarine habitats.*

**Keywords:** alewife, anadromous, bay, estuary, microchemistry, otolith,

## INTRODUCTION

Diadromous fish are uniquely different from other types of teleosts (Gross 1987) in their use of habitats with varying salinities, specifically ocean, lakes and streams, during their life history (Gross 1987, McDowall 1987). Fish that cross habitats of different salinities, such as diadromous species, need flexible osmoregulatory capabilities (Gross 1987). Anadromy represents one type of diadromy. Anadromous species grow at sea and reproduce in freshwater. However, anadromous species can exhibit alternate life histories, some species reproducing and growing entirely within freshwater habitats,

although their osmoregulatory capability to switch habitats persists (Jonsson 1985, Gross 1987). For example, alewife (*Alosa pseudoharengus*) have both anadromous populations and native and introduced land-locked populations persisting within their native range. These contrasting life histories lead to the question, why migrate across salinities, which is energetically costly due to physical exertion during migration and osmoregulatory changes (Gross 1987), when a fish can persist as a resident in fresh water?

Migration costs include physiological mechanisms for osmoregulation, energetic demands of swimming, differences in growth and survival, and increased exposure to predators (Gross 1987, Bauer et al. 2011, Chapman et al. 2012, Zydlewski and Wilkie 2013, Sheaves et al. 2014). However, benefits of diadromy exist, such as increased egg production (up to 3 times higher) in diadromous species compared to nondiadromous species (Gross 1987, Zydlewski and Wilkie 2013). Thus, diadromous species may experience as much as threefold higher mortality than nondiadromous fish, yet diadromy is still favored by natural selection.

The driving factor for evolution of diadromy is hypothesized to be differences in aquatic productivity (Gross 1987). Latitudinal trends in aquatic productivity influence trends in anadromy and catadromy (Baker 1978, Northcote 1978). Northern latitudes provide higher ocean productivity, whereas freshwater productivity at lower latitudes exceeds that of the ocean (Dodson et al. 2009). Thus, anadromy is more prevalent in northern latitudes, whereas catadromy (grow in fresh water, reproduce in the ocean) is more common in tropical latitudes (McDowall 1987).

Juvenile survivorship contributes to the evolutionary success of anadromy (Gross 1987, McDowell 1997). The nursery-role concept states that juvenile fish have a better

chance of maturation in an estuarine environment compared to the ocean, primarily because of lower predation risk due to abundant plant cover and fewer predators in estuaries (Beck et al. 2001). In addition, density of juvenile fish and prey items such as small invertebrates is typically higher in vegetated than in unvegetated habitats (Orth et al. 1984, Able 1999, Heck et al. 1997, Beck et al. 2001). Visual refugia, a lack of predation, and increased vegetation suggest that juvenile anadromous species should favor estuaries over adjacent habitats. However, recent research using otolith microchemistry suggests that variability in the timing of migration and associated habitat use is more variable among juvenile fish than once thought (Limburg and Turner 2016, Payne Wynne et al. 2015, Secor 2015, Turner and Limburg 2011 2016), contradicting the nursery-role concept. Variability in movement of juvenile alewife suggests a benefit to migration that exceeds safety of the estuary.

Like other diadromous species, alewife experienced precipitous declines in the last 200 years (Limburg and Waldman 2009) and have been the subject of research due to dramatic declines after the 1960s (Fay et al. 1983, Mullen et al. 1986, Loesch 1987, Dufour et al. 2005,). Current work focused on alewife movements based on salinity, prey densities, and stable isotope analysis is being conducted within the Penobscot estuary (Amy Webb, unpublished data, Justin Stevens, unpublished data, Rachel Lasley-Rasher, unpublished data). Recent work has found that similar active movements of alewife occur in other watersheds, e.g., the Mohawk and Hudson River systems in New York (Limburg and Turner 2016). Highly migratory behavior of juvenile alewife differs from conventional descriptions of northern river herring life history in the literature. A textbook life history description of juvenile alewife consists of generalized statements

such as, "juvenile alewife move downstream into more saline waters from natal freshwater lakes and eventually into the ocean" (U.S. Fish and Wildlife Service 2010, pp.1).

Historically, the Penobscot River watershed (New England's second largest watershed) was a valuable resource for predators and prey, both on land and aquatic. Estimates from 1869 reveal harvests of over one million river herring from the Penobscot River (Saunders et al. 2006), and, based on area of lake spawning habitat, the Penobscot Estuary was estimated to support over 50 million fish (Hall et al. 2012). In 2015, 589,000 river herring returned to the Penobscot River, a substantial increase from 2009, when only 2,336 river herring returned. Increased river herring return is primarily due to stocking and passage improvements (Maine Department of Marine Resources, 2015).

In this study, I investigated habitat-specific juvenile alewife growth rates in a northeastern estuary-bay system in which alewife of the same size and age were captured from both habitats. Based on the nursery-role concept, I hypothesized that juvenile alewife have faster growth rates in the estuary than in the bay. To test this hypothesis, I compared growth of juvenile alewives by measuring the width of daily growth increments in sagittal otoliths. I also used microchemistry analyses of sagittal otoliths to determine habitat use of the fish during its life span.

To measure growth in teleosts, biologists commonly use growth increments in sagittal otoliths (Campana 2005, Campana and Neilson 1985), a feature discovered by Pannella (1971). Otoliths are incrementally precipitated aragonite structures found in the inner ear of teleost fish (Andrus et al. 2002). Three pairs of otoliths occur in the inner ear (sagittae, lapille, and asteriscii). Sagittal otoliths are the largest ear bones and are used to



determine season of birth, age at death, growth rate, and habitat use (Secor et al. 1995). Otolith formation and growth are biologically controlled and influenced by endogenous factors that correlate with photoperiod, resulting in a 24-hour growth period (Tanaka et al. 1981), which leads to distinctive rings in the bone. A dark band signifies the end of a growth period (Figure 1). Distance between bands is associated with growth rate and corresponds to an increase in fish length. Sismour (1994) validated use of daily increments in otoliths to age juvenile alewives.

Importantly, the chemistry (or elemental composition) of otoliths reflects the elemental composition of the fish's environment. Ions from ambient water are incorporated into blood plasma via branchial or intestinal uptake, cross inner ear membranes into endolymph fluid, and finally precipitate onto the growing surfaces of the otolith (Payan et al. 2004, Sturrock et al. 2012). Microchemistry analysis uses strontium ( $^{86}\text{Sr}$ ) and barium ( $^{138}\text{Ba}$ ) to calcium ( $^{43}\text{Ca}$ ) ratios in otoliths to assess habitat use (Dufour et al. 2005, Elsdon and Gillanders 2005, Arai 2010, Limburg 2011, Reis-Santos et al. 2013, Clement et al. 2014, Smith and Kwak 2014). Sr is recognized as a proxy of water salinity (Elsdon and Gillanders 2003, Gillanders 2005, Reis-Santos et al. 2013, Panfili et al. 2015), whereas Ba is indicative of fresh water (Elsdon and Gillanders 2005). Because materials (including Ca, Ba, and Sr) are laid down sequentially in otoliths, analyzing from the center to the outer edge of the otolith allows evaluation of habitat use through time. By overlapping otolith microchemistry (habitat) and growth increment (growth rates) data, we can measure growth rate for any habitat identified using the elemental composition of otolith growth increments.

## METHODS

Trawl surveys conducted by the National Oceanic and Atmospheric Administration (NOAA) and the Maine Department of Marine Resources (MDMR) demonstrate that juvenile alewife of similar size and age occur in both the Penobscot River estuary and bay. NOAA and MDMR caught juvenile alewife for this study via trawls in the Penobscot Estuary and Bay (Figure 2) in Maine during May and October, 2013 and 2014. Trawls lasted 10–20 min and ranged 1100–2000 m in length due to changes in currents, tidal fluctuations, and wind strength. MDMR bay trawls were conducted at 9–100 m depth, whereas NOAA trawls occurred within the top 6 m of the estuary. Fish were grouped according to trawl month (Spring or Fall), habitat (Bay or Estuary), and year (2013 or 2014) because bay trawls were only conducted during these times. Two trawls caught no juvenile alewife (Spring Bay 2013 and Spring Estuary 2014), for a total of 6 groups. Fish were killed with an overdose of MS-222, frozen immediately in a slurry of dry ice and saline water, and transported to the University of Southern Maine, Gorham, Maine. Prior to analysis, fish thawed for a minimum of 10 min. Fish were weighed using a Sartorius GE812 balance (to the nearest 0.01 g), measured with a Wildco fish board (total and fork length to the nearest 1 mm), and dissected to remove both sagittal otoliths.

Otoliths were placed in a 1:1 bleach and water bath to remove all organic material, and then rinsed twice with deionized water to remove any remaining organic and non-organic materials. Both bleach and deionized water baths lasted 1–2 min. Cleaned otoliths were covered and air-dried in a ventilated chamber at room temperature for 15 min and were mounted sulcus side down with Scotch® Advance Formula

superglue onto a glass cover slide. Cover slides were trimmed with a diamond tip pen to approximately  $5 \times 5$  mm and mounted onto a glass slide with Quickbond mounting glue. Only one otolith was mounted per slide. Prepared otoliths dried for 72 h before polishing. Two polishing techniques were used to polish otoliths to reach the core while revealing readable increments. The first method used an electric lapping wheel (Struers DAP-V) with a 3M 12.7 cm diameter abrasive disc of 5  $\mu$ m grit. The lapping wheel was set at 200–300 rpm with water constantly flowing over the lapping disc. Applying moderate pressure, otoliths were sanded for 8–15 sec. The second method consisted of sanding by hand. A glass plate was thoroughly washed and covered with several lapping papers ranging from 3–5  $\mu$ m grit. To reduce friction, water was added to the lapping paper while sanding. When the daily growth increments were visible using a compound microscope set between 5X–20X magnification, otoliths were rinsed and stored in a protective slide box to avoid contamination.

I used Donohoe and Zimmerman's (2010) multiple otolith mounting method to prepare sanded otoliths for laser ablation inductively coupled plasma mass spectrometry (LA ICP-MS). I applied heat to melt the Quickbond mounting glue, allowing removal of individual otoliths mounted on coverslips. Otoliths on coverslips were grouped in 2 rows of 5 on the top left corner of a petrographic slide labeled with a diamond tip pen to identify otoliths. Grouping otoliths this way allowed for easier transfer into the laser ablation chamber, reduced the need to open the sample chamber and thus increased sample throughput, and reduced instrument run time when performing beam-based microchemical analyses (Donohoe and Zimmerman 2010).

## WATER CHEMISTRY

To establish elemental criteria for distinguishing habitat movement using otolith microchemistry, water samples from the Penobscot watershed were analyzed for Sr, Ba, and Ca. A total of nine water samples were taken, three from each habitat (freshwater, estuary, and bay). Samples were taken in June 2015 from three freshwater sources (Mattamiscontis Lake: 45.475, -68.674, South Branch Lake: 45.369, -68.695, and Pushaw Lake: 44.899, -68.788, the Penobscot Estuary (Bucksport: 44.669190, -68.813271), and the Gulf of Maine (Head Harbor, Isle au Haut: 44.008696, -68.617163). Water samples (50 ml) were filtered through 45-micron nylon filters and fixed in 2% nitric acid in the field in 2% HCl rinsed polypropylene bottles. Salinity was measured in the field at the time of water collections with a calibrated handheld YSI Pro Plus. Samples were analyzed at State University of New York College of Environmental Science and Forestry (SUNY-ESF) for concentrations of Ca, Sr, and Ba via inductively coupled plasma optical emission spectrometry (Perkin Elmer Optima 3300DV). Differences between mean Ba and Sr levels were tested using a one-way analysis of variance (ANOVA) where  $\alpha = 0.05$  (RStudio Version 0.99.896).

Generally, for otolith microchemistry studies, partition coefficients are used to detect the osmoregulatory resistance that an element encounters when transported from the water, through the blood, into the endolymph system, and onto a precipitation site of the otolith (Brown and Severin 2009). A partition coefficient of 1 indicates no resistance relative to Ca, and a value of 0 indicates high resistance (Campana 1999). However, to calculate this value appropriately, fish otoliths need to equilibrate in water of known

elemental composition. For fish moving between estuarine and bay habitats, obtaining this information was beyond our resources.

Sr is positively correlated with higher salinity levels (Secor et al. 1992), whereas Ba is abundant in soils, making it more common in freshwater habitats (Elsdon and Gillanders, 2005). Marine fishes derive 83% and 98% of their otolith Sr and Ba, respectively, from surrounding water (Walther and Thorrold, 2006). Due to the predictable behavior of Sr and Ba, I established a habitat transition criterion capable of distinguishing habitat movements using otolith microchemistry. Mean Ba, Ca, and Sr were calculated for all three-habitat types (Table 1). The Sr cutoff was  $6.32 \text{ Sr: Ca} \times 1000$ , whereas Ba was  $0.004 \text{ Ba: Ca} \times 1000$  (Table 2). Using these cutoffs as transitional cues, I segmented otolith microchemistry data transects into habitat segments (e.g., Figure 3).

## MICROCHEMISTRY ANALYSES

Sr, Ba, and Ca were quantified by laser ablation inductively coupled plasma mass spectrometry (LA ICP-MS) using a PerkinElmer DRC-e ICPMS along with a UP-193 laser ablation system at SUNY-ESF (Appendix A). The laser was run at  $3 \mu\text{m}/\text{sec}$ , with a  $35 \mu\text{m}$  diameter replicate spot, at a distance of  $8.745 \mu\text{m}/\text{replicate}$ , set at 70% power. Before each sample was ablated, the laser warmed up for 60 sec. After a sample was complete, a resting period of  $50\% \cdot \text{runtime}$  was allotted between samples. The laser followed a transect drawn from the edge of the otolith to slightly beyond the core (to ensure the core was properly analyzed), parallel to the same path as growth increment measurements (Figure 3). One standard consisting of a homogenized pellet of freshwater drum otoliths (Table 3) was run before, during, and after ablation to detect instrumental

drift (change in sensitivity), which can impact estimated elemental concentration; therefore, data were adjusted accordingly (Campana and Gagné 1995).

## GROWTH INCREMENTS

To calculate growth rate accurately within a given habitat, growth increments are counted and measured. To count growth increments accurately, I compiled high-resolution photos of each otolith. Otoliths were imaged with an Olympus BX60 compound microscope using 50X, 100X, 200X, or 500X magnification. A Tucsen 5.0 MP camera was mounted to the microscope, and photos were captured with a resolution of  $2592 \times 1944$  pixels using Tsview Version 6.2.3.3. Photos could be enlarged and refined up to an additional 200X with the Tsview program (Klumb et al. 2001). Photos were taken in separate sections and collaged using Adobe Photoshop layout process. After collaging was complete, ImageJ 1.48v software was used to count growth increments, measure distances between increments, and capture images of increments.

An independent observer twice counted each otolith for total number of increments. If counts varied by more than 1 SD, a third reading was done (Durovic et al. 2012, Jenkins and Davis 1990, Turner and Limburg 2011). However, a third reading was not needed for any otolith. Increments were counted in a straight line starting at the core's outer D-matrix (dark rings) and moving towards the posterior edge of the otolith (Figure 1). Transects with the clearest readable path were chosen. Once otolith increment counts were decided, markers were placed at the posterior portion of each incremental D-matrix relative to the core (Campana 1992, Campana and Neilson 1985).

I estimated growth rate by comparing individual growth increments to the mean of all growth increments within the individual otolith (Ralston and Williams 1988):

$$(1) \quad \Delta G_h = \frac{\sum_{i=1}^n \overline{I_{hi}}}{n}$$

where  $n$  is sample size,  $i$  is an individual fish,  $h$  is the habitat (freshwater, estuary, or bay) for fish  $i$ ,  $I$  is the increment width for fish  $i$ ,  $\overline{I_{hi}}$  is mean incremental width within one of the three habitats (freshwater, estuary, and bay) for fish  $i$ , and  $\Delta G_h$  is the mean increment growth rate ( $\mu\text{m}/\text{day}$ ) for each habitat of all fish. Equation 1 standardizes growth increments across all samples, allowing comparisons of growth rates among habitats. Increments were categorized by a specific habitat (freshwater, estuary, or bay) based on the microchemistry analysis of otoliths along the same transect from core to posterior edge of the otoliths.

Similar otolith microchemistry patterns can group fish caught in varying habitats by their natal watersheds, assuming individuals have similar habitat use (Payne Wynne et al. 2015, Turner and Limburg 2011). Otolith microchemistry patterns of Ba:Ca and Sr:Ca among all alewife were visually examined by grouping fish by season, year, age, total length (mm), and weight (g). Otolith microchemistry patterns with homologous Ba:Ca and Sr:Ca ratios and timing in migration were considered to have similar microchemistry patterns.

## STATISTICAL ANALYSES

One-way ANOVAs were used to compare differences in growth rates, weight:length ratios, mean increment width, and increment counts among groups. Significance was set at  $\alpha = 0.05$ . All groups were tested for assumptions of homogeneity of variance and normality. If an ANOVA resulted in a statistically significant result, Tukey's honest significant difference post-hoc test was used to determine which groups

differed in the analyses, again with a significant  $\alpha$  level of 0.05. Also, due to low sample size, an  $\alpha$  level of 0.05–0.10 was considered marginally significant.

I used simple linear regression to determine if mean increment width was related to fish age. Weight:length ratios (W:L) also were compared to growth and mean increment width data using one-way ANOVA, as an indicator of condition (Greenstreet and Stuart 2006). All statistical analyses were done with R 3.2.1 (R Core Team 2015).

## RESULTS

### LENGTH AND WEIGHT PATTERNS

I analyzed 91 juvenile alewife, 46 from Penobscot Bay and 45 from Penobscot Estuary. All alewife fell within the 0–1 age class, which was reflected in growth increment counts as well as total length and weight. Across all groups, mean length was  $104 \pm 18$  (SD) mm (range = 65–155 mm; Figure 4), and mean weight for all alewife was  $9.73 \pm 6.30$  g (range = 2.08–42.66 g; Figure 5). Group Fall Bay 2013 had the largest mean length ( $128 \pm 16$  mm) and weight ( $19.20 \pm 9.08$  g), whereas Fall Estuary 2013 had the smallest mean length ( $90 \pm 19$  mm) and weight ( $6.27 \pm 4.23$  g).

Weight:length (W:L) varied significantly between groups, and groups explained 50% of W:L variation ( $F_{5,85} = 16.33$ ,  $p < 0.001$ ,  $R^2 = 0.49$ ; Figure 6). W:L in Fall Bay 2013 was larger than all other groups (Tukey test:  $p < 0.05$ ), whereas only Fall Bay 2014 and Fall Bay 2013 differed from one another (Tukey tests;  $p < 0.05$ ; Figure 6).



## WATER CHEMISTRY

Fresh water had a salinity of 0 ppt, the upper Penobscot Estuary had a salinity of 5.5 ppt, and the upper Penobscot Bay had a salinity of 27 ppt. Ba levels in water differed significantly among habitats (ANOVA:  $F_{2,8} = 10.16$ ,  $p = 0.012$ ; Table 2). Ba was 2x higher in fresh water compared to estuary and bay (Tukey HSD:  $p < 0.05$ ; Table 2), whereas Ba did not differ between estuary and bay. Sr levels also differed significantly among habitats (ANOVA:  $F_{2,8} = 585.7$ ,  $p < 0.001$ ). Sr levels in the bay were as much as 5x higher than the estuary and up to 300 times higher than fresh water (Table 2).

## HABITAT USE INFERRED FROM MICROCHEMISTRY PATTERNS

Fish spent a minimum of 5 days in fresh water (one individual from Fall Estuary 2014), whereas the maximum amount of time was 251 days (one individual from Fall Bay 2013). Time spent in fresh water differed significantly among groups (ANOVA:  $F_{5,85} = 4.29$ ,  $p = 0.001$ ; Figure 7). However, post hoc tests revealed that Spring Estuary 2013 ( $104 \pm 31$  fresh water days) was significantly greater than Fall Bay 2014 ( $49 \pm 15$  fresh water days, Tukey HSD:  $p < 0.001$ ; Figure 7). Time spent in estuarine habitat did not differ significantly among groups (ANOVA:  $F_{5,81} = 0.95$ ,  $p = 0.45$ ; Figure 7); however, it varied widely among fish, ranging from 2–139 days. Alewife differed in time spent in bay habitat (ANOVA:  $F_{5,36} = 3.07$ ,  $p = 0.02$ ; Figure 7), but only two groups differed significantly, with fish collected in Fall Bay 2013 spending as much as a month more time in the bay than those from Fall Bay 2014 (Tukey HSD:  $p < 0.05$ ; Figure 7). Fish from all groups spent as little as 1 day in the bay or as much as 85 days.

According to microchemistry analysis of otoliths, all alewives spent time in fresh water ( $n = 91$ ), 87 alewives (95.6%) moved to the estuary, and 42 alewives (46.2%)

reached the bay. Mean number of days spent in each habitat differed significantly (ANOVA:  $F_{2,218} = 38.641$ ,  $p < 0.001$ ; Figure 8). Fish spent the most time in fresh water, followed by estuary, and then bay (Tukey HSD: all  $p < 0.001$ ; Figure 8).

No fish re-entered freshwater habitats after migration, and all fish that entered the bay entered from the estuary. Overall, alewife entered the bay after  $65 \pm 19\%$  of their life span was complete, but many fish returned to the estuary after spending time in the bay. Only three fish (7%), after reaching the bay, did not return to the estuary for any amount of time, while 93% of fish that reached the bay migrated to and from bay and estuarine habitats ( $n = 39$ ; Figure 9).

#### HABITAT SPECIFIC GROWTH RATES

Mean increment count for all 91 samples was  $135 \pm 42$  increments (days), corresponding to a June hatch date, which is consistent with the life history of alewives in Maine (Flagg 2007). Increment count varied significantly among groups, ranging from 79–292 (ANOVA:  $F_{5,85} = 5.62$ ,  $p < 0.001$ ; Figure 10). Post hoc comparison showed that all groups differed from at least one other group (Tukey HSD:  $p < 0.05$ ; Figure 10). Individual mean incremental width ranged from 2.1–7.7  $\mu\text{m}$ , with an overall mean of  $4.8 \pm 1.1 \mu\text{m}$  (Figure 10).

For all fish, within the first two weeks, growth increments were smaller (1–6  $\mu\text{m}$ ), and rapidly expanded (10–16  $\mu\text{m}$ ) for nearly one week. Increments then decreased in width and resumed relative uniformity, with occasional outliers, for the remainder of the fish's life span (2–13  $\mu\text{m}$ ).

Incremental width was negatively related to increment count ( $F_{1,90} = 82.75$ ,  $p < 0.001$ ,  $R^2 = 0.66$ ; Figure 11), with a mean incremental width decrease of 0.02  $\mu\text{m}$  for

every additional increment counted. Thus, as fish age, mean incremental width decreased, equating to slower growth.

Growth rates ( $\Delta G_h$ ) among groups within each habitat showed little variation.  $\Delta G$  did not differ among groups in fresh water (ANOVA:  $F_{5,85} = 0.18$ ,  $p = 0.969$ ), estuary (ANOVA:  $F_{5,81} = 0.99$ ,  $p = 0.43$ ), or bay (ANOVA:  $F_{5,36} = 0.57$ ,  $p = 0.720$ ). However, when increment widths were analyzed by habitat (i.e., ignoring groups),  $\Delta G$  differed significantly (freshwater:  $5.23 \pm 1.35$   $\mu\text{m}/\text{day}$ , estuary:  $4.26 \pm 1.75$   $\mu\text{m}/\text{day}$ , bay:  $4.79 \pm 1.72$   $\mu\text{m}/\text{day}$ ; ANOVA:  $F_{2,218} = 18.49$ ,  $p < 0.001$ ; Figure 12).  $\Delta G$  was higher in fresh water compared to bay (Tukey HSD:  $p = 0.017$ ) and estuary (Tukey HSD:  $p < 0.001$ ) and  $\Delta G$  was marginally different between estuary and bay habitats (Tukey HSD,  $p = 0.083$ ).

## DISCUSSION

Alewives captured in the Penobscot Bay-Estuary system grew fastest in fresh water, followed by bay, then estuary. Using otolith microchemistry, regular migrations were made between estuary and bay habitats, but no fish returned to fresh water, suggesting that alewife utilize both bay and estuary habitats during the juvenile (age 0+) life stage.

## WATER CHEMISTRY

To ensure detectability of the presence of a fish at all points within any given habitat, I took estuary and bay water samples from areas with the lowest salinity still considered to be estuary (5 ppt) or bay (27 ppt). Freshwater, estuary, and bay habitats differed distinctly in Ba or Sr values, which indicated that Ba and Sr were suitable for identifying habitat change in otoliths. Because I did not apply a partition coefficient, I

assumed that high levels of Ba or Sr in water chemistry were reflected in otolith composition (Elsdon and Gillanders 2005, Gillanders 2005, Reis-Santos et al. 2013, Panfili et al. 2015). Therefore, the cutoff for Ba:Ca and Sr:Ca levels were chosen based on water samples (Table 2). If cutoff levels for Ba:Ca and Sr:Ca increased, days spent in the estuary would increase, and days spent in the bay would decrease. Conversely, if cutoff levels for Ba:Ca and Sr:Ca decreased, days spent in bay habitat would increase and days spent in the estuary would decrease.

Lag time of otolith elemental composition potentially could delay detection of Ba:Ca and Sr:Ca shifts. Lag time ( $> 15$  d) between changes in water chemistry and subsequent changes in otolith elemental composition exist in other fish species (Elsdon and Gillanders 2005, Lowe et al. 2009, Macdonald and Crook 2010, Miller 2011). A lag time of 15 days would cause delayed habitat shift detection, thus giving an inaccurate representation of time spent in a given habitat. Lag times may explain why the otoliths of some fish caught in the estuary showed the most recent habitat use (i.e., the edge of the otolith) as bay and vice versa. If fish moved back and forth daily between the two habitats, it would be hard to distinguish these movements in the otoliths, even if a lag existed, because the sampling replicate (i.e. spot on otolith sampled by laser) encompassed approximately 2 daily increments. Distinguishing daily habitat movements would require a smaller dot size ( $< 5 \mu\text{m}$ ) when performing laser ablation, to ensure each daily increment was analyzed. If fish moved rapidly between estuary and bay habitats, as it appears some fish did, ratios of Sr:Ca would be expected to reflect movement, but not reach equilibrium with either environment.

## HABITAT USE AS INFERRED THROUGH OTOLITH MICROCHEMISTRY

Even though fish analyzed were all age 0+ and had similar weights and lengths, otolith microchemistry revealed different migration patterns. Sr:Ca and Ba:Ca ratios from otoliths fell within expected ranges for the Penobscot watershed, based on water chemistry data. Within the Penobscot watershed, fine scale movements were undetectable (Turner and Limburg 2011) because we did not have a fine scale water chemistry map. However, large-scale movements across fresh water, estuary, and bay were readily discernable using a combination of Sr:Ca and Ba:Ca ratios. Using the habitat differentiation criteria, many fish migrated back and forth from bay to estuary habitats (20%). Other data showed similar behaviors in alewife (Amy Webb, unpublished data, Justin Stevens, unpublished data, Turner and Limburg 2011). As more otoliths of migratory species are analyzed, I expect juvenile migratory behavior between estuary and bay habitats to be more common than previously thought. By cross examining differences in juvenile movements of various diadromous species using otolith microchemistry, conservation and restorative efforts can become more accurate and holistic, incorporating a slew of species. Furthermore, migratory patterns of diadromous species may become more distinguished from one another. Rather than grouping individuals as anadromous and catadromous, more specific categories may be needed to separate different life histories present within diadromous species.

One aspect of otolith microchemistry that is not well studied is turnover rate of the endolymph pools from which otoliths receive elements. Endolymph pools contain organic matrix precursors, such as Ca, Ba, and Sr. Daily deposition of  $\text{CaCO}_3$  into otoliths corresponds to 7 and 1 time(s) the total content of endolymph pools (Payan et al. 2004), suggesting that renewal time of endolymph fluid should be at most one day (Payan

et al. 2004). If endolymph fluid truly has a turnover rate of one day, receiving new elements from ambient water daily, a lag time for otolith matrix creation would be at maximum one day. However, only a small fraction (1%) of the organic precursors present in the endolymph is used in otolith matrix formation per day (Payan et al. 2004), leading to the question, do precursors not used in matrix formation impact future matrix formation? Currently, turnover time in the otolith matrix using precursors Ca, Ba, and Sr are being investigated for juvenile alewife (Webb et al., unpublished data). If turnover time exceeds one day, then we need to adjust elemental analyses in otoliths for a given lag period.

Even though lag times may exist for Ba:Ca and Sr:Ca, the crystallization process of otoliths is controlled by the rate of matrix protein formation and is inversely proportional to otolith Sr:Ca (Campana 1999). The rate of protein synthesis is correlated with metabolic rate, temperature, and somatic growth rate, which explains temperature and growth rate effects on otolith Sr:Ca (Campana 1999, Radtke 1989, Sadovy and Severin 1992, Townsend et al. 1989). Faster somatic growth is correlated with otolith matrix formation, which is reflected in net protein synthesis that incorporates elements onto the otolith (Campana 1999). Alewife analyzed in this study were all age 0+ and tend to have faster somatic growth rate than their older counterparts (Norden 2011). Due to quick somatic growth for the alewife of this study, there is a high degree of certainty that if any lag period exists, it would be minimal, with no significant impact on otolith microchemistry analysis.

## HABITAT SPECIFIC GROWTH RATES

Patterns of growth increments were consistent among all fish, which were small for the first two weeks (1–6  $\mu\text{m}$ ), then rapidly expanded for approximately one week (10–16  $\mu\text{m}$ ), when the fish lived in fresh water. Once migration occurred, fish growth became relative uniform when the fish used estuary and bay habitats, with occasional bursts and slumps of growth (2–13  $\mu\text{m}$ ). Many factors impact growth rates in juvenile alewife such as age, temperature, prey availability, prey selection, and fish density (Campana 1999, Đurović et al. 2012, Stevenson and Campana 1992). Because all alewives studied were age 0+ and had similar weights and lengths, a lack of variation in growth increments among groups was not surprising. However, between habitats, growth rate differed among individual fish.

For all fish, mean growth rate in the bay (4.74  $\mu\text{m}/\text{day}$ ) was marginally faster than mean growth rate in the estuary (4.24  $\mu\text{m}/\text{day}$ ). With an increased sample size or a larger size range (e.g., Limburg and Turner 2016), particularly for bay fish, estuary and bay growth rates may begin to differ more clearly. Alewife did not enter the bay until approximately 65% of their life was complete, which should correspond to slower bay growth rates. However, significantly slower growth in the bay compared to estuary was not consistent among juvenile alewife, contributing to the growing notion that juvenile alewife easily adjust growth to environmental conditions in early life stages. Fish also re-entered estuarine habitat after spending at least one day in the bay. Migratory behavior between estuary and bay habitats suggests that either alewife may not distinguish estuary and bay habitats from one another or each habitat provides for specific needs at different life stages.

The nursery-role concept is applicable to the Penobscot Estuary, in that the estuary contains fewer predators compared to the bay. Although avian and seal predation may exist in the estuary, the Penobscot Estuary is turbid and may provide refuge from these visual predators. The Penobscot Bay appears to provide a habitat in which juvenile alewife can facilitate growth. Alewife seemingly utilize the estuary and bay not as separate habitats, but as a continuous environment that provides different tools to satisfy different needs, such as growth and protection. Other species, such as blueback herring (*Alosa aestivalis*) and American shad (*Alosa sapidissima*), exhibit similar life history traits as alewife, and juveniles also have been captured in the Penobscot Estuary along with alewife. Atypical migratory behavior has been observed in bluebacks and alewife as far south as New York (Limburg and Turner, 2016), and these movements suggest that connectivity between bays and estuaries is important for these species. To improve connectivity, managers could remove tide gates in small estuaries or dams that restrict tidal waters, or they could monitor bycatch at river mouths where juveniles migrate between habitats (Bethoney et al. 2013).

#### PATTERNS WITHIN HABITAT USE

Patterns of Sr:Ca and Ba:Ca over the life span of 89% of examined fish were similar, showing characteristic Ba:Ca transitioning to low levels, and low Sr:Ca transitioning to higher levels associated with a migration to more saline waters. However, four fish from two groups, Fall Estuary 2013 and 2014, showed unusual otolith microchemistry patterns, where Ba:Ca and Sr:Ca were constant until approximately 400  $\mu\text{m}$  (unusually late) from the core, where Sr:Ca began to rise and Ba:Ca began to drop. These unusual fish spent minimal time in freshwater (2–18 days) relative to other fish



(mean number of days in fresh water =  $75 \pm 39$  days). These patterns are inconsistent with the general life history of juvenile alewives (Flagg 2007, Figure 13). However, similar patterns were observed in blueback herring adults that originated in natal lakes that exited immediately into salt marshes with no transition zone (Payne Wynne et al. 2015). Further studies on Penobscot water chemistry and juvenile alewife movement are needed to fully understand what these unique alewife movements mean.

## CONCLUSION

Growth rates and habitat use of juvenile alewife appear to have a connected relationship that may play a role in dictating juvenile alewife migrations. Findings of natural variation and non-text book migratory behavior in juvenile alewife support recent findings (Turner and Limburg 2016, Limburg 2001, Payne Wynne et al. 2015, Secor 2015). Clearly, juvenile diadromous species utilize habitat transition zones more frequently than the literature has previously portrayed. As conservation efforts continue to reconnect historic watersheds, transition zones should be a primary focus, especially for systems containing diadromous species.

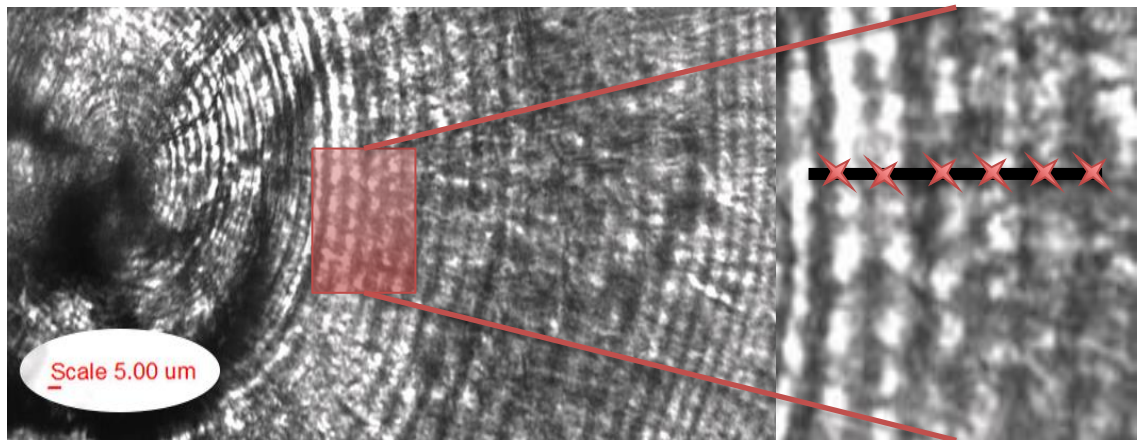


Figure 1. A magnified section of otolith from juvenile alewife ID 1064, group Spring Bay 2014. Stars along the black transect indicate measuring points used to calculate incremental width ( $\mu\text{m}$ ), mean incremental width ( $\mu\text{m}$ ), and increment counts. For all otoliths, points are measured on the posterior edge of the d-matrix (dark lines).

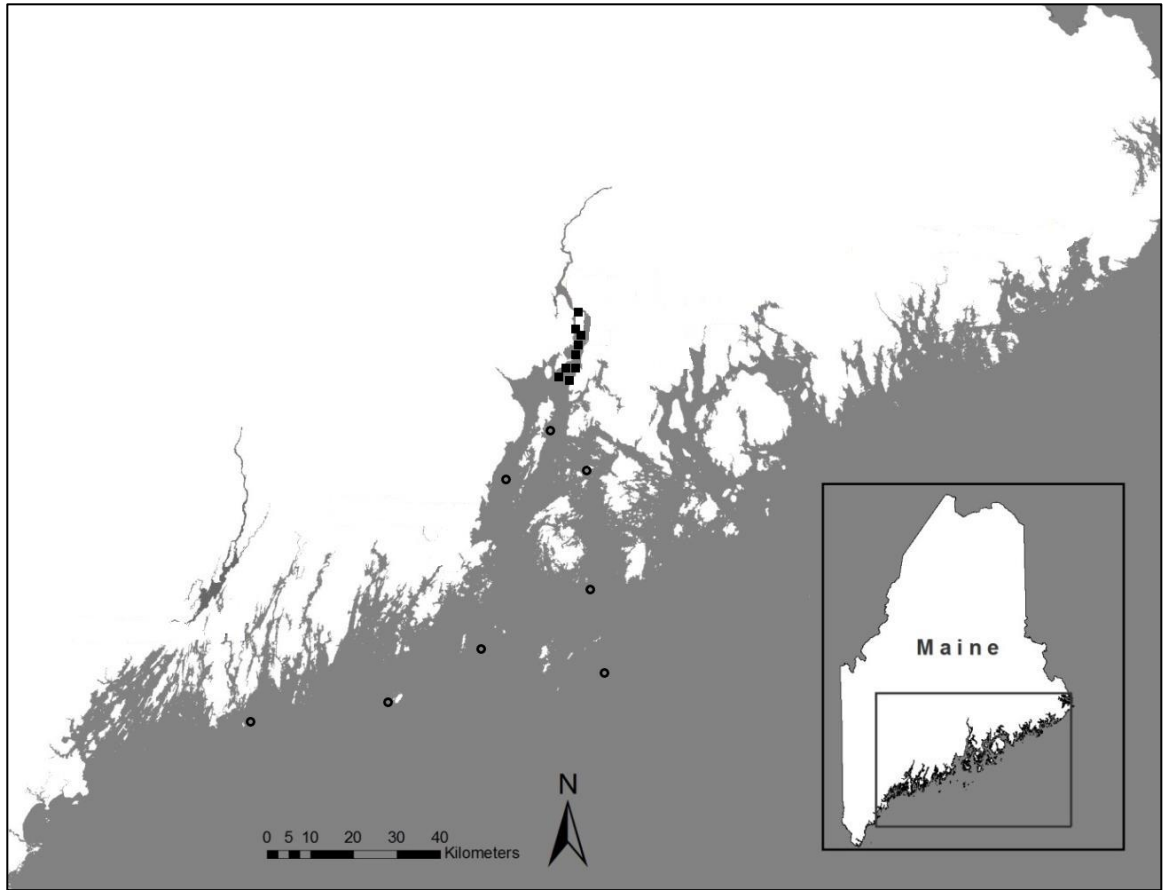


Figure 2. Location site of Penobscot Estuary (square) and Bay (circle) trawls for alewife conducted by NOAA and MDMR during October and May, 2013 and 2014.

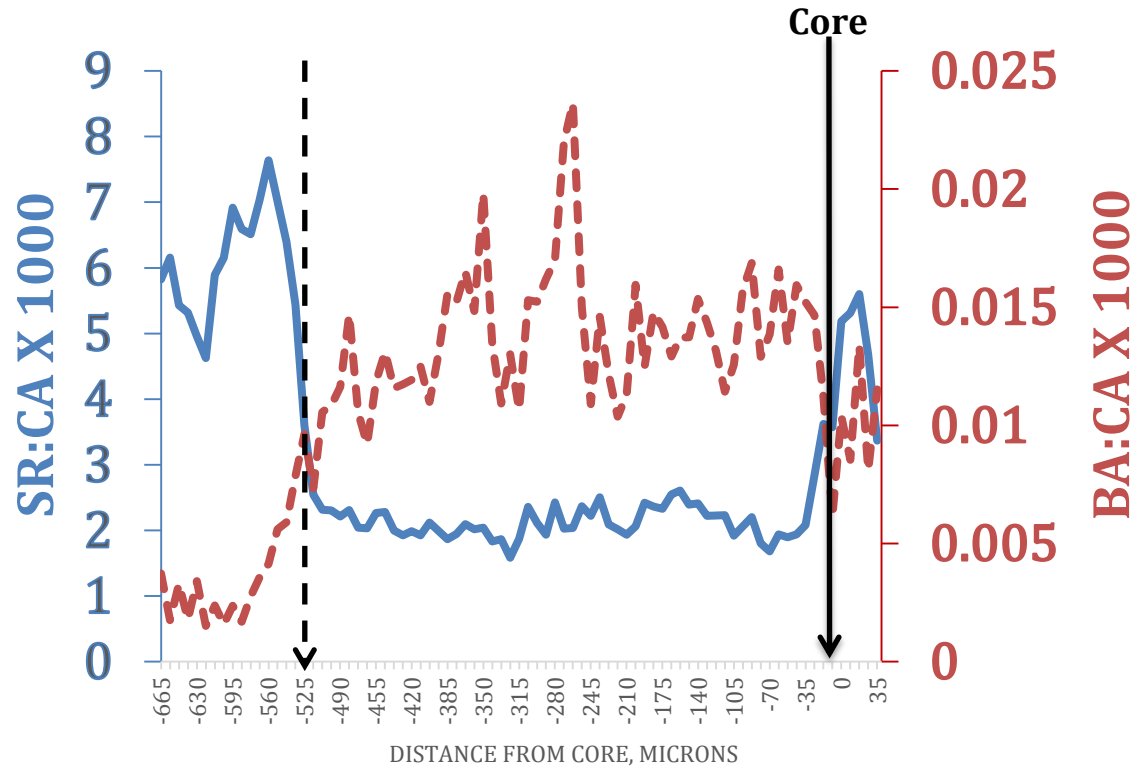


Figure 3. Sr:Ca (solid line) and Ba:Ca (dashed line) ratios starting from the core (solid arrow) moving to the posterior edge of the otolith (665  $\mu\text{m}$ ), showing transitional movement (dashed arrow) from freshwater to an estuarine habitat. This alewife (ID 79) was caught during spring 2013 in the Penobscot Estuary, Maine.

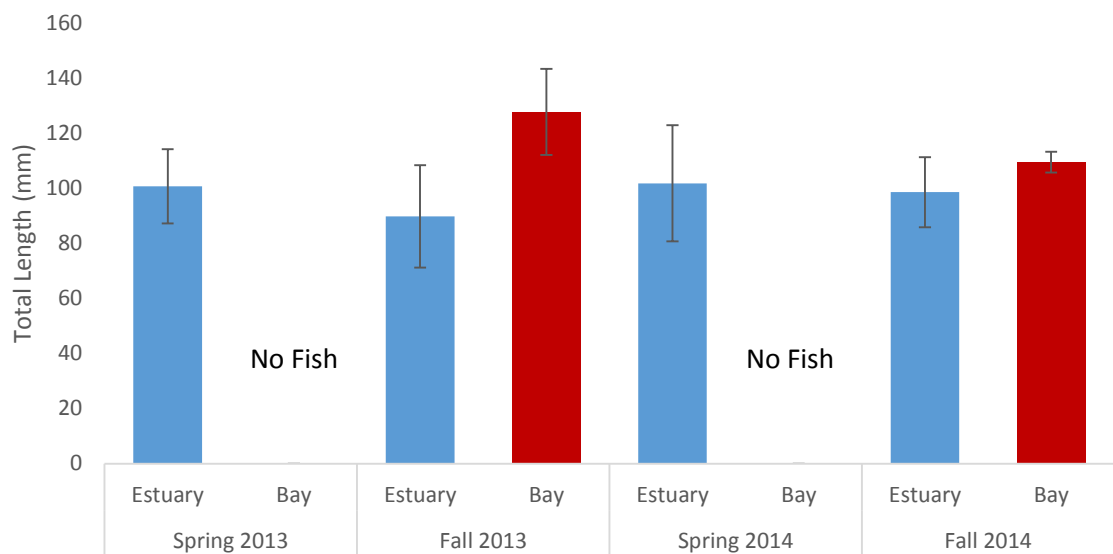


Figure 4: Mean ( $\pm$  SD) length (mm) of juvenile alewife caught via trawl in May (Spring) and October (Fall), 2013 and 2014 in the Penobscot Estuary and Bay, Maine. Trawls conducted in Spring Bay 2013 and Spring Bay 2014 caught no juvenile alewife.

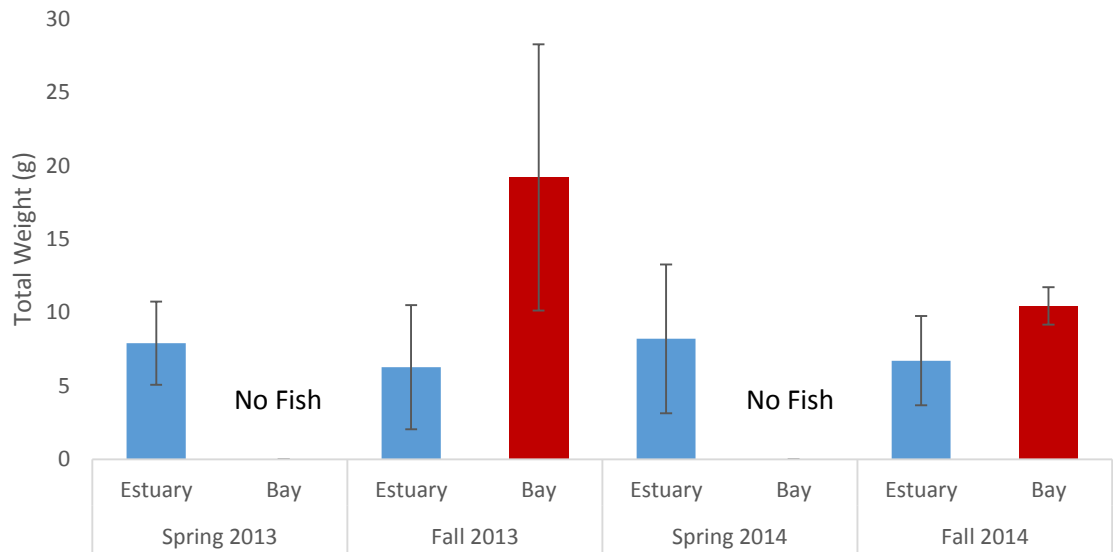


Figure 5: Mean ( $\pm$  SD) weight (g) of juvenile alewife caught via trawl in May (Spring) and October (Fall), 2013 and 2014 in the Penobscot Estuary and Bay, Maine. Trawls conducted in Spring Bay 2013 and Spring Bay 2014 caught no juvenile alewife.

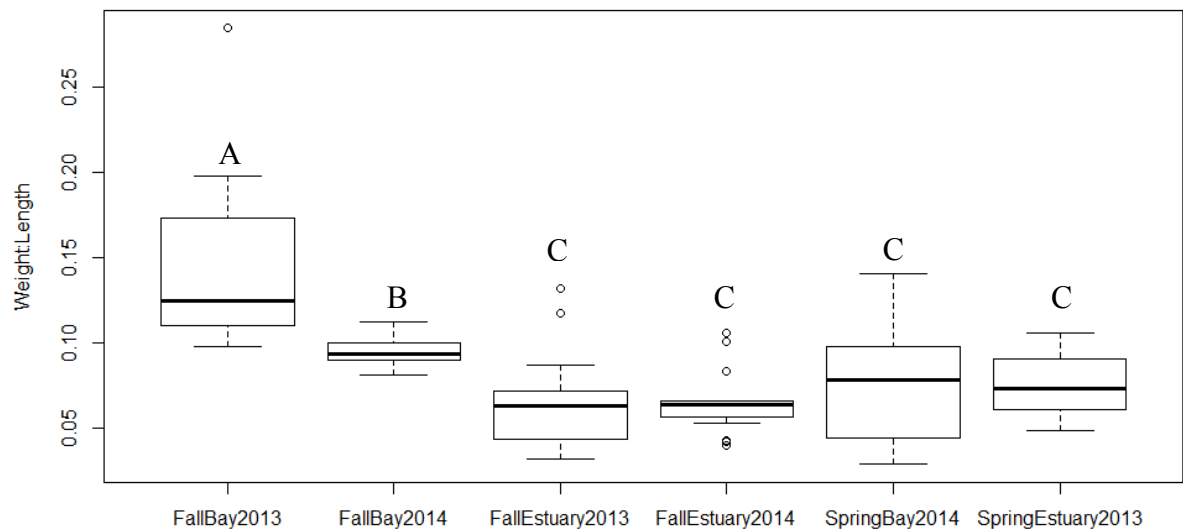


Figure 6. Box and whisker plot for weight (g):length (mm) ratios among groups of alewife caught in October (Fall) and May (Spring), 2013 and 2014 from the Penobscot Bay and Estuary, Maine. Different letters indicate statistical differences in means (Tukey HSD,  $p < 0.05$ ).

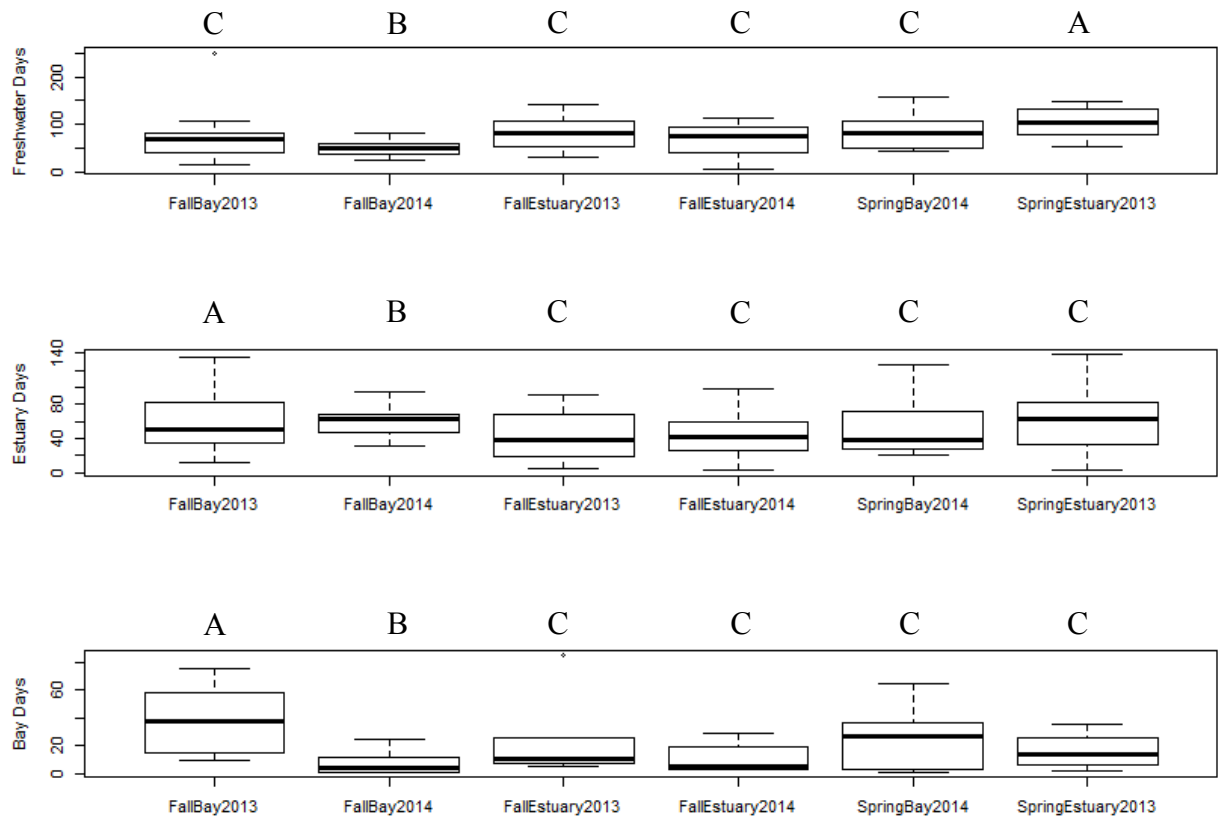


Figure 7. Box and whisker plots for number of days spent by juvenile alewife within each habitat among each group. Statistical differences within groups for mean freshwater days ( $n = 91$ ), estuary days ( $n = 89$ ), and bay days ( $n = 42$ ) are designated by different letters. All fish experienced freshwater days, 98% of fish spent time in the estuary, and 46% of all alewife spent time in the bay.



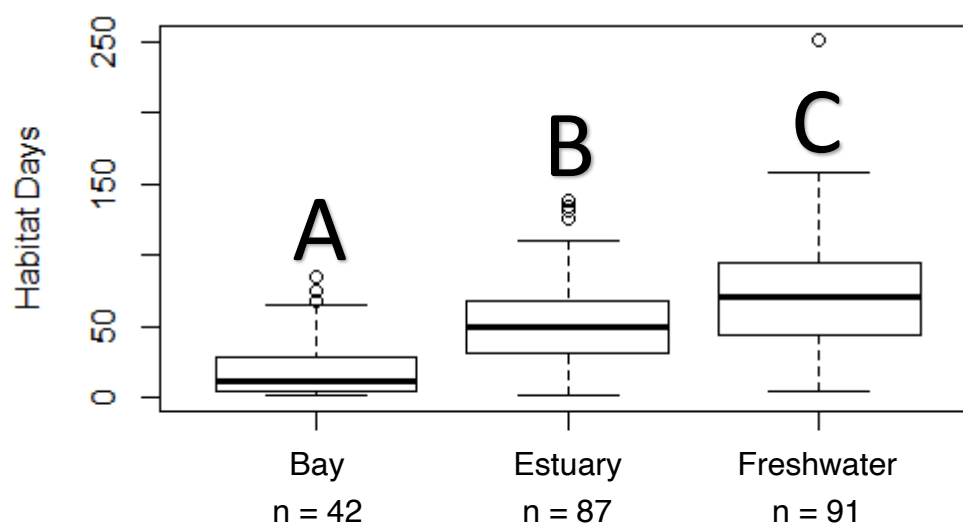


Figure 8. Box and whisker plot showing days spent by juvenile alewife in bay, estuary, and freshwater habitats regardless of group. Overall, fish spent the most time in fresh water and the least amount of time in the bay. Different letter indicate statistical differences in means (Tukey HSD,  $p < 0.05$ ).

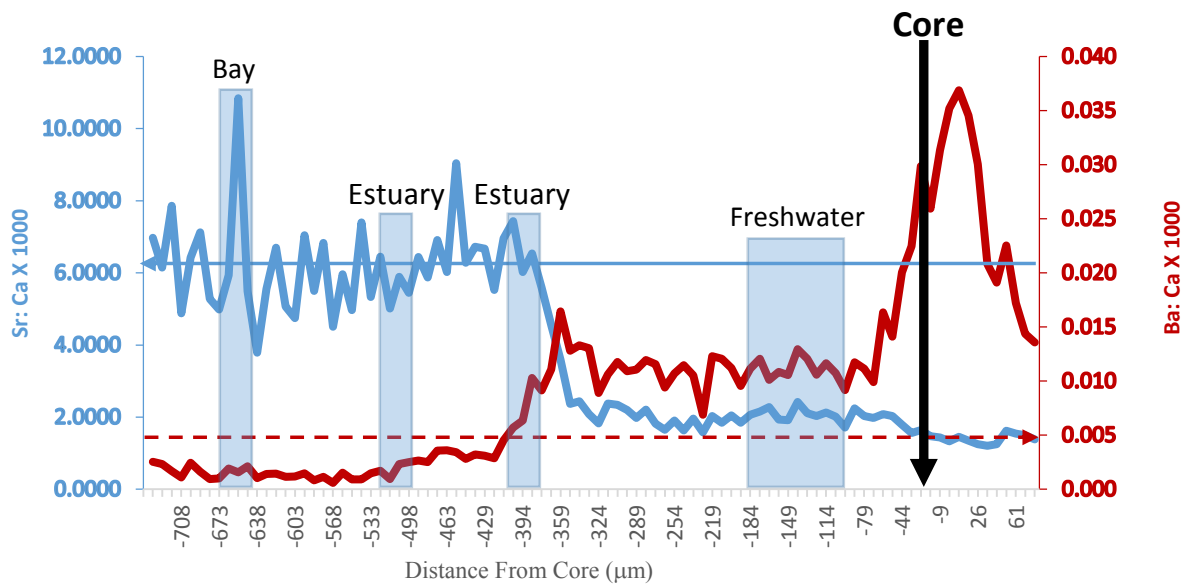


Figure 9. Otolith microchemistry of alewife ID 669 caught in the Penobscot Estuary during October, 2013. The core of the otolith (thick arrow) shows the starting point for microchemistry analysis. Sr cutoff (6.32 Sr: Ca x 1000; thin horizontal arrow) and Ba cutoff (0.004 Ba: Ca x 1000; dashed horizontal arrow) were used to distinguish habitat changes. Examples of habitats are designated by transparent rectangles. This fish had large fluctuations of Sr:Ca ratios during the last 60 days of its life, interpreted as migrations between estuary and bay habitats.

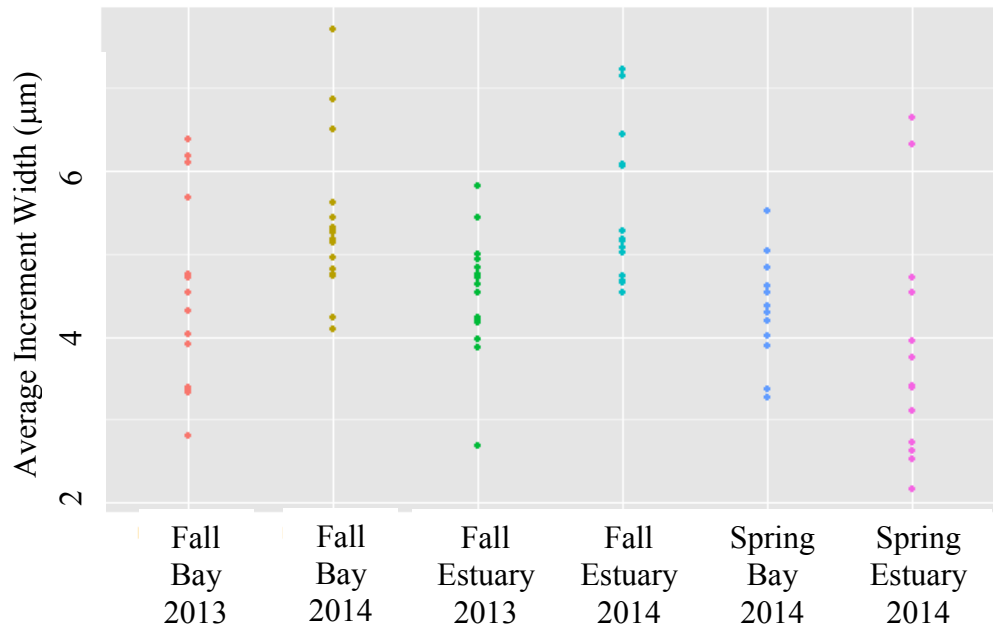


Figure 10. Mean incremental width ( $\mu\text{m}$ ) of juvenile alewife within their respective groups, where each dot represents an individual fish. Individual mean increment width ranged from 2.1 to 7.7  $\mu\text{m}$ , with an overall mean of  $4.75 \pm 1.1 \mu\text{m}$  ( $n = 91$ ).

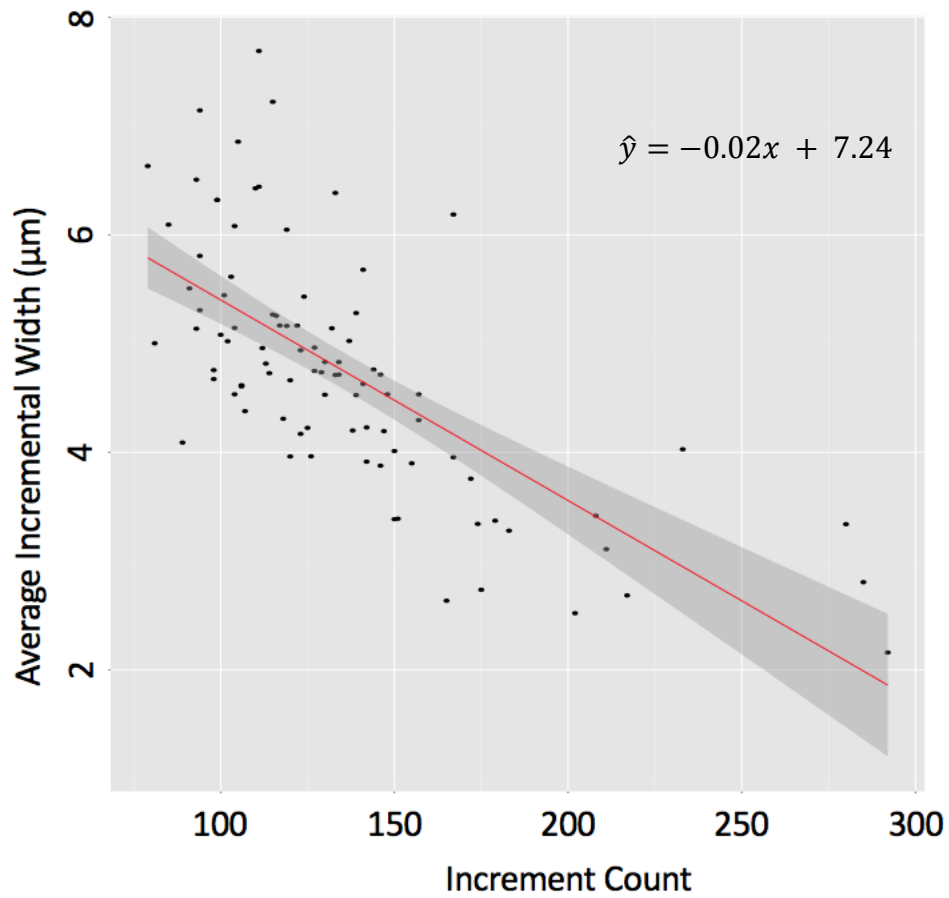


Figure 11. Mean increment width versus increment count for all fish. Fish grew more slowly (increment width) as they aged (increment count). The red line denotes the predicted mean increment width (μm) based on increment count and shading represents 95% CI.

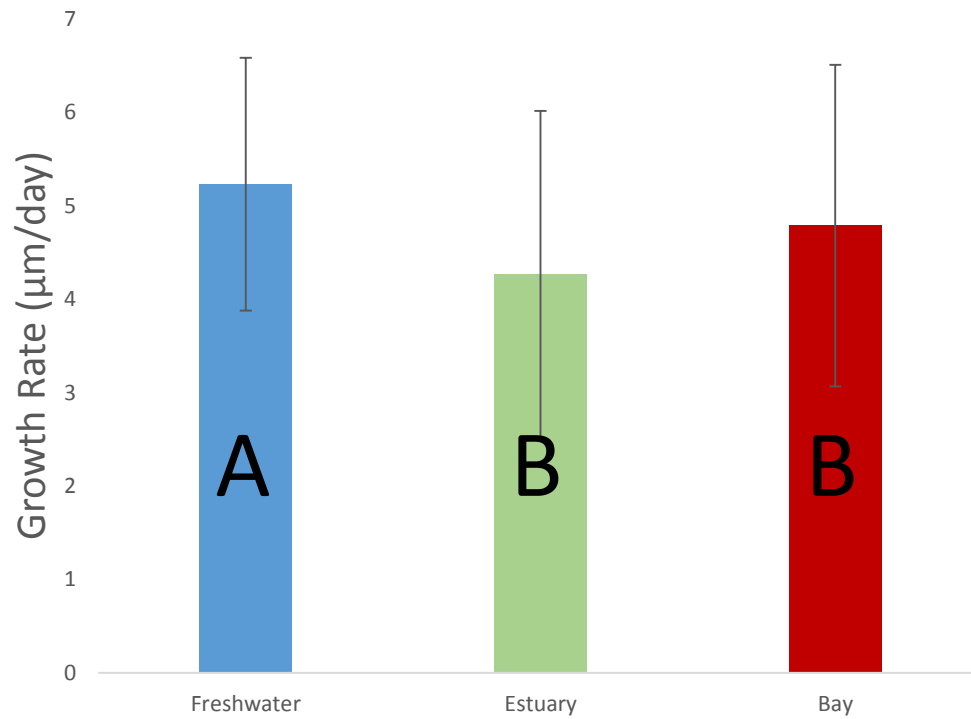


Figure 12. Mean ( $\pm$  SD) growth rates ( $\mu\text{m/day}$ ) within each habitat for all juvenile alewife ( $n = 91$ ) caught in the Penobscot Bay and Estuary, Maine. Different letter indicate statistical differences in means (TukeyHSD,  $p < 0.05$ ).

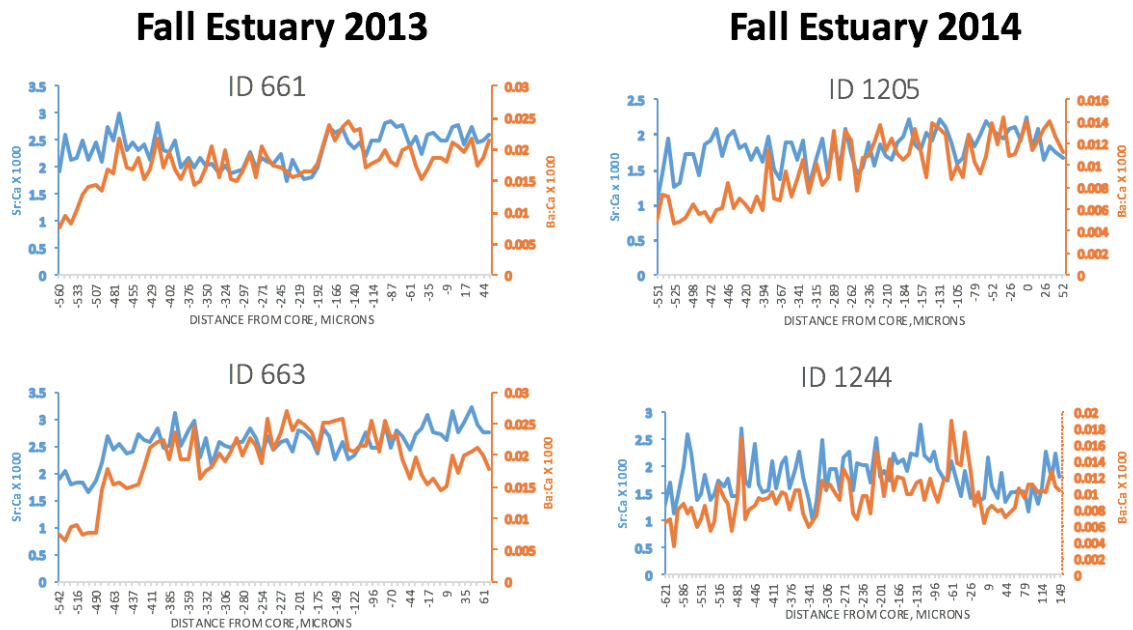


Figure 13. Sr:Ca and Ba:Ca patterns of four alewife caught in the Penobscot Estuary, Maine, during Fall 2013 and Fall 2014. These fish exhibited similar otolith transect lengths, Ba:Ca and Sr:Ca levels, and peaks and falls of Ba:Ca and Sr:Ca, suggesting that these fish come from the watershed and possibly the same natal water system.

Table 1. Mean ( $\pm$  SD) elemental composition (mg/L) for Ba, Sr, and Ca of water samples, taken in June 2015, from freshwater (Mattamiscontis, South Branch, and Pushaw) , estuary (Penobscot River, Bucksport) and bay (Head Harbor, Isle au Haut), habitats in Maine.

Habitat	Barium	Strontium	Calcium
Freshwater	$0.008 \pm 0.0020$	$0.02 \pm 0.02$	$3.25 \pm 2.44$
Estuary	$0.004 \pm 0.0002$	$1.24 \pm 0.06$	$72.10 \pm 3.02$
Bay	$0.004 \pm 0.0005$	$6.33 \pm 0.41$	$335.15 \pm 13.69$

Table 2. Habitat transition criteria, showing values for Sr:Ca and Ba:Ca necessary to be considered freshwater, estuary, or bay habitat.

Habitat	Sr: Ca x 1000	Ba: Ca x 1000
Freshwater	< 6.32	> 0.004
Estuary	< 6.32	> 0.004
Or		
Estuary	> 6.32	< 0.004
Bay	> 6.32	< 0.004



Table 3. Elemental concentrations of standard solids (homogenized otolith pellet) used to detect drift of the laser ablation unit.

<b>Element</b>	<b>ppm</b>
Ca	386733
Sr	1140
Mn	8
Ba	22
Mg	21

## APPENDIX A

An example of otolith microchemistry data (Sr:Ca and Ba:Ca) corrected for instrumental drift of alewife ID 901 caught in the Penobscot Estuary, Maine.

<b>Distance from core (<math>\mu\text{m}</math>)</b>	<b>Corrected Ca</b>	<b>Sr: Ca x 1000</b>	<b>Ba: Ca x 10000</b>
0	433450.89	5.13	0.01
9	364820.49	4.87	0.01
17	412579.18	6.41	0.01
26	402384.15	8.43	0.01
35	325881.67	5.38	0.01
44	419457.22	6.46	0.01
52	421643.14	4.62	0.01
61	386040.73	4.86	0.02
70	315159.02	8.94	0.02
79	414531.63	5.78	0.03
87	303930.01	6.78	0.02
96	270189.18	6.95	0.05
105	320589.31	5.73	0.04
114	292308.63	7.37	0.04
122	353974.65	7.21	0.04
131	322633.17	4.87	0.06
140	396136.76	3.73	0.07
149	344042.26	5.18	0.07
157	392947.82	5.27	0.06
166	336186.93	7.66	0.07
175	386139.28	4.95	0.06

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## CHAPTER 2: TURNOVER RATES OF $\delta^{15}\text{N}$ AND $\delta^{13}\text{C}$ IN LIVER AND MUSCLE OF JUVENILE ALEWIFE (*ALOSA PSEUDOHARENGUS*)

**Abstract Ch2:** *Ecologists are increasingly employing stable isotope turnover rates as an indicator of recent habitat use. Literature is lacking concerning isotopic turnover rates in juvenile alewife. By conducting a diet-switch experiment, I was able to compare juvenile alewife turnover rates of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  between muscle and liver. My initial hypothesis was that liver tissue would turnover fastest and reach equilibrium before muscle tissue, due to the higher metabolic rate of liver tissue. This change was expected to show a non-linear relationship, where the rate of turnover is fastest immediately after the diet switch and gradually slow as the isotopic signature reaches equilibrium with the diet source. I found that liver had a faster turnover rate than muscle and reached equilibrium before muscle with the saltwater food (SWD). However, the saltwater diet was nutrient poor and led to minimal growth through the first 84 days. This may have slowed turnover rate of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in both muscle and liver. An experiment that stabilized nutritional value of diets could be conducted to determine more accurate turnover rates of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  between muscle and liver.*

**Key Words:** Alewife, Diet Switch, Stable Isotopes, Tissue Turnover Rate

### INTRODUCTION

Stable isotopes, particularly nitrogen and carbon, are often used to determine trophic structure within a community (Post 2002). Carbon isotopes reflect sources of carbon (e.g., freshwater vs. marine, benthic vs. pelagic) and therefore location.

Incorporating isotopes through a food web allows the use of carbon to predict diet composition and location of consumption, whereas nitrogen indicates trophic level (Fry 2006). Within the past five years, researchers have begun focusing on the length of time required for stable isotopes to reach equilibrium in various tissues of the organism (Buchheister and Latour 2010, Heady and Moore 2013, Nelson et al. 2011). Experimental validation of turnover rates is important for strong ecological inferences on resource use, habitat use, and trophic interaction (Chen et al. 2012, Heady and Moore 2012).

Because stable isotopes reflect prey selection, stable isotope values may reflect recent habitat use rather than the current habitat due to distinctly different isotopic values of food sources during migration. For example, freshwater  $\delta^{13}\text{C}$  values for a benthic invertebrate range from  $-25$  to  $-35$   $\delta^{13}\text{C}$ , whereas values for marine benthic invertebrate range from  $-10$  to  $-20$   $\delta^{13}\text{C}$  (Post 2002). Primary consumers fractionate environmental stable isotope ratios according to environmental conditions, such as  $\text{CO}_2$ , temperature, growth rate, and salinity (Nelson et al. 2011). Fractionation rate is the changing isotopic ratio due to a chemical reaction (e.g., digestion) or change in physical state (Fry 2006). Environmental factors and fractionation ultimately cause carbon and nitrogen stable isotope values to differ in the tissues of consumers. As diets or habitats change, and cells in tissues are replaced, stable isotope values will change.

Thus tissue turnover rates can be particularly crucial for understanding highly migratory fish (Heady and Moore 2012). For example, rainbow trout (*Oncorhynchus mykiss*) can migrate between freshwater, estuarine and marine habitats during the entirety of their life (Hayes et al. 2011). This is a species where the application of stable isotope turnover rates may answer important ecological questions, such as time spent in a given

habitat, leading to more precise understanding of life history strategies (Heady and Moore 2012). Many studies examining various fish species have used stable isotopes as a way to track fish movements (Cambiè et al. 2016, Huijbers et al. 2015, Ofukany et al. 2014, Reis-Santos et al. 2015). Understanding life history with more certainty can lead to improved management and fishing regulations.

Stable isotopes turnover in different tissues depending upon tissue turnover rate, which is faster in tissues with high metabolic rates, like liver, and slower in tissues such as muscle (Fry 2006). These differences in tissue turnover rates can be used to infer recent diet change or recent habitat movement between habitats (Fry 2006).

The objective of this study was to compare turnover rates of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  in muscle and liver tissue of juvenile alewife (*Alosa pseudoharengus*). Tissues differ in metabolic rates leading to variation in turnover rates (Fry 2006, Fry and Arnold 1982, Tieszen et al. 1983). No studies have been published involving juvenile alewife turnover rates, but preliminary work (Amy Webb 2013, unpub. data) suggests differences in turnover rates in liver and muscle tissue of alewife. This study was a diet-switch experiment, starting with a saltwater-based diet, switching to a freshwater diet. I predicted that liver tissue would have the faster turnover rate, whereas muscle tissue would take longer to reach equilibrium, and have a slower turnover rates for  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ . Based on previous studies, I expected liver tissue to reach equilibrium in approximately 50 days in an exponential pattern, initially changing rapidly within the first 30 days (Buchheister & Latour 2009, Nelson et al. 2011, Xia et al. 2013).

## MATERIALS AND METHODS

### FISH COLLECTION

Scientists from the National Oceanic and Atmospheric Administration (NOAA) National Marine Fisheries Service, Orono, Maine, collected 50 juvenile alewives on June 31, 2014, via pelagic trawl on the Penobscot River near Searsport, Maine (44.426065, – 68.848743, Figure 1). Fish were transported from the boat's live well and placed into an insulated circular cooler, filled with ambient water from the Penobscot River and equipped with aeration and circulating water. Fish were immediately moved to holding tanks at the Ira C. Darling Marine Center for Research, Walpole, Maine.

Alewife were held in circular tanks (1.5 m diameter; 1,667 L) with filtered flow through water held all alewife. Water pulled from Lowes Cove in South Bristol, Maine, to emulate natural conditions. A data logger tracked temperature and salinity fluctuations throughout the study period. A dark-light period recreated natural conditions (13L:11D based on summer sunrise and sunset times of Bristol, Maine).

### FEEDING EXPERIMENT

Fish acclimated for one day to their new environment to allow emptying of the gut and to ensure normal behavior before feeding began (Nelson et al. 2011, Chen et. al 2012). All fish were fed diet-1 (SWD) initially and switched to diet-2 (FWD) after 83 days (Table 1). SWD consisted of salt-water raised brine shrimp from San Francisco Bay Brine Shrimp Company with an isotope signature of 9.62  $\delta^{15}\text{N}$  and –26.98  $\delta^{13}\text{C}$ . Initially, before feeding began, 3 fish were sampled to get a baseline for stable isotope values of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  from the Penobscot Estuary, Maine. Fish were fed *ad libitum* for the



duration of the experiment. At days 45, 66, and 84 three fish were sacrificed to determine if fish tissues reached equilibrium for SWD. After 83 days, half of the remaining fish were randomly selected and transferred into tank-2. Tank-2 had the same parameters as tank-1. These fish were fed FWD (freshwater mysid), which had a distinct isotopic signature from SWD for  $\delta^{13}\text{C}$  (Table 2). Fish in tank-2 were sampled at days 1, 3, 8, 14, and 19 after the diet switch. Fish in tank-1 continued to be fed SWD and were sampled again at day 98 and 103 to ensure consistent isotopic values (Kiljunen 2006, Nelson et al. 2011). All fish fed actively during the experiment and all sacrificed fish had full stomachs.

## COLLECTION AND PROCESSING OF SAMPLES

One to three fish were selected randomly at each sampling event to obtain isotope values of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  in dorsal muscle and liver (Chen et. al 2012, Church et. al 2009, Heady and Moore 2013, Nelson et al. 2011). Fish were killed with an overdose of MS-222, weighed using a Sartorius GE812 balance to the nearest 0.01 g, and measured using a Wildco fish board (total and fork length, to the nearest 1 mm). Approximately 1 cm<sup>3</sup> of muscle was taken from behind the dorsal fin and above the lateral line (Figure 2) and placed in a vial, the liver was removed and placed in a separate vial. Liver and muscle were thoroughly washed with deionized water and frozen at -20°C until processing.

## STABLE ISOTOPE ANALYSIS

All samples were dried at 55°C for a minimum of seven days in a Fisher Scientific Isotemp oven to ensure all moisture was removed. Samples were homogenized with mortar and pestle. Samples were placed into tin capsules (5 x 8 mm) at a mean weight of

1.2 ± 0.3 mg per sample.  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  were measured for each sample at the University of California Davis Stable Isotope Laboratory, using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer.

All isotope ratios were reported in the conventional standard (Fry 2006):

$$(1) \quad \delta X = \frac{(R_{\text{sample}} - R_{\text{standard}})}{R_{\text{standard}}} \times 1000$$

where  $X$  is the stable isotope value of C or N, and  $R$  is the mass ratio of the heavy to light stable isotope for the sample and the standard. The standard used for N is air and the standard for C is PeeDee Belemnite standard (Fry 2006).

## LIPID CORRECTION

The Kiljunen et al. (2006) model for lipid-normalization was used to correct for lipid concentration in fish liver without chemical lipid extraction. Kiljunen et al. (2006) created the model using herring (*Alosa*), Atlantic salmon (*Salmo salar*), and eel (*Anguilla rostrata*), making this model a good choice for this study. The model is defined by two equations:

$$(2) \quad L = \frac{93}{1 + (0.246 * (C : N) - 0.775)^{-1}}$$

$$(3) \quad \delta^{13}\text{C}' = \delta^{13}\text{C} + D * \left( I + \frac{3.90}{1 + 287 / L} \right),$$

where  $L$  is the proportion of lipid content of the sample and  $\delta^{13}\text{C}$  is the lipid-normalized carbon value of the sample. The C:N ratio and  $\delta^{13}\text{C}$  is obtained from the sample.  $D$  is the isotopic difference between protein and lipid and is equal to  $D = 7.018$  (Kiljunen et al.

2006) and  $I$  is a constant equal to  $I = 0.048$  (Kiljunen et al. 2006). Both  $D$  and  $I$  are estimated parameters.

Lipid corrections were applied using the equations above for C and N. Levels shifted  $< 1$  SD for all samples, which suggested that lipid levels did not impact N or C levels in liver or muscle tissues. Therefore, lipid corrections were not used during final analysis of liver and muscle tissues.

## RESULTS

Fish were all the same age-class (0 +), similar in length ( $112 \pm 7$  cm) and weight ( $9.9 \pm 2.4$  g) at the beginning of the experiment. Alewife were measured for total length ( $\pm 1$  mm) at the start and end of the experiment and all fish fell within 1 SD of the starting mean length. A total of 25 alewives were sacrificed during the experiment. Sample sizes were limited by number of surviving fish, limiting statistical power. Initially, isotopes for captured alewife were  $\delta^{13}\text{C} = -20.36\text{‰}$  and  $\delta^{15}\text{N} = 11.37\text{‰}$  for liver, whereas muscle levels were  $\delta^{13}\text{C} = -20.85\text{‰}$  and  $\delta^{15}\text{N} = 12.17\text{‰}$ . The similar values for liver and muscle suggest ample time spent in an estuarine environment.

At the end of the experiment,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  levels in muscle tissue had not changed and were similar for fish feeding on both SWD and FWD (Figures 3 and 4). Fish also did not grow during the experiment. Although lack of growth was unexpected, the experiment, has provided turnover rates of fish that are not growing, as many do in the winter season in the Penobscot watershed, Maine.

In contrast,  $\delta^{13}\text{C}$  values for liver in the FWD treatment deviated from liver levels in the SWD treatment by more than 1 SD (Figure 5).  $\delta^{13}\text{C}$  values for liver samples of

fish feeding on SWD finished higher at  $\delta^{13}\text{C} = -24.57\text{‰}$  compared to livers of fish feeding on FWD,  $\delta^{13}\text{C} = -26.76\text{‰}$ . Liver values of  $\delta^{15}\text{N}$  for fish feeding on both diets did not differ statistically from another (Figure 6). There was no trend in N data for either muscle or liver.

## DISCUSSION

Changes in stable isotopes for liver and muscle tissues were not as profound as expected due to slow growth. However, many species of fish in northern latitudes experience minimal growth due to cold temperatures (Pannella 1971, Houde 1989). After reviewing fat and protein levels of the SWD, it became apparent that it was nutrient depleted compared to FWD, which undoubtedly caused deleterious effects on growth and therefore stable isotope incorporation. However, since all fish were subjected to the same nutrient depleted diet, the growth and turnover rate of all sampled fish ingesting SWD is reminiscent of a fish experiencing high levels of stress or cold temperatures. Despite no growth and little change in muscle  $\delta^{13}\text{C}$  values, liver  $\delta^{13}\text{C}$  values did change overtime to more closely resemble the SWD treatment, suggesting liver is a good indicator of diet despite no somatic growth (Figure 5).

Feeding on the SWD, muscle tissue never reached equilibrium for  $\delta^{13}\text{C}$  in 102 days, which suggests that low somatic growth halts turnover rates for  $\delta^{13}\text{C}$  in muscle (Figure 3). In 19 days, there was no observable trend of muscle tissue  $\delta^{13}\text{C}$  moving towards equilibrium with the FWD. Fish that were transferred to FWD did consume SWD for 83 days, which may have influenced metabolic activity while consuming FWD, thus creating a similar outcome for  $\delta^{13}\text{C}$  turnover in muscle. Other studies have found

fish to have a turnover rate of 4 – 20 days for  $\delta^{13}\text{C}$  in muscle tissue after switching to a new diet (Bosley et al. 2002, Weidel 2011). However, their food selection was nutrient rich, and expedited the turnover rate process. Although, a nutrient depleted diet may not be the only factor to consider, muscle tissue has a slower metabolic rate compared to other tissues such as blood and liver (Buchheister and Latour 2010, MacNeil et al. 2006, Phillips and Eldridge 2006), which may also have attributed to the slow turnover rate of  $\delta^{13}\text{C}$  in muscle tissue. Because the fish in this experiment did not grow, we cannot estimate turnover time for muscle tissue in 0 + alewife. However, liver values did change over time to resemble SWD  $\delta^{13}\text{C}$ , suggesting that liver is a good indicator of diet, even in poor growth conditions.

There were no observable trends for  $\delta^{15}\text{N}$  turnover rate in muscle or liver tissue. Wild juvenile alewife tend to feed on zooplankton (Janssen 1976, 1978), which occupy a similar position in an aquatic food web as saltwater brine shrimp (SWD) and freshwater mysids (FWD). Occupying a similar position in a food web would present similar levels of  $\delta^{15}\text{N}$  (Cabana and Rasmussen 1994, Hussey et al. 2014), causing little change in  $\delta^{15}\text{N}$  levels.

In conclusion, poor growth due to inadequate nutrition led to minimal change in  $\delta^{13}\text{C}$  muscle tissue with the introduction of a new diet in this experiment. However,  $\delta^{13}\text{C}$  in the liver did change to more closely resemble diet  $\delta^{13}\text{C}$  levels, suggesting even during poor growth such as overwintering, liver  $\delta^{13}\text{C}$  may be a reliable indicator of diet and habitat use.

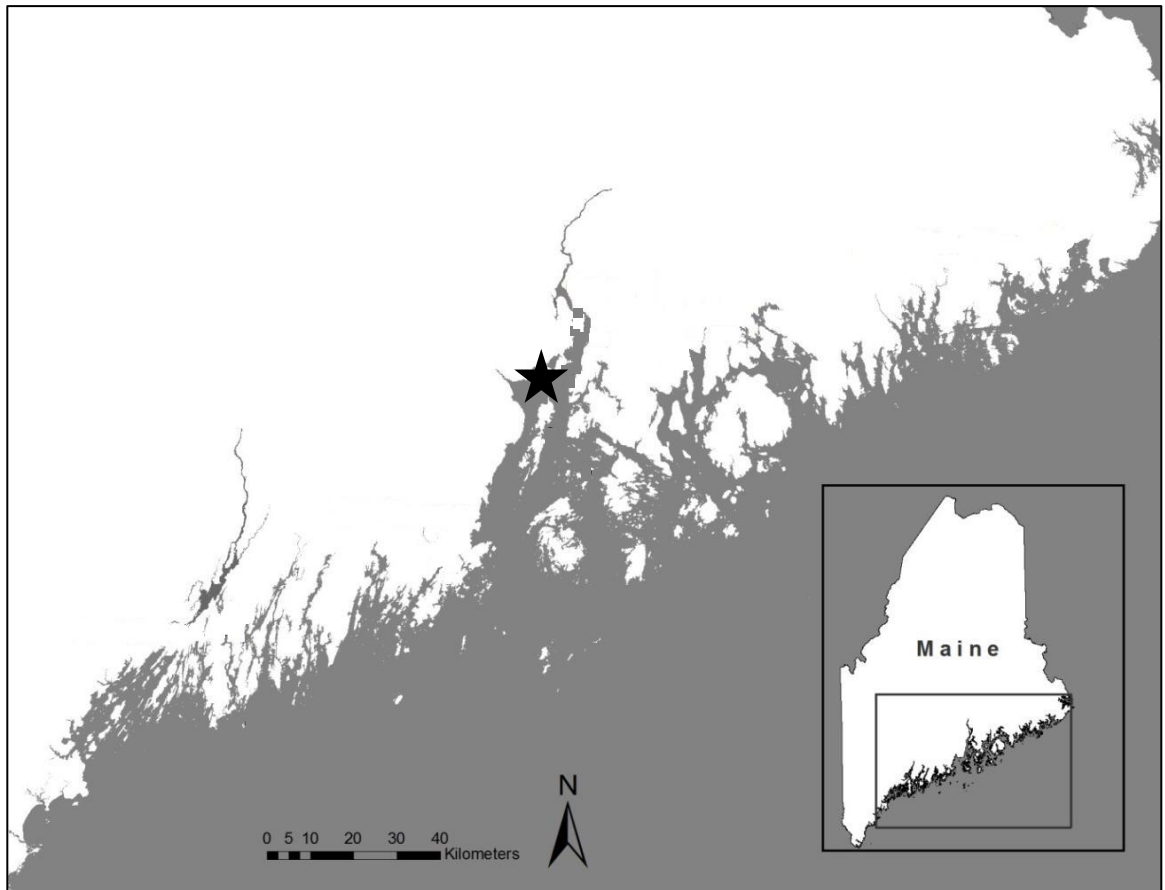


Figure 1. Pelagic trawl site (star) conducted by NOAA on the Penobscot River, Maine, United States, where alewives (*Alosa pseudoharengus*) were collected in June, 2014.



Figure 2. Dissection locations for liver (left) and muscle (right) tissue circled in black for all alewife in this study.

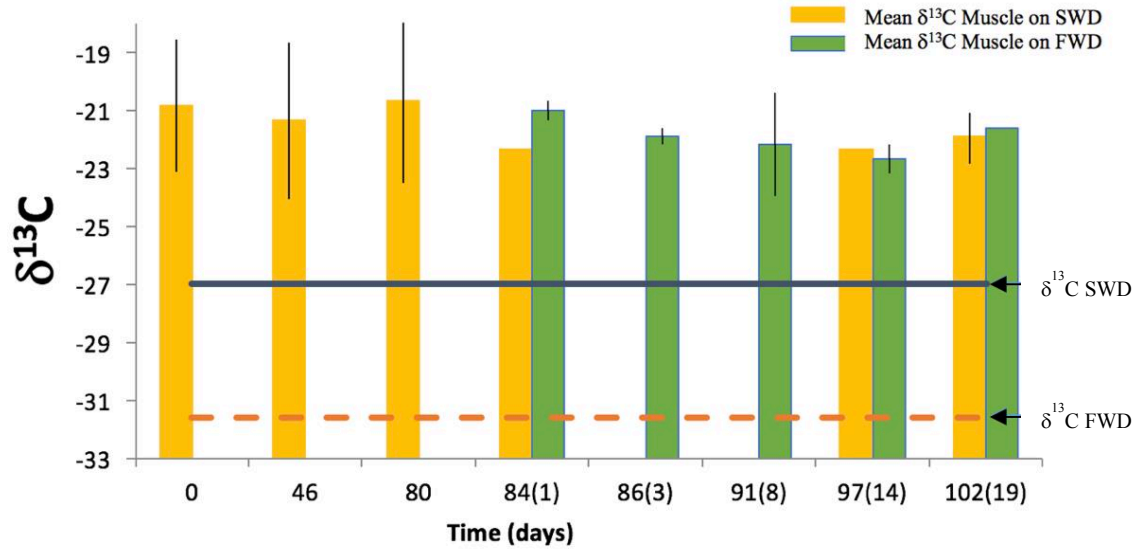


Figure 3. Mean ( $\pm 1$  SD)  $\delta^{13}\text{C}$  value in the muscle of juvenile alewife over the duration of the feeding experiment. Between 1 – 3 fish were sacrificed during each sampling event. The solid line represents SWD  $\delta^{13}\text{C}$ , and the dashed line represents FWD  $\delta^{13}\text{C}$ . The freshwater diet started at day 84 in the experiment and is represented by days enclosed by ( ). Neither diet caused muscle tissue to reach equilibrium for  $\delta^{13}\text{C}$ .



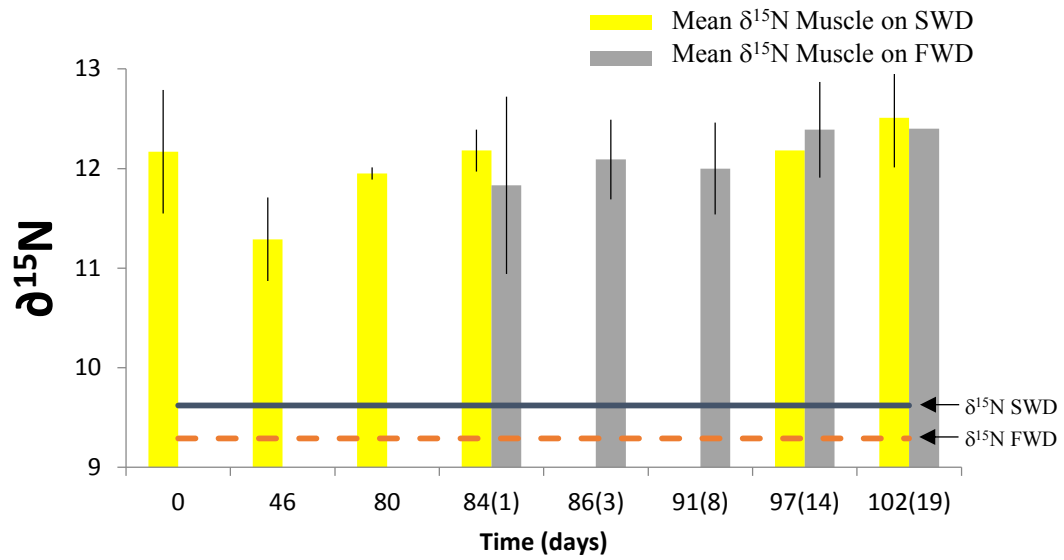


Figure 4. Mean ( $\pm 1$  SD)  $\delta^{15}\text{N}$  value in the muscle of juvenile alewife. Between 1 – 3 fish were sacrificed during each sampling event. The solid line SWD  $\delta^{15}\text{N}$ , and the dashed line represents FWD  $\delta^{15}\text{N}$ . The freshwater diet started at day 84 in the experiment and is represented by days enclosed by ( ).

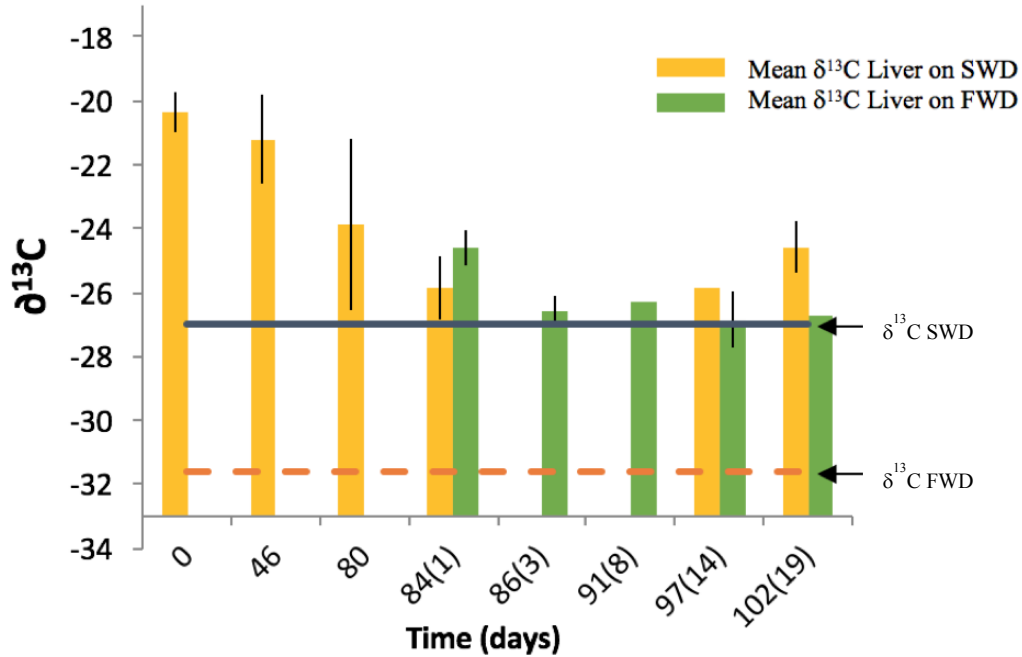


Figure 5. Mean ( $\pm 1$  SD)  $\delta^{13}\text{C}$  value in the liver of juvenile alewife. Between 1 – 3 fish were sacrificed during each sampling event. The solid line represents SWD  $\delta^{13}\text{C}$ , and the dashed line represents FWD  $\delta^{13}\text{C}$ . The freshwater diet started at day 84 in the experiment and is represented by days enclosed by ( ). Liver tissue for SWD approached equilibrium for  $\delta^{13}\text{C}$  by day 84, but liver tissue for FWD never approached FWD  $\delta^{13}\text{C}$  values due a limited amount of time.

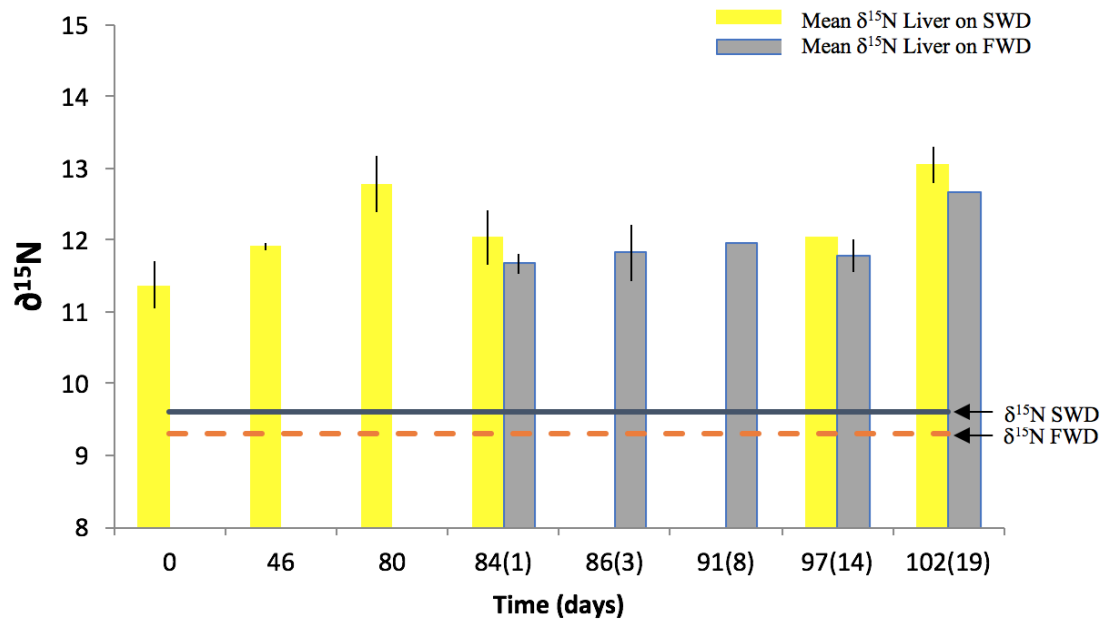


Figure 6. Mean ( $\pm 1$  SD)  $\delta^{15}\text{N}$  content in the liver of juvenile alewife. The solid line represents SWD  $\delta^{15}\text{N}$ , whereas the dashed line represents FWD  $\delta^{15}\text{N}$ . The freshwater diet started at day 84 in the experiment and is represented by days enclosed by ().

Table 1. Number of juvenile alewife sampled for nitrogen and carbon stable isotope analysis of liver and muscle tissue. Days in ( ) are the number of days fish were fed diet #2.

Day	0	45	66	84	86	92	98	103
				(1)	(3)	(8)	(14)	(19)
Tank 1- Diet 1	3	3	3	3	N/A	N/A	2	2
Tank 2- Diet 2	N/A	N/A	N/A	2	2	2	2	1

Table 2. The  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  levels of both SW (saltwater brine shrimp) and FW (freshwater mysids) diets obtained from University of California Davis Stable Isotope Laboratory. Protein, fat, ash, and moisture levels of diets one and two obtained from San Francisco Bay Brine Shrimp Company. Both diets were fed to the juvenile alewife in this study.

	<b>SWD</b>	<b>FWD</b>
	<b>Saltwater</b>	<b>Freshwater</b>
$\delta^{15}\text{N}$	9.62	9.29
$\delta^{15}\text{C}$	-26.98	-31.61
Protein	4.7%	69.5%
Fat	0.8%	8.35%
Ash	N/A	5.5%
Moisture	92.0%	N/A

Table 3. Mean  $\delta^{15}\text{N}$  &  $\delta^{13}\text{C}$  levels for liver and muscle tissue of alewife on the given day of the experiment. SW  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  for liver and muscle on days 86 and 91 are missing due to the absence of sampling on those days.

Diet	Sample Day	Tissue Type	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
SW	0	Liver	-20.36	11.37
SW	46	Liver	-21.21	11.91
SW	80	Liver	-23.87	12.78
SW	84(1)	Liver	-25.86	12.04
SW	86(3)	Liver	—	—
SW	91(8)	Liver	—	—
SW	97(14)	Liver	-25.86	12.04
SW	102(19)	Liver	-24.57	13.05
SW	0	Muscle	-20.85	12.17
SW	46	Muscle	-21.35	11.29
SW	80	Muscle	-20.69	11.95
SW	84(1)	Muscle	-22.34	12.18
SW	86(3)	Muscle	—	—
SW	91(8)	Muscle	—	—
SW	97(14)	Muscle	-22.34	12.18
SW	102(19)	Muscle	-21.87	12.51
FW	84(1)	Liver	-24.59	11.68
FW	86(3)	Liver	-26.58	11.83
FW	91(8)	Liver	-26.29	11.97
FW	97(14)	Liver	-26.84	11.78
FW	102(19)	Liver	-26.76	12.66
FW	84(1)	Muscle	-21	11.83
FW	86(3)	Muscle	-21.9	12.09
FW	91(8)	Muscle	-22.17	12
FW	97(14)	Muscle	-22.68	12.39
FW	102(19)	Muscle	-21.6	12.4

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