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Juvenile alewife *(Alosa pseudoharengus)* feeding habits, movement and residency in a northern temperate estuary

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A THESIS

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Introduction

River herring is a term applied collectively to both alewife, *Alosa pseudoharengus*, and blueback herring*, A. aestivalis,* anadromous fish that spend most of their life in the ocean and migrate upriver to freshwater to spawn each spring (NEFSC 2006). Juveniles spend their first summer in freshwater and are thought to migrate to the ocean in fall, where they remain until they are sexually mature and ready to spawn (three to five years; ASMFC 2012).

River herring have experienced dramatic declines throughout their range. In Maine, there has been a large-scale restoration effort on the Penobscot River aimed at improving access to historic habitat for diadromous species including alewife. As part of this project scientists at the National Oceanic and Atmospheric Administration (NOAA) have been monitoring use of the Penobscot estuary by alewife and other fish species. This work presented an important opportunity to learn more about early life stages of alewife. In 2012, and again in 2013, researchers from the NOAA Orono Field Station unexpectedly found a large number of juvenile age 1+ alewife from April through November in the Penobscot estuary (Stevens et al. 2021). These juvenile alewife are interesting because they are too young to be returning upriver to spawn, and most of them are too old to be recent freshwater emigrants. It is not clear how these fish use the estuary.

The main objective of my first chapter was to determine if juvenile alewife feed in the Penobscot estuary and what they eat (using standard diet content analysis). Once this information was determined, I created a $\delta^{13}C$ isoscape or isotope map of the Penobscot system from the Penobscot Bay to 4 Penobscot lakes using alewife and alewife prey collected in each habitat (bay, freshwater lakes and estuary). I used this $\delta^{13}C$ isotope map to

infer information about juvenile alewife movement patterns based on the $\delta^{13}C$ values of their liver and muscle tissue. These results are described in Chapter 2.

Juvenile alewife fed extensively on estuarine calanoid copepods, barnacle larvae and mysid shrimp, which were used to create the estuary isotope map. The $\delta^{13}C$ values of estuary caught juvenile alewife liver and muscle tissue fell into one of three habitat use patterns: recently from the bay, recently from freshwater, and estuarine occupants. Juvenile alewife from all three habitat use patterns ranged in size from 51-180 mm fork length and were identified across spring, summer and fall, with the exception of fish that had recently fed in freshwater. The fish that were from freshwater were only present in the estuary in fall.

Estuarine use of the Penobscot by juvenile alewife occurred over time and a range of fish sizes. The results from this research suggest that the Penobscot estuary provides significant feeding habitat for juvenile alewife that reside in and frequently move between the estuary and bay.

A better understanding of movement patterns, feeding and habitat occupancy of juvenile alewife in estuaries will help with decision making around river restoration efforts. This research is particularly important at this time when wide scale dam removals and restorations have been and continue to be pursued across the East Coast of North America.

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Chapter 1. Diet and prey selectivity of juvenile alewife *(Alosa pseudoharengus)* in a northern temperate estuary

Abstract

Alewife have experienced dramatic declines throughout their range and are the subject of numerous restoration efforts. However, little is known about critical early life histories of these fish. This study examined feeding habits and prey selectivity of juvenile alewife in the Penobscot estuary, Maine over time in spring (May), summer (July), fall (September/October) and across space (upper and lower estuary; low and high salinity, respectively) using diet content analysis. A high percentage of fish (97%) had identifiable prey in their stomachs. Juvenile alewife consumed mostly crustaceans with 61% of prey genera identified as copepods. Diets were dominated by barnacle larvae, *Balanus spp*., and an estuarine calanoid copepod, *Eurytemora affinis,* which were positively selected over time even though they were not always the most abundant food resource. Diets were also characterized by temporal changes, with barnacle larvae decreasing in importance from May to September while mysid shrimp, *Eurytemora* and *Calanus* copepods increased in importance. These results demonstrate that the estuary is a significant feeding habitat for these fish during the time period sampled.

Introduction

As "nurseries of the sea," estuaries support diverse and abundant populations of both invertebrate and vertebrate species (Hildebrand and Schroeder 1928). As essential transitional habitat for diadromous fish species, estuaries also connect marine and freshwater ecosystems, making estuaries among the most productive ecosystems and biomes in the world (Day et al. 1989; Beck et al. 2001; Able 2005). Habitats within estuaries, including tidal flats, salt marshes, sea grass and oyster beds, are typically associated with high productivity (Beck et al. 2001). In the United States, estuaries along the Northeastern coast have been identified as essential fish nursery habitats (Able and Fahay 1998) because they typically support large numbers of juvenile fish and provide food resources and protection from predators (Blaber and Blaber 1980; Boesch and Turner 1984; Hoss and Thayer 1993; Able 1999).

Smaller anadromous fish species commonly known as "bait fish" (e.g., shad *Alosa sapidissima***,** river herring *Alosa pseudoharengus* and *Alosa aestivalis*, and smelt *Osmerus mordax*) are critical links in both the marine and freshwater food chains as foraging fish for commercially important fish species (Pikitch et al. 2014). Like most anadromous fish, these species utilize a wide range of habitats during their life cycle (Durbin et al. 1979; Schindler et al. 2005; O'Higgins et al. 2010). On the Atlantic coast, alosines such as alewife *(Alosa pseudoharengus)* support substantial commercial fisheries during their spawning runs in rivers (Neves 1981).

Along the East Coast, anadromous alewife are highly mobile with complex life histories. They spend most of their lives at sea, and they migrate through or use estuaries

for spawning and nursery habitats (Fay et al. 1983). Most work on early life stages of alewife and other alosine species focus on reproduction and population emigration from lakes (Kosa and Mather 2001; Yako et al. 2002; Gahagan et al. 2010) and growth rates as they relate to recruitment or movement and migration history (e.g., Limburg 1996, 1998, 2001; Baltz et al.1998; Turner and Limburg 2012). Much less attention has been paid to use of estuaries by juvenile alewife (but see Stone and Daborn 1987; Grabe 1996).

Alewife have experienced declines throughout much of their range, with spawning runs a mere fraction of what they once were (Limburg and Waldman 2009; NOAA 2009; Hall et al. 2012). In 2006 alewife were listed as a NOAA species of concern due to large decreases in populations from being caught as bycatch in commercial fishing, pollution and poor water quality (particularly in the past before the Clean Water Act was established) and from dam construction ultimately blocking access to essential spawning habitat. In Maine, the Penobscot River has undergone a large-scale restoration effort involving a series of dam removals and stocking efforts in watershed lakes as part of the Penobscot River Restoration Project (Trinko Lake et al. 2012). From 2012 to 2013, two dams were removed from the Penobscot River estuary, opening up 1000 mi^2 of habitat to sea-run fish. As part of this project, scientists from the National Oceanic and Atmospheric Administration (NOAA) Fisheries Division monitored use of the Penobscot estuary by alewife and other fish species. In 2012 and 2013, NOAA researchers unexpectedly found a large number of juvenile alewife (ages 0 - 2^+ years) from April through November in the estuary (O'Malley et al. 2017; Stevens et al. 2021), suggesting that these species spend considerable time in the estuary as juveniles. Given the importance of alewife as a forage fish and limited knowledge of their early life stages, it is important to study

the diets of juvenile alewife in the estuary because diets are critical for regulating growth, which could ultimately affect recruitment (Craig and Helfrich 2002; Nunn et al. 2011).

Does the Penobscot estuary provide critical feeding habitat for juvenile alewife? To my knowledge, this is one of the first studies investigating diets of juvenile alewife in a northern temperate estuary since 1987 (Stone and Daborn 1987) and the first study comparing juvenile alewife prey choice to prey availability to determine selectivity in a northern temperate estuary. Specific objectives of this study were (1) to characterize the diets of juvenile alewife (*Alosa pseudoharengus*) over time (May, July and September) and space (upper and lower estuary), (2) to quantify diet selectivity by comparing prey taxa consumed relative to prey available in the water column over time and space, and (3) to determine temporal or spatial patterns in fish condition and stomach fullness (an estimate of the amount of food eaten).

Methods

Study Area and Field Collections

The Penobscot River is the second largest river system in New England and the largest river located entirely within Maine (Trinko Lake et al. 2012). Running from Penobscot Lake in Somerset County to Maine's southeast coast near Searsport, the river drains over 25% of the state (22,196 km^2) and falls 488 m (Penobscot River Restoration Project n.d.). The basin of the Penobscot River historically held significant numbers of diadromous fish species, including alewife (Hall et al. 2012), with estimates of annual commercial harvests of alosines in the millions in the 19th century (Foster and Atkins 1869).

Juvenile alewife and zooplankton samples were collected concurrently by the NOAA Fisheries Maine Field Station's pelagic fish survey from May through September 2013 in the lower tidal section of the Penobscot River that runs approximately 40 km from Eddington Bend (45°14′12″N, 68°38′57″W) to the mouth of the river near Fort Point (Lipsky et al. 2019; Figure 1). The upper estuary, which ranged in salinity from 0 to < 20 ppt, included three collection sites from Oak Point downstream to just upstream of Bucksport, and the lower estuary, which ranged in salinity from 20 to 30 ppt, contained four collection sites from Bucksport downstream to Fort Point. Samples were collected via systematic sampling of seven fixed collection sites within the Penobscot estuary (Figure 1). A "Mamou Trawl" was used (Innovative Net Systems, Milton, Louisiana). The net was towed with an 11 m Duffy-style lobster boat. Tows were conducted at 20-min intervals at a speed around 2-4 knots during a daylight flood tide. Sampling began at low tide in the lower estuary and moved upstream with the flood tide, ending in the upper estuary such that each collection site was sampled at a similar period in the tidal cycle for each sampling day.

Subsamples of at least 20 alewife (when available) were killed with an overdose of MS-222 (Ethyl 3-aminobenzoate methanesulfonate) and immediately frozen on dry ice in the field to reduce post mortem digestion (see Storch et al. 2007). In the lab, fish were defrosted, total length and fork length were measured to the nearest 1.0 mm, and weight was measured using an electronic balance (Sartius GE812; ± 0.01 g). Alewife identification was confirmed by examination of peritoneum color with pink to gray assumed to be alewife and black assumed to be the closely related blueback herring (*Alosa aestivalis;*

Loesch 1987; but see Berlinsky et al. 2015). Only alewife were used in this study. Alewife stomachs were removed and stored in F13 preservative (Warmington et al. 2000).

Stomach Content Analysis

Stomach contents of individual fish were examined through a dissecting microscope under magnifications ranging from 10x to 40x, and prey items were identified to the lowest possible taxon using established keys (Gerber 2000; Smith and Johnson 1996; Todd et al. 1996). Diet analyses were largely focused at the genus level (and hereafter only genera are named) due to partially digested prey and inherent difficulties in identifying zooplankton to the species level. Stomachs were weighed (wet weight ± 0.01 g) before and after contents were removed to calculate an estimate of the amount of food eaten (i.e., stomach fullness).

Prey Availability

To assess zooplankton prey selectivity by juvenile alewife, zooplankton samples were collected simultaneously with fish at collection sites 2, 4, 5 and 8 (Figure 1). Zooplankton were collected using a 0.5 m diameter ring net with a flowmeter affixed to the opening and a 250 mm mesh. Vertical tows were used to collect animals throughout the water column. The net was slowly released to a depth of 1 m above the substrate and immediately pulled to the surface by a motorized 'pot hauler' at a rate of approximately 1 m s^{-1}. To collect mysid shrimp and other large zooplankton, a ring net (1000 mm mesh, 1 m dia. opening, and flowmeter) was allowed to sink to 1 m above the substrate and to remain at depth for approximately 1 min before the net was pulled up quickly using a motorized 'pot hauler'. This method allows time for animals that were disturbed by the net's descent to redistribute themselves. The speed of the net (approximately 2-3 m s^{-1}) was

necessary to combat net evasion by the mysids. This method has been used successfully to catch other small cardioid species (Benoit-Bird, personal communication). Animals were immediately preserved in 4% neutrally buffered formaldehyde solution.

To enumerate and identify zooplankton and mysids from net samples, at least 100 individuals were counted in three replicate aliquots. To quantify species composition, a subsample of 100 individuals was identified to the lowest taxonomic resolution possible (usually species) using established keys (Gerber 2000; Smith and Johnson 1996; Todd et al. 1996). Zooplankton were classified into the same categories used for diet analysis. These data were used to calculate selectivity indices for each prey item.

Statistical Analyses

To test for differences in feeding, Kruskal Wallis with Dunn post hoc tests were used to test differences in the mean prey number per fish (MPN) as a function of the independent variables, i.e., month collected, fish size (small, < 100 mm fork length, or large, ≥ 100 mm fork length) and collection site (upper estuary, lower estuary). Mean number of prey per fish did not differ statistically ($\chi^2 = 13.756$, df = 1, $P = 0.08$) between small and large juvenile fish, and size of fish collected (total length) was not affected by month ($\chi^2 = 0.1956$, df = 2, $P = 0.91$) or collection site ($\chi^2 = 1.0414$, df = 1, $P = 0.31$). Therefore, I pooled all sizes of fish for analysis.

To characterize the diets of juvenile alewife (*Alosa pseudoharengus*) over time (May, July and September) and space (upper and lower estuary), I reported monthly diet composition by location as a percentage of prey by number (%N) and by frequency of occurrence (%FO). These calculation methods are similar to Hyslop (1980). Percentage of prey by number (%N) was calculated as the total number of prey identified by month or

space divided by the total number of prey identified in all fish. Frequency of occurrence (%FO) was calculated as how often a specific prey item was identified out of the total number of diets examined. I did not include unidentified material (animal, plant or inorganic debris) in the calculation of %N or %FO because enumeration of these items was not possible.

Juvenile alewife diet selectivity (alpha) was calculated by month and collection site for all prey that occurred in ≥ 5% of diets or the environment using Chesson's selectivity index. The index (i.e., alpha index [α_i]) was calculated using paired fish-diet and plankton samples for each fish according to equation 1:

$$(1)\quad \alpha_i = \frac{r_i/p_i}{\sum_{j=1}^{m}(r_j/p_j)}, i = 1, \ldots, m$$

Where α_i is the selection index for prey type *i* for alewife from a given month-collection site, r_i and p_i are the proportions of prey type *i* in the diet (*r*) and the environment (*p*) for that month-collection site, and *m* is the number of prey taxa available, based on diet and concurrent zooplankton samples. Values of α_i were normalized so α_i ranged from 0 to 1.

To assess and interpret selectively, I used confidence intervals for Chesson's alpha values and compared them against random feeding (1 / number of prey taxa *k* identified within the stomach contents). A value of 1/k indicates the random feeding line where alpha values above this number (mean ± 95% CI) are interpreted as significant positive selection for that prey item; alpha values overlapping the random feeding line are interpreted as neutral selection; and alpha values below this value are considered significant negative selection (Graeb et al. 2005; Rudershausen et al. 2005). In this dataset, 1/k = 0.055, as 18 prey taxa were identified within stomach contents.

Once diet selectivity was quantified, a two-way ANOVA followed by Tukey's HSD for multiple comparisons was performed to determine if prey selectivity differed over time (May, July and September) and space (upper and lower estuary).

As a representative of body condition, Fulton's condition index (K) was calculated as follows (originally used but not explicitly stated by Fulton, 1904) in equation 2:

$$(2)\ K = 100{,}000 \times M_B / L_S{}^3$$

where fish total length is L_s (in mm) and wet body mass is M_b (in g).

Fish condition was normally distributed (Shapiro Wilk test, P = 0.83) and variance between groups (site and month collected) were equal (Bartlett test, P = 0.63 and 0.30, respectively). Therefore, an ANOVA followed by a Tukey's post hoc test was used to determine if there were temporal or spatial patterns in fish condition by month and a t-test was used to determine if fish condition differed by collection site.

To estimate stomach fullness, the weight of stomach contents was used, standardized for differences in body size.

Stomach fullness (% BW) was calculated based on equation 3 (Brodeur and Percy 1987):

$$(3)\ \%\ BW = \frac{Stoma\quad content\ weight}{Total\ fish\ weight - Stomach\ content\ wei} \times 100$$

Fullness data violated the normality assumption of parametric testing (Shapiro Wilk test: P < 0.0001). Instead, I used nonparametric Mann–Whitney U and Kruskal Wallis tests (Zar 1999), to test for significant ($\alpha < 0.05$) differences in fullness between collection sites and across months.

All analyses were conducted in R (R Core Team 2018). The significance threshold for all statistical tests was set at 0.05.

Results

Stomach Content Analysis

A total of 132 alewife, ranging from 47–200 mm TL, were examined for diet, condition and fullness. Of the alewife examined, 123 (93%) stomachs contained at least one prey item in the gut. Only 6 alewife (4%) had either no food or no identifiable prey in their stomachs, and 4 alewife (3%) contained prey items that were unidentifiable and were not included in analyses. Juvenile alewife consumed mostly crustaceans. A total of 18 different prey were identified in the stomach contents, and the majority of prey were copepods (11 genera). Prey items that occurred in < 5% of stomachs included: *Acartia*, Amphipoda, Chaetognath, Decapoda, Gastropoda, isopods, *Paracalanus, Parvocalanus,* Polychaeta*,* and *Sagitta.* The 8 prey types that occurred in ≥ 5% of stomachs included barnacle larvae, bivalve larvae, Mysidae, cladocerans, and the four copepods *Calanus, Eurytemora, Harpacticoida,* and *Temora*.

Diets were dominated by a few species; barnacle larvae (*Balanus*) contributed 51.6% of all diets by numbers followed by the calanoid copepods *Eurytemora* (39.4%) and *Temora*. *Eurytemora* dominated the diet in terms of frequency of occurrence, present in 80% of diets.

The diet of juvenile alewife exhibited spatial and temporal variation with respect to specific prey items (% of diets in which that item was found). In May barnacle larvae were the most abundant prey item seen in alewife caught in both the upper and lower estuary (47.7% and 93.4% of fish contained at least one barnacle larvae in their stomachs) (Figure 2, Table 3 and Table 4). In July and September *Eurytemora* was the

most abundant prey item in both the upper and lower estuary. Over time (May to September) the percentage of *Eurytemora* identified in stomachs (12.2% to 78.2%), as well as mysids (1.0% to 7.2%), and *Temora* (1.2% to 8.6%) increased whereas barnacle larvae decreased (70.5% to 48.7%). Across space, mysids and *Harpactacoida* were highest in the upper estuary versus the lower estuary (8.0-0.1% and 2.5-0.2%).

Prey Availability in the Environment

A total of 38 zooplankton genera were collected from the water column (Table 1). The six most common taxa (representing ≥ 5% individuals/m^3 by number) collected from the environment across all time periods and locations included *Acartia* spp., *Balanus* spp., *Eurytemora affinis, Oithona* spp., *Pseudocalanus* spp. and gastropods. Zooplankton samples were dominated by copepods, and among these samples, across all time periods and sites sampled, *Acartia spp.* and *Eurytemora spp*. represented between 12.5% and 44.7% of zooplankton composition by number. In the lower estuary, from May to September, *Acartia, Eurytemora* and *Oithona* were the dominant species collected, representing 15-54%. In the upper estuary, from May to September*, Acartia, Eurytemora* and gastropods were the dominant species collected, representing 15-63%.

Over time and space, the abundance of barnacle larvae and mysids (Mysidae) available within the estuary changed notably (Table 1). Numbers of barnacle larvae were highest in May and declined in July and September (14.6, 9.5 and 8.8 individuals/m^3, respectively), whereas abundance of mysids was lowest in May and increased in July and September (0.1, 0.3 and 0.9 individuals/m^3, respectively). Mean mysid abundance was 52 times greater by number in the upper estuary versus the lower estuary, whereas barnacle larvae were twice as abundant in the lower estuary.

Prey Selectivity

Barnacle larvae and *Eurytemora* were positively selected across the entire time and space analyzed (Figure 3). Even though bivalves and cladocerans were identified in ≥ 5% of stomachs, they were negatively selected across all locations and dates sampled. During the majority of months and locations sampled, *Calanus* copepods and harpacticoids were neutrally selected. *Acartia*, *Oithona* and *Pseudocalanus* copepods were highly abundant in the environment but not a dominant food item in alewife diets (< 5%). From May to September, juvenile alewife went from positively selecting barnacle larvae and *Eurytemora* to positively selecting barnacle larvae, *Calanus*, *Eurytemora*, and mysids (Figure 3).

Condition and Fullness

Fish condition and fullness by month and collection site are listed in Table 2. There was no interactive effect between month and collection site on fish condition ($F_{(2,117)} = 0.173$, $P = 0.841$), and condition was not affected by collection site ($t_{(121)} = 0.8558$, $P = 0.3938$). Fish condition was affected by month collected ($F_{(2,120)} = 14.35$, $P < 0.0001$). Post hoc tests showed that fish collected in July were significantly higher in condition (7.4% and 11.6% greater) than fish collected in May and September ($P < 0.0001$, $N = 46$ and $P = 0.0092$, $N = 27$, respectively). Fish collected in May and September did not differ in condition ($P = 0.2964$, $N = 50$).

Stomach fullness did not differ between fish collected in the upper estuary (0.66 ± 0.07, mean ± SE, N = 68) vs lower estuary (0.73 ± 0.05, N = 55; Mann Whitney U = 1.6234, df = 1, P = 0.1072). Stomach fullness did not differ by month collected between

May (0.63 ± 0.06, N = 46), July (0.63 ± 0.06, N = 50) and September (0.92 ± 0.12, N = 27; χ^2 = 4.4313, df = 2, P = 0.1091).

Discussion

Fish used in this study were collected as early as May and as late as October. Stomach content analyses demonstrated that, during this time, juveniles fed extensively in the estuary on a diet of estuarine calanoid copepods, barnacle larvae and mysid shrimp, with over 97% of fish containing identifiable prey in their stomachs. Less than 4% of these fish had empty stomachs, indicating that alewife were feeding heavily during daylight hours on the rising tide in both the upper and lower estuary. These results suggest that the estuary is an important feeding area for juvenile alewife and used for more than just movement between fresh water and marine habitats.

Pelagic fish such as alewife are important connections in marine food webs. Alewife are one species of fish known to feed on both benthic and pelagic food. Subadult alewife in Minas Basin, a turbid macrotidal estuary in Nova Scotia, consumed larger benthic prey as opposed to smaller pelagic prey (Stone and Daborn 1987). In the continental shelf waters from North Carolina to Nova Scotia, adult alewife fed mainly on pelagic euphausiids and calanoid copepods (Holland and Yelverton 1973; Edwards and Bowman 1979; Neves 1981; Bowman 1986, Simonin et al. 2007; Hanson 2018). Although fish analyzed in this study were juveniles, the majority of prey identified consisted of estuarine copepods and barnacle larvae, which are mostly pelagic prey; however, benthic prey were identified in lower numbers. Mysids, for example, perform diel vertical migrations and can be benthic or pelagic by spending most of their time in the benthos during the day in deeper waters (Mauchline 1980).

Differences between available prey and consumed prey demonstrated that juvenile alewife do not always select the most abundant food resources. Juvenile alewife in this study both fed on and avoided prey in high and low concentrations within the water column. Of the two most abundant prey within the environment (*Acartia* and *Eurytemora*), alewife fed heavily on only one (*Eurytemora*) even though they are both copepods, nearly equivalent in size, and found in the same area of the water column based on zooplankton tows. *Oithona* and *Pseudocalanus* copepods were also abundant prey within the environment but not a dominant food item in alewife diets. Perhaps certain prey species digest faster than others. However, given that copepods were of similar size, composition and identified in other diets (just in much lower numbers), this explanation is unlikely. A more plausible explanation for selection of one dominant species over another is that some copepods (such as *Acartia* and *Calanus*) have different escape strategies that could be more effective (Burdick et al. 2007). *Calanus* have avoidance speeds averaging 160 mm sec^{-1} (Loren et al. 1980). The average escape speed of *Calanus finmarchicus* has been shown to be 18% faster in the light than in the dark (Fields et al. 2012). All fish in this study were collected during daylight hours. *Acartia* copepods use short quick bursts of speed to escape predators with *A. hudsonica* exhibiting escape jumps up to 59 mm sec^{-1} (Suchman 2000) with acceleration up to 255 and 319 ms^{-2} for *Acartia tonsa* and *A. lilljeborgii* (Buskey et al. 2002). *Eurytemora affinis* copepods exhibit slow lazy movements (personal observation) with an average speed of < 4 mm sec^{-1} in the presence of predators for both male and female *E. affinis* (Mohamed-Sofiane 2011). Barnacle larvae are less motile prey with a passive form of transportation, using the currents to move. Although not the focus of this study, the tradeoff between feeding on higher energy prey with rapid

escape responses and slower, lower energy, motile prey may be a factor in alewife prey preferences.

Alewife feeding habits are flexible, matching prey availability with selectivity (Janssen 1980; Janssen et al. 1995). Alewife can feed selectively on zooplankton by "picking" individual prey from the water column or non-selectively by filtering prey through their gills (Janssen 1976). Alewife are also opportunistic feeders, which may account for seasonal changes in diet (Davis and Foltz 1991). This study shows a shift in prey preference from solely barnacle larvae in May to a preference for both barnacle larvae and *Eurytemora* in July to selecting barnacle larvae, *Eurytemora* and mysids in September. This shift could be reflected by different feeding modes of alewife, switching from an opportunistic feeding mode consisting mostly of barnacle larvae in spring when less motile larvae are in high abundance to feeding selectively on more motile copepods later in the season. Because barnacle larvae lack the escape abilities that copepods have, it makes sense that barnacle larvae would be eaten in proportion to their abundance.

The importance of mysids and *Eurytemora* in the diet may have increased over time. Barnacle larvae (the most dominant food source by numbers in May) were typically most abundant in the environment in May (when adult barnacles reproduce), suggesting that barnacle larvae may alleviate predation pressure on other preferred prey such as *Eurytemora*, or maybe even mysids, which appear to increase in importance over time. Freshwater feeding habit studies in particular have shown that alewife are generally considered size selective, active particulate, and passive filter feeders that can change zooplankton communities (Crowder et al. 1987; Hewett and Stewart 1989; Stone and Jessop 1994). With alewife feeding mode depending on a number of factors that include prey

density, size, visibility and size of the alewife (Janssen 1976, 1978; Durbin et al. 1979), these different feeding modes enable alewife to consume a large size range of prey (Stone and Jessop 1994). My study also shows that juvenile alewife consume a large size range of prey, from small copepods to mysids. However, I did not examine alewife-driven changes in the zooplankton community.

This study adds to the literature showing that juvenile alewife feed primarily on zooplankton. The quantity and availability of different zooplankton are important for juvenile survival, with competition for food a limiting factor of juvenile alewife recruitment (Post et al. 2008). The preference for different prey types over time highlights different feeding modes of juvenile herring as they feed selectively (by picking from the environment) or randomly (filter feeding from the environment). Benthic organisms, e.g., amphipods, were also observed in stomachs of these fish. These feeding modes and ability to feed on both pelagic and benthic prey are important survival tactics that allow these fish to avoid direct competition from fish that feed on similar species such as blueback herring, a sister species of alewife. One study in North Carolina found that juvenile alewives ate more types of prey than blueback herring (Davis and Cheek 1966), which could be an important factor for sustained survival of this species. During a time when habitat restoration is ongoing, particularly in the Penobscot estuary where dams have been removed, expanding historical access to spawning habitat, this information is important for understanding how changes in season or location affect feeding.

Prey selection by piscivorous fish can affect the structure of fish communities (Hambright 1994). For example, laboratory experiments mimicking temperate estuarine ecosystems with and without piscivorous predators have shown that piscivorous predators

can change prey fish size distribution and community composition (Wright et al. 1993). A better understanding of prey selectivity and preference for certain species can be important for predicting community changes in response to changes in predator numbers that can happen after large scale restoration efforts.

Conclusion

Understanding prey availability and preference for individual fish is important for early life history feeding success and ultimately recruitment of marine fishes (Mullin 1993). While my study focuses on juvenile alewife collected from the Penobscot estuary, the results can provide insight into life history and recruitment of alewife in similar systems. In Maine, the Penobscot River has undergone a large-scale restoration effort that has improved access to 3,218 km of river and stream habitat for alewife and other sea-run fish (Trinko Lake et al. 2012). These results emphasize the importance of the Penobscot estuary as habitat for juvenile alewife and add to a growing body of research highlighting the essential function of estuarine ecosystems to primary and secondary production (Beck et al. 2001). However, estuaries can be highly impacted by human activities. For centuries, humans developed and altered estuarine ecosystems for housing, energy production, and recreation, significantly degrading these systems (Edgar et al. 2000). Identifying the importance of estuaries to forage and commercially-important species such as alewife is critical for understanding and managing the different functions and services that these ecosystems provide. Recovery of the river herring population, a fundamental food source for many fish species, could result in the subsequent recovery of other species such as nearshore groundfish. A better understanding of the early life history of these fish could aid in answering fundamental questions surrounding recovery efforts of these fish

(i.e., where they stay, how long, how often they feed). This study provides valuable insight into feeding ecology of juvenile alewife, a species that once supported major fisheries along the Atlantic coast (Hall et al. 2012; Bethoney et al. 2013). Information derived from such studies could indirectly help to improve commercial and recreational fishing and increase tourism, along with recovery of predators that rely on a river herring diet.

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Tables & Figures

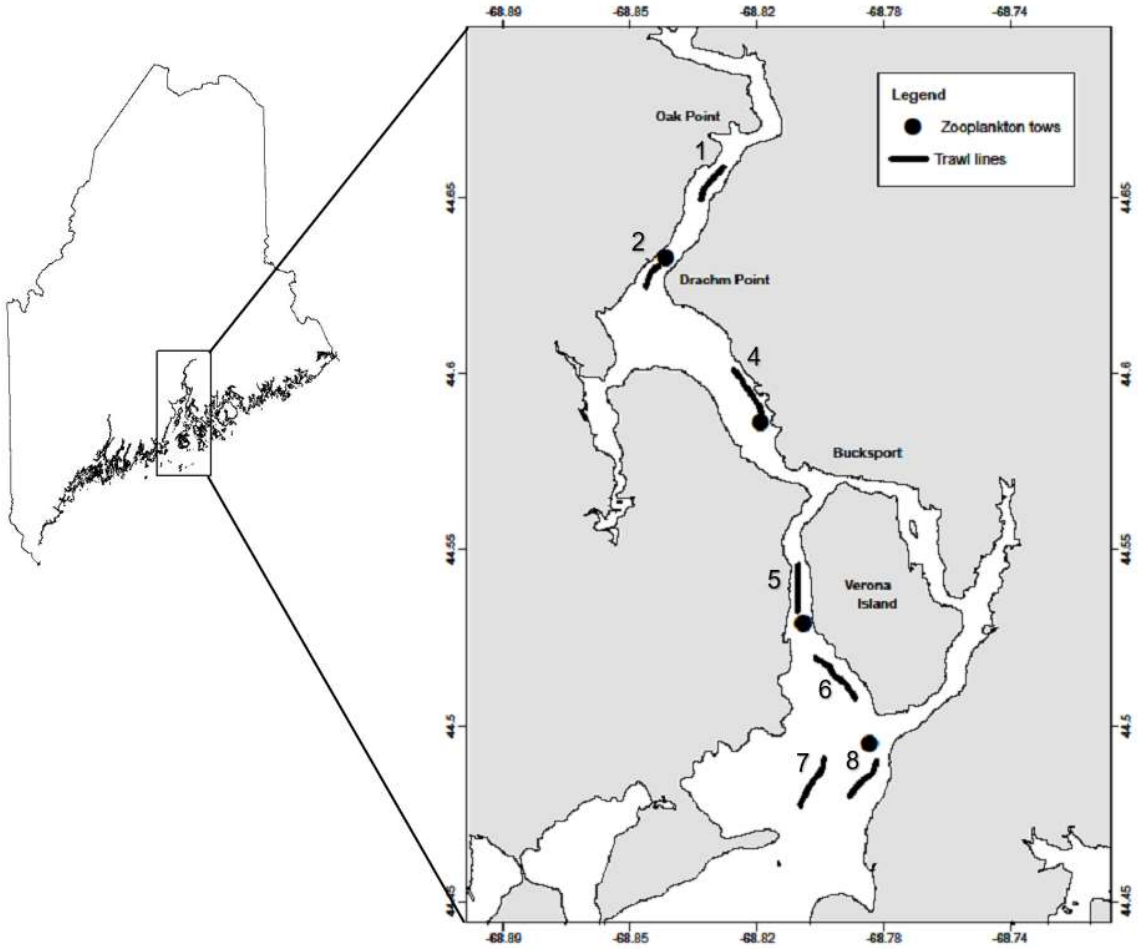

Figure 1. Penobscot estuary, Maine. The thick black lines indicate sites from which fish were collected in May, July and September/October 2013. Black circles indicate zooplankton tow locations. Collection sites 1- 4 (furthest north) fall within the upper estuary, whereas site 5-8 fall within the lower estuary. There is no site 3.

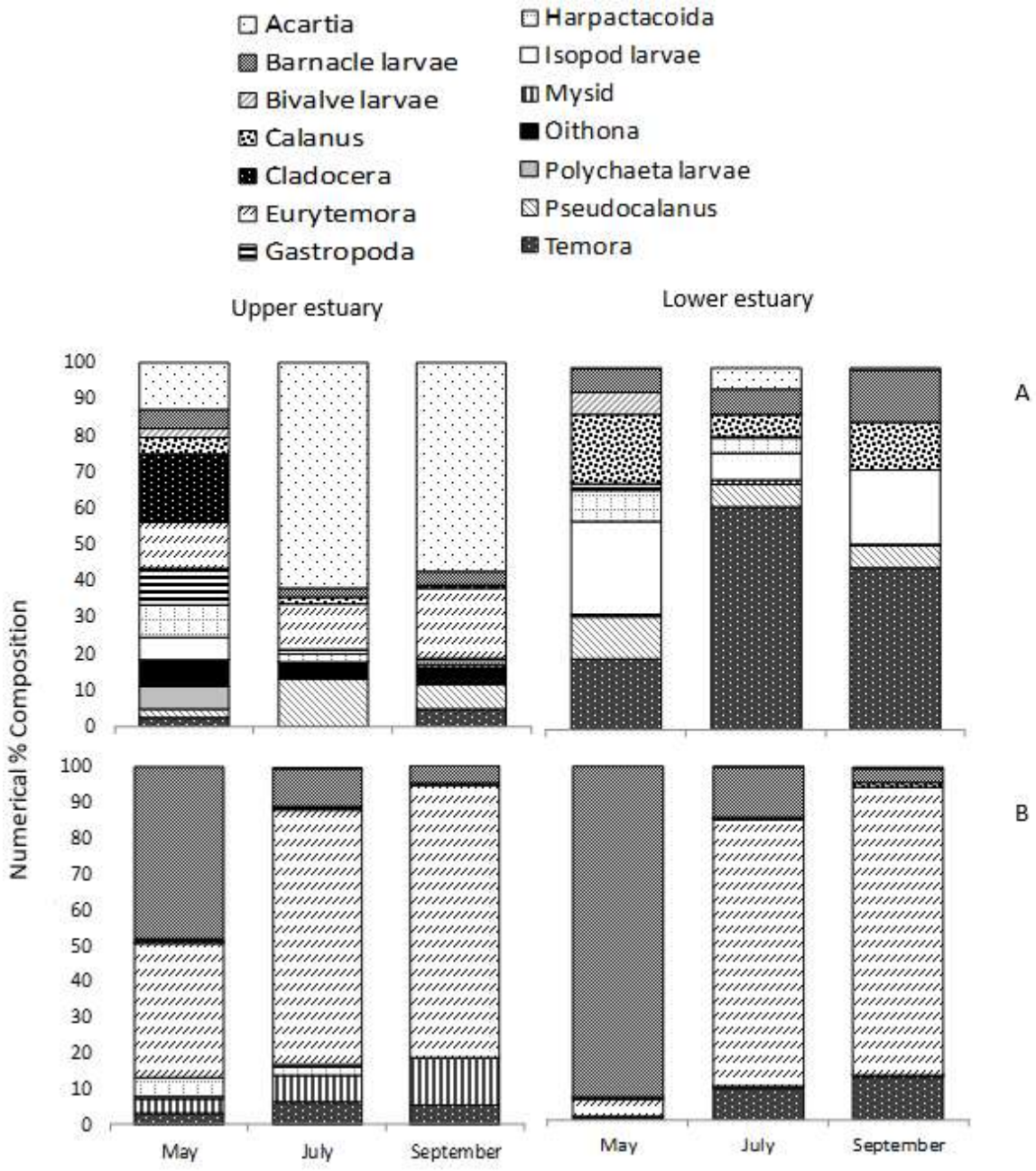

Figure 2. Stacked bar graphs show the percent by number (%) of prey items identified in > 5% of (A) the environment/estuary and (B) prey items identified in >5% of juvenile alewife diets from fish caught in the upper and lower estuary in May, July and September, 2013. Prey items and alewife were collected from the Penobscot estuary environment and from stomachs of juvenile alewife caught in the Penobscot estuary.

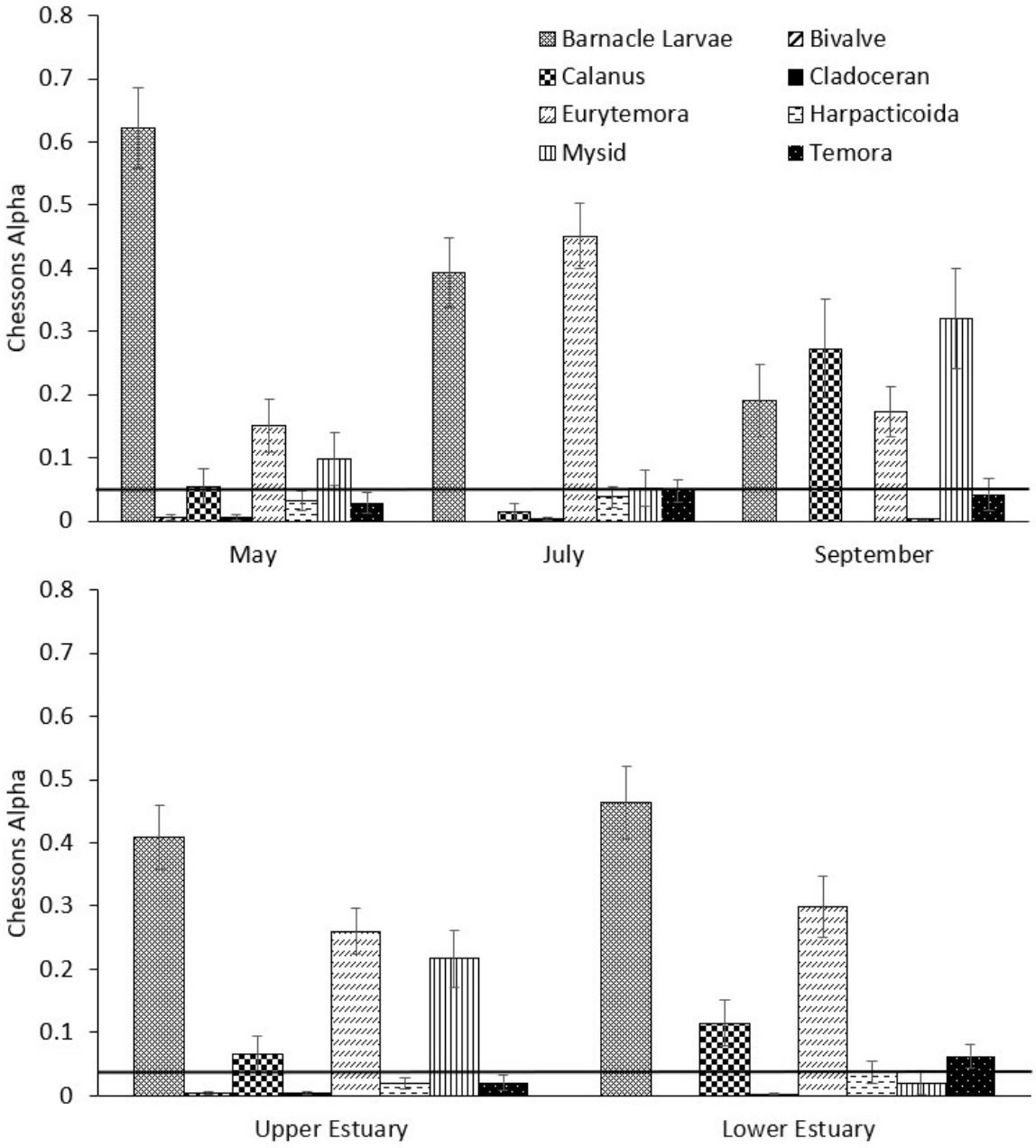

Figure 3. Mean (± SE) Manly-Chesson's alpha selectivity index over time (top panel) and by location collected in the estuary (bottom panel) for diet items that occurred in > 5% of juvenile alewife stomachs. The horizontal line indicates the random feeding line where alpha values above this value were interpreted as positive selection for that prey item, values overlapping the random feeding line were interpreted as neutral selection, and values entirely below the line were interpreted as negative selection.

Table 1. Abundance of zooplankton collected from the Penobscot estuary grouped by site and month. Numbers represent individuals/m^3, and are estimates based on subsampling.

	Lower Estuary			Upper Estuary		
Genus	**May**	**July**	**September**	**May**	**July**	**September**
Acartia	1086.77	13786.59	2152.26	436.19	4133.72	2280.43
Alosa	0.00	0.00	0.00	0.00	0.31	0.00
Amphipoda	0.00	0.00	0.00	0.00	0.13	0.00
Amphipod eggs	0.00	0.00	0.00	41.28	0.00	0.00
Balanus	455.85	455.85	1705.09	168.00	86.36	210.29
Balanus larvae	0.00	0.00	0.00	0.00	0.00	0.00
Balanus naupllii	203.84	203.84	273.22	0.00	0.00	0.00
Bivalvia	89.87	89.87	866.13	53.04	455.07	0.00
Bivalve larvae	0.00	0.00	0.00	82.56	0.00	0.00
Bosmina	0.00	0.00	0.00	454.08	0.00	0.00
Calanus	83.40	83.40	0.00	0.00	202.25	0.00
Centropages	335.41	335.41	409.83	53.04	0.00	278.02
Cladocera	0.00	0.00	478.14	151.28	0.00	31.56
Cnidaria	83.40	83.40	0.05	0.00	0.00	0.00
Ctenophora	0.00	0.00	0.20	0.00	0.00	0.00
Cytia	0.00	0.00	68.66	0.00	0.00	0.32
Decapoda	0.16	0.16	0.16	0.00	1.16	0.57
Eurytemora	1614.14	1614.14	1742.49	645.35	781.95	865.95
Fish larvae	0.00	0.00	0.16	0.96	0.53	0.00
Gastropoda	350.87	451.96	0.00	636.46	50.56	39.72
Harpactacoida	107.49	136.61	0.00	347.75	202.25	43.26
Isopoda	0.00	0.00	0.00	206.40	0.00	0.00
Malacostraca	0.11	0.00	0.00	2.76	0.00	0.00
Maxillopoda	364.17	546.45	0.00	583.43	75.84	0.00
Membranipora	0.00	0.00	160.48	53.04	0.00	71.28
Mysida	0.81	0.68	0.90	5.86	8.28	30.32
Oithona	1195.33	1924.18	575.23	954.70	229.10	197.51
Paracalanus	0.00	0.00	0.00	0.00	0.00	23.40
Parvocalanus	0.00	0.00	0.00	41.28	0.00	79.43
Podon	0.00	108.27	0.00	0.00	50.56	0.00
Polychaeta	144.52	0.10	0.00	0.33	0.00	0.19
Polychaeta larvae	667.22	0.05	0.00	683.75	0.00	0.00
Pseudocalanus	348.36	2086.58	578.21	318.23	660.45	834.07
Sagitta	250.21	0.05	17.58	0.00	0.00	0.13
Temora	48.17	1773.39	33.35	100.20	0.00	234.01
Tortanus	203.84	216.53	0.00	106.08	0.00	39.72
Unknown	0.00	273.22	35.00	0.44	0.00	0.00

Table 2. Number of juvenile alewife and mean (± SE) condition, fullness, total length and weight collected in May, July and September 2013 from the upper and lower Penobscot estuary.

	Lower Estuary			Upper Estuary		
	May	**July**	**September**	**May**	**July**	**September**
Sample size	26	22	7	20	28	20
Condition	0.75 (±0.02)	0.83 (±0.01)	0.76 (±0.04)	0.72 (±0.02)	0.81(±0.01)	0.76 (±0.02)
Fullness	0.66 (±0.07)	0.75 (±0.07)	0.94 (±0.11)	0.58 (±0.09)	0.54 (±0.10)	0.91(±0.16)
Total Length (mm)	105.92 (±5.61)	99.50 (±5.08)	89.43 (±5.30)	105.85 (±4.70)	102.89 (±7.74)	116.95 (±8.18)
Weight (g)	10.87 (±2.05)	9.24 (±1.08)	5.63 (±0.92)	9.53 (±1.70)	12.50 (±2.04)	15.46 (±3.45)

Table 3. Diet of juvenile alewife captured during 2013 collections in the lower estuary of the Penobscot River. Diets are grouped by month collected (May, July and September). Prey count represents the total quantity of each prey type identified within stomachs of each group, %N and %FO are percentage by number and frequency of occurrence, respectively, within each group. Chesson values are Chesson selectivity means ± SE for each prey type within a given group. UnID = unidentified.

LOWER ESTUARY												
	MAY				JULY				SEPTEMBER			
Prey Type	**Prey count**	**%N**	**%FO**	**Chesson**	**Prey count**	**%N**	**%FO**	**Chesson**	**Prey count**	**%N**	**%FO**	**Chesson**
Balanidae												
Balanus spp.	7859	93.5	80.77	0.71±0.13	460	14.1	100.00	0.32±0.07	73	4.1	100.00	0.03±0.01
Cladocerans	20	0.2	11.54	0.00±0.00	6	0.2	18.18	0.01±0.00	0	0.0	0.00	0.00±0.00
Copepods												
Calanus spp.	47	0.6	30.77	0.10±0.02	14	0.4	9.09	0.00±0.00	24	1.4	71.43	0.52±0.20
Eurytemora affinis	417	5.0	80.77	0.14±0.03	2466	75.4	86.36	0.51±0.11	1449	81.7	100.00	0.15±0.06
Harpacticoid copepods	17	0.2	15.38	0.02±0.00	14	0.4	13.64	0.06±0.01	2	0.1	14.29	0.00±0.00
Temora spp.	44	0.5	15.38	0.03±0.01	292	8.9	27.27	0.07±0.02	219	12.4	42.86	0.15±0.06
Mollusca (larvae)												
UnID bivalvia larvae	5	0.1	11.54	0.00±0.00	6	0.2	18.18	0.00±0.00	0	0.0	0.00	0.00±0.00
Mysids												
UnID mysids	0	0.0	0.00	0.00±0.00	1	0.0	4.55	0.00±0.00	2	0.1	14.29	0.14±0.05
Total:	8409				3259				1769			

Table 4. Diet of juvenile alewife captured during 2013 collections in the upper estuary of the Penobscot River. Diets are grouped by month collected (May, July and September). Prey count represents the total quantity of each prey type identified within stomachs of each group, %N and %FO are percentage by number and frequency of occurrence, respectively, within each group. Chesson values are Chesson selectivity means ± SE for each prey type within a given group. UnID = unidentified.

UPPER ESTUARY												
	MAY				JULY				SEPTEMBER			
Prey Type	**Prey count**	**%N**	**%FO**	**Chesson**	**Prey count**	**%N**	**%FO**	**Chesson**	**Prey count**	**%N**	**%FO**	**Chesson**
Balanidae												
Balanus spp.	1160	48.4	80.00	0.52±0.10	80	11.6	71.43	0.43±0.08	105	4.9	80.00	0.25±0.06
Cladocerans	4	0.2	10.00	0.01±0.00	1	0.1	3.57	0.00±0.00	0	0.0	0.00	0.00±0.00
Copepods												
Calanus spp.	11	0.5	20.00	0.00±0.00	5	0.7	10.71	0.03±0.00	12	0.6	20.00	0.18±0.04
Eurytemora affinis	907	37.5	85.00	0.16±0.03	549	71.5	82.14	0.38±0.07	1635	75.8	90.00	0.18±0.04
Harpacticoid copepods	129	5.3	25.00	0.04±0.01	18	2.3	17.86	0.02±0.00	3	0.1	5.00	0.00±0.00
Temora spp.	84	3.5	15.00	0.03±0.01	49	6.4	10.71	0.03±0.00	121	5.6	15.00	0.01±0.00
Mollusca (larvae)												
UnID bivalvia larvae	16	0.7	15.00	0.01±0.00	0	0.0	0.00	0.00±0.00	0	0.0	0.00	0.00±0.00
Mysids												
UnID mysids	97	4.0	25.00	0.22±0.04	57	7.4	10.71	0.09±0.02	281	13.0	55.00	0.38±0.09
Total:	2408				759				2157			

Chapter 2. Juvenile alewife (*Alosa pseudoharengus*) movement and residency in a northern temperate estuary

Abstract

Information on juvenile alewife movement and habitat use of estuaries is limited to a few watersheds on the East Coast. The main purpose of this study was to use carbon isotopes to examine movement of juvenile alewife between the Penobscot Bay, estuary and fresh water and to estimate the amount of time spent in the estuary. Fish were collected in spring (May), summer (July) and fall (September and October) 2013 and 2014 at 7 fixed collection sites in the estuary. Based on the $\delta^{13}C$ signatures of muscle and liver tissue, juvenile alewife were identified as freshwater transient (recently from freshwater), bay transient (recently from the bay) or estuarine occupant (spent extended time in the estuary). Of 88 juvenile alewife analyzed for carbon isotopes, 34% were identified as estuarine occupants and had spent extended time periods in the estuary, whereas 66% of juvenile alewife had recently moved from the bay or freshwater over the time period analyzed, regardless of where they were caught in the estuary. Mean number of days that juveniles spent in the estuary was highest in spring and declined over time, with more movement occurring in fall. Estuarine occupants were significantly smaller than fish identified as recently from the bay or fresh water. This result suggests that movement of juvenile alewife between bay and estuary occurs frequently from May to October with movement partially explained by fish size. Juvenile alewife may use estuarine and nearshore marine habitats for extended time periods and migration between estuarine and bay habitats may not follow typical life history strategies.

Introduction

In the United States, estuaries along the northeastern coastline have been identified as essential fish nursery habitats (Able and Fahay 1998). These areas offer a range of habitats that can support large numbers of juvenile fish by providing food resources and protection from predators (Hoss and Thayer 1993; Strus and Hurley 1992; Boesch and Turner 1984; Blaber and Blaber 1980). As highly productive and critical nursery habitats for age 0 juveniles, estuaries are particularly important for diadromous fish species that spend their early life in those areas (Hoss and Thayer 1993).

In their native range, anadromous alewife (*Alosa pseudoharengus*) move from freshwater to marine habitats (and vice versa) as young of year and spawning adults, using highly productive estuaries during the transition. The majority of their life is spent at sea before returning to freshwater as adults to spawn. Although some adults die after spawning, typically they make their way back to sea and return to their natal streams to spawn the following year (NEFSC 2006). After hatching (2-15 days, depending on water temperature; Klauda et al. 1991), young fish spend 1-3 months in a nursery area before migrating through the estuary to the ocean. Alewife spend the next 3-5 years at sea before returning to their natal habitat to spawn (Jessop 1993).

As a diadromous species, juvenile alewife life history is well described (see Greene et al. 2009 for review), with complex use of (and movement across) marine, estuarine and freshwater habitats. As a critical link between upper and lower trophic levels, juvenile alewife transport nutrients between freshwater and marine systems. However, information on estuarine habitat use and movement between freshwater and marine systems by juvenile alewife is limited, and it is typically described with generalized statements

such as age-0 fish move downstream from natal freshwater lakes to more saline waters from mid-summer to late fall (Fay et al. 1983). In North America, alewife habitat ranges from Newfoundland to South Carolina. Among the more southern latitudes of their distribution, their use of estuaries has been studied more frequently, and some studies suggest complex movement patterns between marine areas and estuarine nurseries during the first year (Hoffman et al. 2008; Gahagan et al. 2012; Payne Wynne et al. 2015; Turner and Limburg 2016). This research suggests that alewife are opportunists and potentially move freely between marine and estuarine habitats, but this behavior has not been documented in more northern temperate estuaries.

In Maine, the Penobscot River has undergone a series of dam removals and alewife stocking as part of a large-scale effort to restore diadromous fish populations (Day 2006). As part of this project, National Oceanic and Atmospheric Administration (NOAA) scientists have monitored use of the Penobscot estuary by alewife and other fish species since 2012. In 2012 and again in 2013 researchers from the NOAA Orono Field Station unexpectedly found a large number of juvenile age 1+ alewife from April through September in the Penobscot estuary (Trinko Lake et al. 2012; O'Malley et al. 2017; Stevens et al. 2021). This observation was significant because these fish were too young to be spawning adults and too old to be recent young of year immigrants from fresh water, which suggested that juvenile alewife used the Penobscot estuary as more than a transitional habitat between freshwater and the ocean.

To study movement patterns, researchers measure stable isotope concentrations in animal tissues (see reviews by Hobson 1999; Rubenstein and Hobson 2004; Trueman et

al. 2012). Carbon isotopes are particularly useful in movement and food web studies because they vary by habitat and the primary photosynthetic pathway used by primary producers in the system, and they undergo minimal (0-2%) fractionation from prey to predator (Vander Zanden et al. 1999). Thus, the isotopic composition of carbon in tissues reflects the isotopic composition of carbon in the animal's prey. The isotope composition of carbon generally differs at the base of the food web, which creates a marked gradient of depleted to enriched ^{13}C values between freshwater and marine systems (Peterson and Fry 1987; Fry and Sherr 1989; Fry 2002). Researchers use these gradients to track movement patterns between habitats with different base carbon values (see reviews by Herzka 2005; Rubenstein and Hobson 2004; Hobson 1999). The rate of the shift from one carbon value to the next depends on tissue type and age of the fish, i.e., tissue carbon turnover time (Trueman et al. 2012).

Other stable isotopes useful in food web and movement studies are nitrogen and sulfur. Nitrogen isotopes can be used to infer the trophic position of an organism (Post 2002). Sulfur can be used to distinguish among marine, estuarine and freshwater food webs because the sulfur isotope distribution is dictated primarily by the salinity gradient (Rees et al. 1978).

In addition to inferring movement, carbon isotopes can estimate the amount of time (i.e., days) any given fish has resided in the habitat of interest by equating the incorporation or elimination of stable isotopes to "clocks". Laboratory experiments showed that juvenile fish tissue can follow a predictable shift over time when fish switch to a new diet or habitat (see review by Herzka 2005). When juvenile alewife change habitats and feed on prey with a significantly different isotopic value, that new value is incorporated

into the tissue over time. Eventually the tissue reaches a new equilibrium, reflecting the new habitat value. Carbon isotopic turnover times differ by tissue type, with muscle tissue typically taking longer to turn over than organs such as liver (Boecklen et al. 2011). For example, in a lab experiment estimating turnover rates of liver and muscle in juvenile salmonids following a diet switch, turnover rates of liver were faster (16 ± 4.8 days, mean ± SE) than muscle (39 ± 3.2 days; Heady and Moore 2013). With an estimate of turnover rates for the tissue of interest, the degree to which stable isotopes in the tissue(s) reflect the new habitat value can be used to estimate the timing of the diet shift (i.e., how long that fish fed in that habitat). Fishes with tissues that differ in carbon isotope values (i.e., muscle versus liver) can be assumed to have recently moved to a new habitat, whereas fish with similar stable isotope values in tissues can be inferred to have spent extended time in the same habitat.

I used $\delta^{13}C$ stable isotope values collected from habitat specific juvenile alewife and their food sources to create a map or isoscape of the different habitats (freshwater, bay and estuarine) that juvenile alewife experience. Using this map, juvenile alewife captured in the estuary were identified as estuarine occupants or transients based on where the $\delta^{13}C$ stable isotope values of their muscle and liver tissues fell within the carbon isotope map (criteria for assigning the $\delta^{13}C$ cut off points for each habitat are outlined in the methods below). These values were then used to estimate the amount of time that juvenile alewife fed and resided in the estuary of the Penobscot River. If juvenile alewife were transitioning through the estuary, I assumed that alewife would exhibit stable isotope values in both slow and fast turnover tissues that would indicate fully freshwater or fully marine habitat use. If juvenile alewives were feeding in the estuary, I expected that

tissues would reflect a more intermediate isotope value, and that the difference between fast and slow turnover tissues would indicate the amount of time the juvenile had spent in the estuary. Clarification of migration dynamics of juvenile alewife may contribute to a better understanding of estuarine use by these fish and assist in estuarine management, which is particularly important given the significance of alewife as a forage fish for species of high economic value.

Methods

Collection of target fish

Juvenile alewife were collected by the NOAA Fisheries Maine Field Station's pelagic fish survey (Stevens et al. 2021) from May, July and September (herein referred to as spring, summer and fall) in 2013 and 2014. Samples were collected via systematic sampling of 7 fixed sites within the Penobscot estuary representing a range of salinities from 0 to 28 ppt (Figure 1). The trawl net was a Mamou Trawl (Innovative Net Systems, Milton, Louisiana), a custom designed two-seam shrimp trawl constructed from two 19-mm diamond stretch mesh panels of high-density polyethylene (HDPE). The cod end (6.35 mm nylon mesh) was fitted with a rigid, live-capture aquarium that was a 1/3 scale version of the aquarium described by Sheehan et al. (2011). The net was towed with an 11-m Duffy-style lobster boat. Tows were conducted at 20-min intervals at a speed of ~2-4 knots. Sampling began in the morning at low tide in the lower estuary and moved upstream with the flood tide, ending in the upper estuary such that each site was sampled at a similar period in the tidal cycle for each sampling day. All sampling was conducted during daylight hours.

Once onboard, fish were identified and sorted by species. When available, a subsample of at least 20 juvenile alewife from each habitat type for each time period collected were retained for stable isotope and stomach content analyses. Fish were euthanized with a lethal dose of tricaine methanesulfonate (MS-222) and immediately frozen on dry ice to reduce post-mortem digestion. Animal care protocols were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Southern Maine (IACUC #050913-02).

Creating the isoscape

To create the $\delta^{13}C$ habitat range (isoscape) of the study area, prey items from the estuary were identified via stomach content analysis (Webb 2021), and the main prey identified in the stomach contents were collected from the estuary and used to characterize estuarine habitat endpoints. Juvenile alewife were used to characterize the freshwater and marine habitat endpoints.

Collection of estuarine prey items to map estuary endpoints

The expected carbon isotope range of the estuarine habitat was characterized by preferred prey items identified from stomach contents (zooplankton and mysid shrimp). Zooplankton and mysid shrimp were collected from the water column in spring and summer 2013 at sites 2, 4 and 8 in tandem with collection of juvenile alewife from the estuary (Figure 1). Zooplankton were collected using a 0.5-m diameter ring net with a flowmeter affixed to the opening and a mesh size of 250 mm towed vertically from 1 m off the bottom substrate through the water column. Larger zooplankton and mysid shrimp were collected using a ring net with a 1000-mm mesh size and a 1-m diameter opening with a flowmeter attached. Vertical tows were conducted by allowing the net to sink to a depth

of 1 m above the substrate and then allowing the net to remain at depth for approximately 1 min before pulling the net up quickly using a motorized 'pot hauler'. This method allows time for animals that were disturbed by the net's descent to distribute themselves in their original position. The speed of the net (approximately 2-3 m s-1) is necessary to combat net evasion by mysids. This method has been used successfully to catch other small cardioid species (Benoit-Bird personal communication). Individuals were identified to the lowest taxonomic resolution possible (usually species) and separated for stable isotope analysis. Animals used in stable isotope analysis were frozen (-20°C).

To further characterize the food web and to add additional isoscape verification data in each habitat, I collected resident benthic organisms. At estuarine intertidal sites, barnacles, crabs, mussels and periwinkles were collected by hand. Blue mussels only were collected from the bay, and Unionide freshwater mussels were collected from the four lakes (Table 1). Barnacles, crabs, mussels and periwinkles in the estuary were collected in spring and summer 2013, and mussels in the lakes were collected in spring 2014 for comparison purposes only and are not included in the habitat range (as they are not alewife prey). All samples were rinsed with deionized water before they were stored in the freezer (-20°C).

Collection of freshwater fish to map freshwater habitat endpoints

To characterize the carbon isotope habitat range in fresh water, young of year alewife were collected as they first exited four freshwater lakes in the Penobscot River watershed during fall 2014 (September and October; Table 1). Fish were transported to the lab on ice and the ^{13}C isotope values from their muscle tissue were used to determine the freshwater habitat designation.

Collection of bay fish to map marine habitat endpoints

To characterize the expected carbon isotope range of the bay habitat, alewife were collected from Penobscot Bay by the Maine Department of Marine Resources during the regular spring and fall 2013 and 2014 Maine-New Hampshire Inshore Trawl Surveys (Figure 1-B; Sherman et al. 2005). Fish were transported to the lab on ice. I designated six juvenile alewife as "bay residents" based on: (1) at least 30 consistent days in bay habitat prior to death, as inferred via otolith microchemistry (LaBonte 2016), (2) similarities in ^{13}C isotope values of both liver and muscle tissue (mean difference 0.17 ± 0.12‰ mean ± SD, n = 6), and (3) ^{13}C isotope values similar to adult alewife caught in the bay in previous years before returning to spawn (-18.61 ± 0.49‰, n = 13; Karen Wilson, unpublished data). All fish were euthanized with a lethal dose of tricaine methanesulfonate (MS-222) and transported to the lab on ice.

Treatment of samples for stable isotope analysis

Tissue samples were dried and homogenized for analysis using a mortar and pestle. However, depending on the sample type, analysis of stable isotope values required different pre-treatments. In the lab, fish were defrosted, total length and fork length were measured to the nearest 1.0 mm, and fish were weighed using an electronic balance (Sartius GE812; ± 0.01 g). Alewife identification was confirmed by examination of peritoneum color with pink to gray assumed to be alewife and black assumed to be the closely related blueback herring (*A. aestivalis;* Loesch 1987; but see Berlinsky et al. 2015). For fish the white dorsal muscle above the lateral line and the liver tissue were dissected and used for isotopic analysis. Zooplankton and mysid samples were processed whole; several individuals grouped by collection location were pooled for analysis. Soft tissues of

the benthic samples (crab [muscle], periwinkles [foot] and barnacles ([entire animal]) and the foot muscle of bivalves were used for isotopic analysis. All samples were rinsed three times with deionized water before they were dried at 60 °C for 24 hr. Dried samples were ground to a fine powder before encapsulation into 5 × 9 mm tin cups (CE Elantech, Inc.). Samples were weighed to the following specifications: liver and muscle tissue: 0.1 – 0.2 mg; mussels, periwinkles, barnacles, and crabs: 0.2 – 0.3 mg. Dissection tools, mortar and pestle and other materials used for sample preparation were washed with 2% HCl and rinsed with deionized water between each sample.

All samples were sent to the University of California–Davis Stable Isotope Facility for analysis. Samples were processed using a Europa Scientific Hydra 20/20 continuous-flow isotope ratio mass spectrometer with an analytic precision within 0.1% for C using Pee Dee belemnite as a standard. Sample precision fell between 0‰ and 0.41‰ as measured by duplicates. Stable isotopic ratios are given in the ‘‘delta (δ)’’ notation and calculated using the following formula:

$$\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000,$$

where X is ^{13}C and R is the ratio of $^{13}C/^{12}C$ for the sample and the international standard (Peterson and Fry 1987).

Lipid correction

High lipid tissues such as liver tend to have lower (i.e., more negative) $\delta^{13}C$ values relative to key biochemical compounds (DeNiro and Epstein 1978). Thus, lipids in tissue samples have the potential to elevate C:N ratios, which could ultimately bias the isotopic results. The C:N value of proteins typically ranges between 3.5 and 4.0 (Graham

et al. 2013). In this study the C:N ratio for all muscle tissue analyzed was < 4.0, suggesting minimal lipid effects in muscle tissue. The C:N ratio in liver, however, ranged from 3.47 to 6.89 (4.43 ± 0.57 SD). Typically, many researchers remove lipids prior to stable isotope analyses in tissue samples with C:N ratios > 3.5 (Post et al. 2007), or $\delta^{13}C$ results are corrected mathematically (McConnaughey and McRoy 1979; Post et al. 2007). Removal of lipids prior to analysis was not feasible for this study due to time and cost constraints; therefore, to determine if lipid corrections were required, the Kiljunen et al. (2006) model for lipid-normalization was calculated for liver and muscle tissue. The model was created using Atlantic herring (*Clupea harengus*), Atlantic salmon (*Salmo salar*), and eel (*Anguilla rostrata*), which was appropriate for this study because Atlantic herring belong to the same family as alewife.

I mathematically corrected for lipids using the following formula (Kiljunen et al. 2006) then compared lipid corrected tissue to uncorrected tissue:

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

Where L is the proportion of lipid content of the sample and $\delta^{13}C'$ is the lipid-normalized carbon value of the sample. D is the isotopic difference between protein and lipid and, based on laboratory experimentation, is equal to D = 7.018 (Kiljunen et al. 2006). I is a constant equal to 0.048 (Kiljunen et al. 2006). All lipid corrected $\delta^{13}C$ values shifted in a slightly positive (enriched in $\delta^{13}C$) direction, ranging from 0.78‰ to 1.13‰. $\delta^{13}C$ values and lipid corrected $\delta^{13}C$ values in muscle or liver did not differ statistically (muscle: t_{164} = 1.5148, p = 0.250; liver: t_{142} = 1.3026, p = 0.195). The habitat designation for each fish (see below) remained similar using the lipid corrected values and did not affect the final

analysis (i.e., 96% of designations were the same). Because minimal lipid correction shifts occurred, final analyses are presented without lipid corrections.

Statistical analyses

To test for differences between habitats, a one-way analysis of variance (ANOVA) followed by a Tukey's HSD post hoc test was performed on the three habitat types (i.e., the $\delta^{13}C$ stable isotope values from alewife muscle tissue collected in freshwater and marine habitats and from whole zooplankton and mysids collected from the estuary).

The isotope habitat map was created from the mean $\delta^{13}C$ stable isotope values (± 1 SD) of alewife muscle tissue and prey from each habitat (Table 2). I assigned each juvenile alewife captured in the estuary to one of three habitat use categories based on where its muscle and liver $\delta^{13}C$ values fell along the carbon map (Table 3): freshwater transients (recently moved from freshwater), bay transients (recently moved from the bay) and estuarine occupants (extended time residing in the estuary). For example, if a fish caught in the estuary had a liver value that fell within the range of the estuary designated endpoints (-25.58 to -20.23) and a muscle value that fell within the bay endpoints range (≥ -18.97), then that individual was identified as a transient from the bay because liver tissue turns over faster than muscle tissue and therefore liver should reflect the most recent feeding habitat.

Calculating residence time

Once each fish was assigned to a habitat, the amount of time that individual alewife spent in the estuary (i.e., residence time) was estimated using an equation by Hesslein et al. (1993; rearranged and transformed by Guelinckx et al. 2006). Following a diet

(i.e., habitat) switch, tissue replacement is affected by growth rate and to a smaller extent metabolic activity. The rate of metabolic turnover in tissue replacement for juvenile clupeids is unavailable and not feasible due to high mortality rates of juvenile clupeids in laboratory experiments (Guelinckx et al. 2006). Metabolic turnover (M) is assumed to be zero in the calculation of residence time because the rate of metabolic turnover is minimal relative to the fast growth rates of juvenile fish (Hesslein et al. 1993; McAvoy et al. 2001). Juvenile alewife specific growth rates were not available; however, the mean growth rate of juvenile fish from the same family (clupeids) was available. Therefore, to calculate residence time, I used a yearly average growth rate of 0.0163 day^{-1} calculated from young of year Atlantic herring (*Clupea harengus*; Guelinckx et al. 2006).

Using the following equation (Hesslein et al. 1993) transformed by Guelinckx et al. (2006) into a function of time (with M = 0), residence time (i.e., turnover time) for juvenile alewife was estimated:

$$t = -\frac{1}{k}\ln\left((C_t - C_f)/(C_i - C_f)\right)$$

where C_t is the $\delta^{13}C$ value of a fish at any time after switching to a new diet (actual value of the tissue of interest during the time analyzed), C_i is the initial $\delta^{13}C$ value of a fish (expected value of the fish in its prior habitat), C_f is the expected $\delta^{13}C$ value of a fish in equilibrium with its new diet (expected value based on where the fish was caught), k is the specific growth rate constant (estimated at 0.0163 per day) and t is time (days). Time estimates were calculated for both muscle and liver tissues, and the mean residence time of muscle and liver was used to improve precision for estimated time since diet shift (Heady and Moore 2013).

Assigning estuary fish as occupant or transient based on the isoscape

Once target fish collected from the estuary were assigned to one of three habitat use categories (estuarine occupant, bay transient or freshwater transient) and estimated number of days in the estuary was calculated, differences in habitat use patterns were analyzed by month collected, fish size and fish condition. To determine if time spent in the estuary and habitat use designation were related to month collected and fish size, individual $\delta^{13}C$ stable isotope values were plotted by month against fish length and coded by tissue type.

Data for time spent in the estuary by month were not normally distributed (Levene test) and could not be corrected using log transformations. For these data, a Kruskal Wallis nonparametric test, followed by Dunn post hoc analysis, was used on individual fish to determine if the amount of time spent in the estuary differed by month collected.

Fish size (fork length and weight) was normally distributed; thus, a t-test was used to determine if there was a difference in fish size by designation (transient or occupant).

Fulton's condition index

As a measure of body condition, Fulton's condition index (K) was calculated as follows (originally used but not explicitly stated by Fulton, 1904):

$$K = 100{,}000 M_b L_S^{-3},$$

where fish standard length is L_s (in mm) and wet body mass is M_b (in g).

To determine if fish condition differed based on whether the fish had recently moved into the estuary from another habitat (freshwater or bay transient) or had remained

in the estuary (estuarine occupant), a t-test was performed between transient and occupant fish.

Model residuals were checked for normality and homoscedasticity by visual inspection of residual plots. All statistical analysis were conducted in R (R Core Team 2018) based on a 0.05 significance level.

Results

Stomach content analysis

Stomach contents from 132 alewife were identified. Of those, 123 (93.2%) stomachs contained at least one prey item. Only 6 alewife (4.5%) had no food and 3 alewife (2.3%) had no identifiable prey within their stomachs. Diets were dominated by a few species; barnacle larvae (*Balanus*) contributed 51.6% of all stomachs by numbers followed by the calanoid copepods *Eurytemora* (39.4%) and *Temora longicornis. Eurytemora* spp. dominated the diet in terms of frequency of occurrence (80% of stomachs) and Mysidae dominated the diets in terms of prey weight.

Target fish collection

Out of 132 alewife that had stomach contents analyzed, 88 juvenile alewife ranging in size from 62 mm to 166 mm were analyzed for carbon isotopes. Of those fish, 45 alewife were collected from the lower estuary (sites 5 - 8) and 43 from the upper estuary (sites 1 - 4), with a mean fork length of 98 ± 0.63 mm (SE) and 100 ± 0.67 mm, respectively. Nine young of year alewife with a mean fork length of 88 ± 1.65 mm were collected from four lakes. Six juveniles from the bay (110 ± 5.06 mm) had otolith microchemistry indicating residence in full strength seawater (Labonte 2016); thus, they were identified as bay residents and used in the bay habitat use designation. These bay fish also

had $\delta^{13}C$ values consistent with bay habitat use with minimal differences between liver and muscle tissue $\delta^{13}C$ values (range = 0.03 - 0.34‰, mean difference = 0.17‰, SD = 0.12‰). Neither size (Kruskal Wallis: $\chi^2 = 4.30$, df = 2, p = 0.12) nor weight (Kruskal Wallis: $\chi^2 = 4.74$, df = 2, p = 0.09) of fish differed across habitats.

The isoscape

$\delta^{13}C$ values from resident organisms collected in the three habitats were distinct (ANOVA: $F_{2,32} = 70.86$, $p < 0.001$; Table 2). Post hoc comparison of $\delta^{13}C$ values showed that all bay, estuary and freshwater habitats differed significantly from each other (Tukey HSD: adjusted $p < 0.0005$). Mean ± 1 SD $\delta^{13}C$ values from each habitat were used to assign estuarine-caught fish into their respective habitat (Table 2). I also examined stable isotopes for nitrogen (for all samples) and sulfur (in muscle tissues of a subset of 17 samples) to confirm habitat designations: Transients from the bay had 58% higher sulfur (Kruskal Wallis: $\chi^2 = 14.62$, df = 2, $p < 0.0001$) and 22% higher nitrogen (Kruskal Wallis: $\chi^2 = 45.77$, df = 2, $p < 0.0001$) values than both estuarine occupants and freshwater transients (Figure 2).

Movement of individual fish was inferred by the difference between muscle tissue (relatively slow turnover) and liver tissue (relatively fast turnover) (Figures 3-5). Isotope values of livers were more similar to the estuarine habitat in which the fish were caught compared to muscle tissues, with 72% of transient fish livers showing a shift toward estuarine values (Figure 3-5). Both transient and resident fish were captured in the estuary in all three seasons across all fish sizes and from all collection sites.

Assigning estuary fish to resident or transient classification

Overall, most fish captured in the estuary were categorized as bay transients (52%), followed by estuarine residents (34%) and freshwater transients (14%). The proportions of habitat use patterns changed seasonally. The majority of juveniles (59%) in the spring were estuarine residents, but declined to 45% in summer and 10% in fall. Juveniles recently from the bay increased from 41% to 55% to 59% from spring to summer to fall. Freshwater transients were only identified in fall, when they represented 31% of all fish.

Differences in residence time

Estimates of residence time from 22 fish were based on muscle tissue only because the liver was too small to sample accurately. A plot of estimated residence time using just muscle tissue versus the average of muscle and liver showed that the estimated number of days in the estuary based on one tissue type followed a similar pattern as when residence time was calculated using two tissue types (Figure 6). Therefore, these fish were included in the mean residence time calculations.

On average, fish identified as estuarine residents spent 103 ± 8 days (SE) in the estuary, while transients spent 15 ± 2 days in the estuary (regardless of season). Spring estuary residents spent over 100 ± 11 days in the estuary prior to capture, which would place the fish in the estuary as early as February (Figure 7).

Variables associated with habitat use patterns

The amount of time fish spent in the estuary differed by season, with fish collected in spring (Kruskal Wallis: $\chi^2 = 19.16$, df = 2, p = 0.0002) and summer (Kruskal Wallis: $\chi^2 = 19.16$, df = 2, p = 0.0025) spending 3 and 1.2 times more days in the estuary

than fish collected in fall (Figure 7). Fork length of alewife identified as estuarine residents was 13% shorter than alewife identified as bay transients (Kruskal Wallis: $\chi^2 = 16.62$, df = 2, p = 0.0002) while there was no difference in size between freshwater and bay transients or between freshwater transients and estuarine residents. Estuarine resident fish were 32% lighter than transient fish ($t_{(96)} = -3.1429$, P = 0.0022). Condition of fish did not differ across the three habitats (Kruskal Wallis: $\chi^2 = 2.96$, df = 2, p = 0.227).

Discussion

In contrast to their traditionally accepted life history strategy, this study shows at least two different habitat use patterns for juvenile alewife: (1) juveniles remain and grow in the estuary or further upstream for an extended time period that can include overwintering in the estuary, and (2) juveniles move between the estuary and bay habitat frequently, a result also inferred from the examination of otolith microchemistry of some of these same fish (LaBonte 2016). Across all months sampled, some fish spent extended time periods in the estuary and others frequently immigrated from the bay. This observation of a more complex early life history of juvenile alewife in northern temperate estuaries is supported by findings of juvenile alewife and their sister species, blueback herring, in more southern latitudes (Limburg 1998; Turner and Limburg 2012, 2016).

Based on estuary residence times, length of time spent in the estuary varied widely, which can be partially explained by fish size. Fish collected in the estuary that I identified as recently coming from the bay were significantly larger than estuary occupants, with no difference in condition. Early life history strategies of fish have shown that growth rates and condition can be factors in migratory behavior (Jonsson and Jonsson 1993). Growth rates measured for a small number of these fish were lowest in the estuary

(Labonte 2016), in agreement with estuary residents being significantly smaller. In some cases, slow growing fishes migrate to another habitat, e.g., Seabass (*Lateolabrax japonicus*), Atlantic Salmon (*Salmo salar*) and White Perch (*Morone americana*; Bujold et al. 2004; Kraus and Secor 2004; Fuji et al. 2011), whereas in other cases fish classified as residents show faster growth (Mohan et al. 2015). Larger individuals in better condition when leaving the natal habitat or emigrating may have better survival odds and ultimately enhanced fitness (Limburg 1996; Sogard 1997). Given that these strategies are species specific and appear to vary individually, there is most likely a tradeoff between residing in or between nursery and marine habitats and frequent movement between habitats. Although fish condition did not differ between the two strategies, one could question which strategy contributes more than the other to the adult population, i.e., is there an advantage to either strategy? Analyzing growth rates over the entire lifespan of adult alewife using otolith microchemistry could reveal which strategy provides enhanced fitness and overall recruitment to the adult population (Secor and Rooker 2000).

Based on residence time, smaller juvenile alewife spent extended time in the estuary. Nearly half of the fish caught in spring were identified as spending extended time in the estuary (i.e., estuarine occupants). Although my sample size is limited, and residence time is an estimate based on growth of other juvenile species, this result suggests that nearly half of juvenile alewife residing in the estuary could have overwintered estuary or further upriver. These results complement research in more southern latitudes inferred from otolith microchemistry which also demonstrate that alosines overwinter in estuaries and further upriver in different river systems (Hoffman et al. 2008; Gahagan et al. 2012;

Turner and Limburg 2016). Additional focus on individuals captured in the estuary in early spring might further clarify the prevalence of overwintering in the estuary.

Adult alewife spawn from March to May in Maine. Juvenile alewife could potentially follow adults into the estuary during this time, which could explain why juvenile alewife from the bay (bay transients) were identified in spring (May). However, most bay transients were identified in fall (September and October), when young of year may be transiting via the estuary out to sea, not returning from sea. Fish recently moving in from the bay were collected throughout the estuary across all months with no patterns or grouping by collection site. A more plausible explanation might be that the estuary provides an enhanced feeding ground or refuge from predators. Over 97% of stomachs analyzed contained estuarine prey, and most stomachs (~75%) were full to engorged, suggesting that the estuary is significant feeding habitat for a wide size range of juvenile alewife.

There are limitations to using stable isotopes to identify movement patterns. Alewife must feed in that habitat long enough to incorporate habitat-specific carbon isotope values. I confirmed fish were indeed feeding in the estuary because 97% of fish stomachs contained estuarine organisms. By comparing a relatively slow turnover muscle tissue to a fast turnover liver sample, I minimized the possibility of not identifying a recent habitat switch. Turnover rates slow down as the fish gets older and their growth slows. Although there is a small range of sizes for fish analyzed in this study, the pattern is still maintained, with larger fish showing large and small differences between isotopic values of liver and muscle tissue, respectively. Furthermore, both resident and transient individuals were identified during a time when juveniles should exhibit significant growth. Fish may

assimilate isotope values from two different habitats if they move frequently between two habitats and feed in both habitats. However, I did not identify any marine prey in stomachs. If these fish move frequently between isotopically distinct habitats and do not feed there, isotopic values will not reflect the different habitats. Becker et al. (2016) found large numbers of fish moving between the estuary and marine system over short tidal periods (hours) from late winter to early spring, with more fish moving out of the estuary with the ebb tide (versus into the estuary). All fish in this study were collected on the rising tide; therefore, if fish were moving in with the rising tide, I should have seen marine species in their diets if they were feeding there. The wide range in liver and muscle differences between carbon isotope values clearly shows feeding on two isotopically distinct food sources, which I inferred as movement between habitats.

I used species specific samples for freshwater and marine baselines across multiple seasons. A clear pattern emerging from this study was the presence of highly significant differences in ^{13}C signatures between freshwater and marine alewife and prey from the estuary. The strong gradient in ^{13}C suggests that distinct ^{13}C based on habitat was incorporated into alewife tissue. Within each baseline, isotope values varied little across individuals and time collected. Different freshwater bodies of water differed slightly in isotope values. In this case, differences were not large enough to affect interpretation of the results. However, ^{13}C is a marker of terrestrial influence, with low values indicating terrestrial inputs related to the ^{13}C depletion of C_3 plants (Peterson and Fry 1987). When precipitation is low, terrestrial inputs decrease, potentially affecting the ^{13}C gradient between seasons. The zooplankton and mysids used to identify the estuarine ^{13}C signature were collected in spring (May) and fall (September and October), and the estuarine ^{13}C

signature for either species did not differ between May and October, suggesting no seasonal change in estuarine signature based on these prey items.

Conclusion

Movements of juvenile alewives are far more complex than indicated by the classical outline of anadromy. Distinct ^{13}C gradients have been used increasingly to study fish migration (see reviews by Hobson 1999; Herzka 2005; Graham et al. 2010; Trueman et al. 2012). Individuals moving between distinct isotopic habitats incorporate and carry with them information about previous feeding locations (Hobson 2008). The clear ^{13}C signature incorporated by juvenile alewife during their movements from the Penobscot Bay to the Penobscot River System provides a good example.

This research indicates that species specific movements may be individually based as opposed to population based. Movement among different nursery areas is a key strategy used by juvenile fish to increase growth (i.e., utilize higher quality or more abundant food sources) as well as the probability of survival to recruitment (i.e., to decrease predation risk). Pollution, over fishing eutrophication and development have led to habitat loss and degradation, particularly in estuaries. With increasing anthropogenic pressure on estuaries, understanding estuary use and movement of fish within estuaries is important for effective conservation and management of fish species.

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Tables & Figures

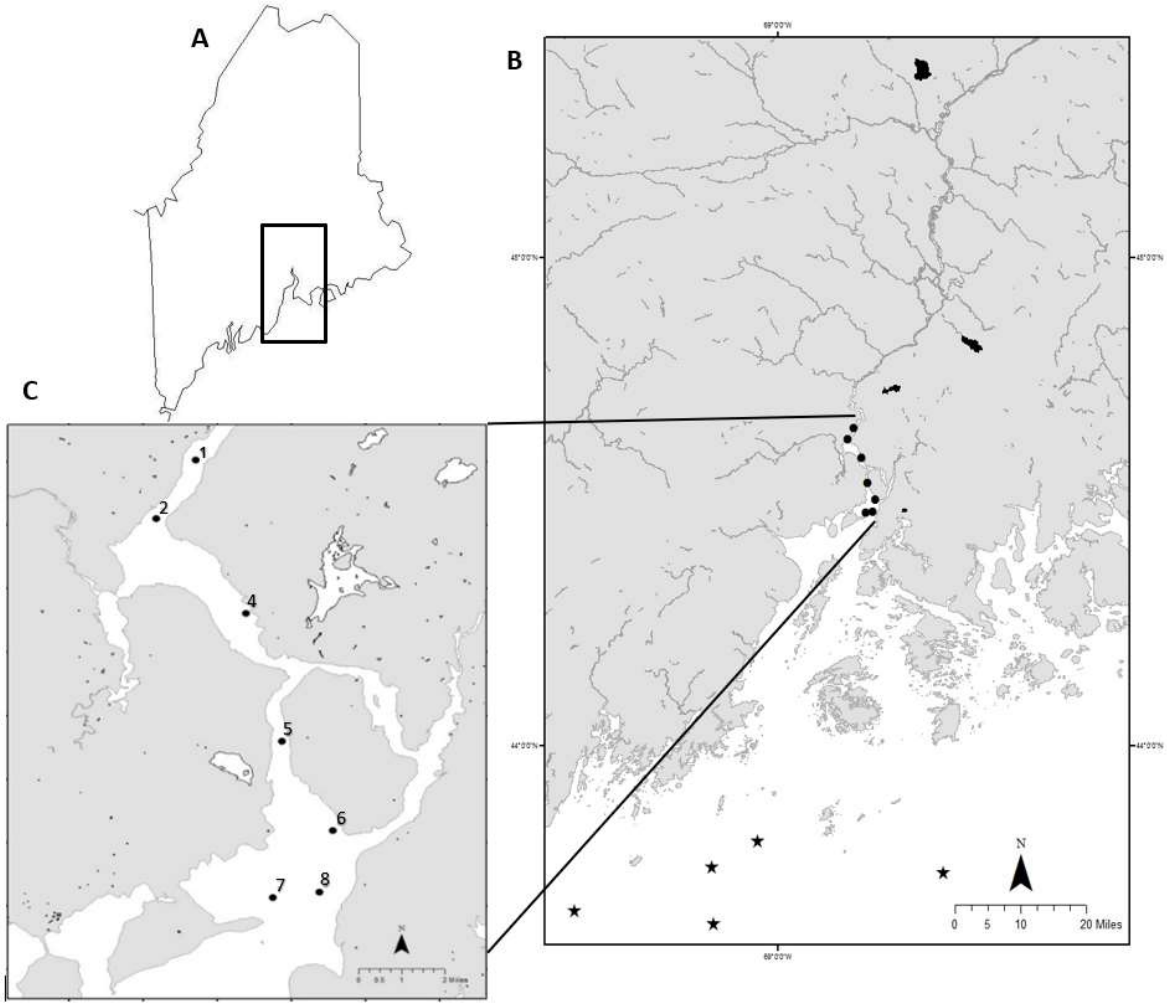


Figure 1. Sample sites where alewife were collected within the State of Maine (A). Penobscot lakes, estuary and bay collection sites are indicated on map B. The estuary discharges into Penobscot Bay. Bay collection sites are indicated by stars, estuary sampling sites are indicated by circles and lake collection sites are filled in black. Map C is a close-up map of the Penobscot estuary study area. Black numbered circles indicate sites from which fish were collected. Collection sites 1-4 (furthest north) fall within the upper estuary, whereas collection sites 5-8 fall within the lower estuary (note: there is no collection site 3).

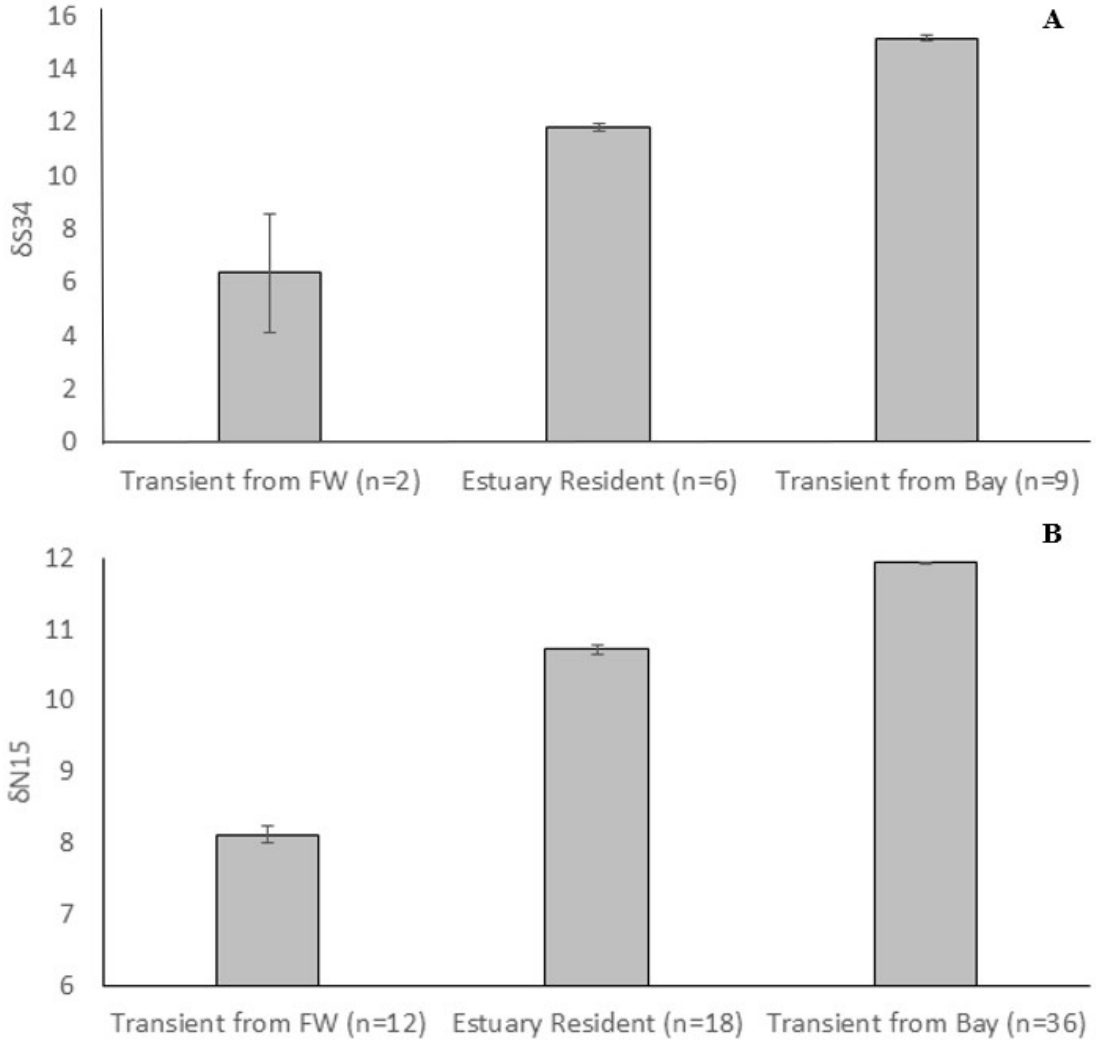


Figure 2. A) Mean ± SE sulfur isotope values ($\delta^{34}S$) of muscle tissue from juvenile alewife collected from the Penobscot Estuary for each habitat use designation. B) Mean ± SE nitrogen isotope values ($\delta^{15}N$) of muscle tissue from juvenile alewife collected from the Penobscot Estuary for each habitat use designation.

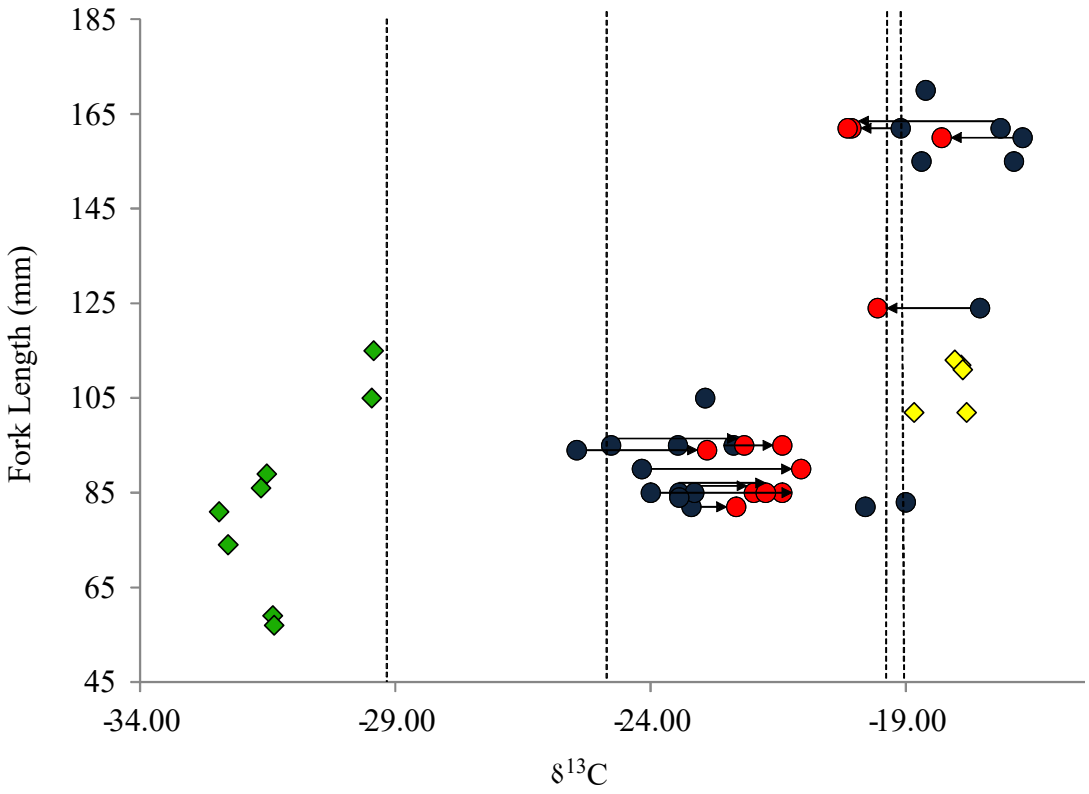


Figure 3. Relationship between ^{13}C of tissue and total length of juvenile alewife caught in the Penobscot estuary in spring. Each individual fish's muscle tissue is shown as a dark blue circle connected by its liver (red circle) to show direction of the diet shift. Individual green diamonds represent young of year alewife collected from freshwater lakes before exiting to the Penobscot River system, and individual yellow diamonds represent juvenile alewife collected from the Penobscot Bay that were identified as bay residents. Habitat isotopic ranges and transition areas are shown on each graph separated by vertical black dotted lines.

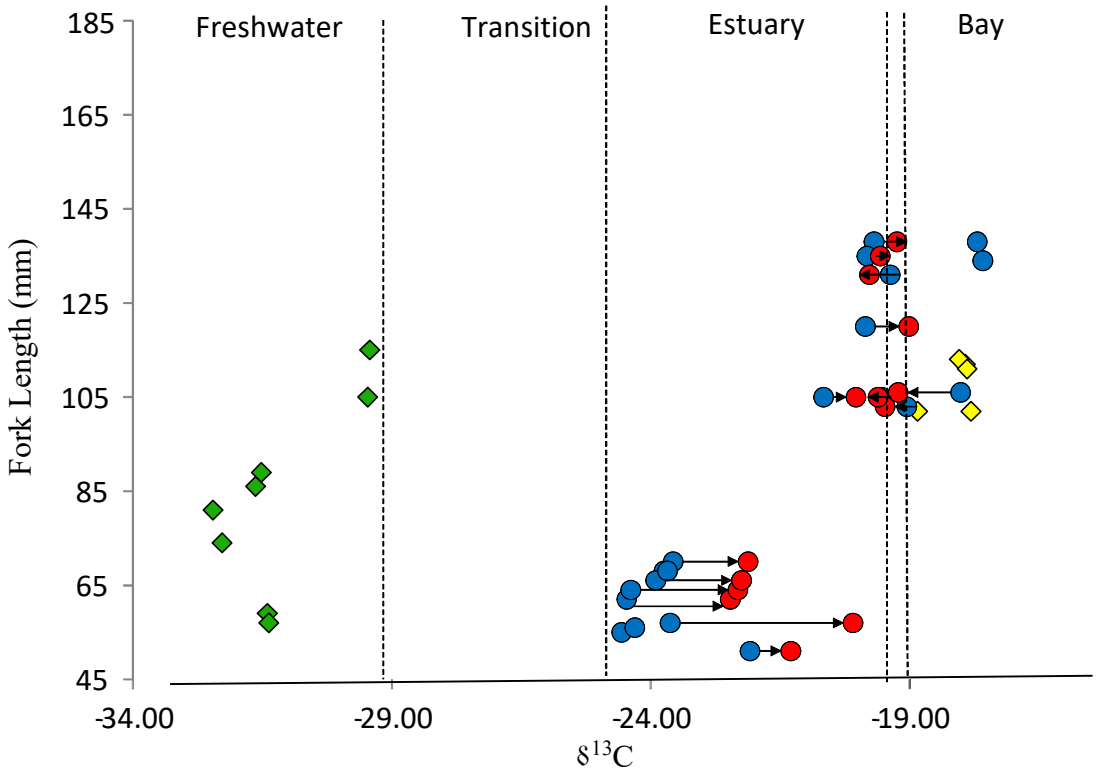


Figure 4. Relationship between ^{13}C of tissue and total length of juvenile alewife caught in the Penobscot estuary in summer. Each individual fish's muscle tissue is shown as a blue circle connected by its liver tissue (red circle) to show direction of the diet shift. Individual green diamonds represent young of year alewife collected from freshwater lakes before exiting to the Penobscot River system, and individual yellow diamonds represent juvenile alewife collected from the Penobscot Bay that were identified as bay residents. Habitat isotopic ranges and transition areas are shown on each graph separated by vertical black dotted lines.

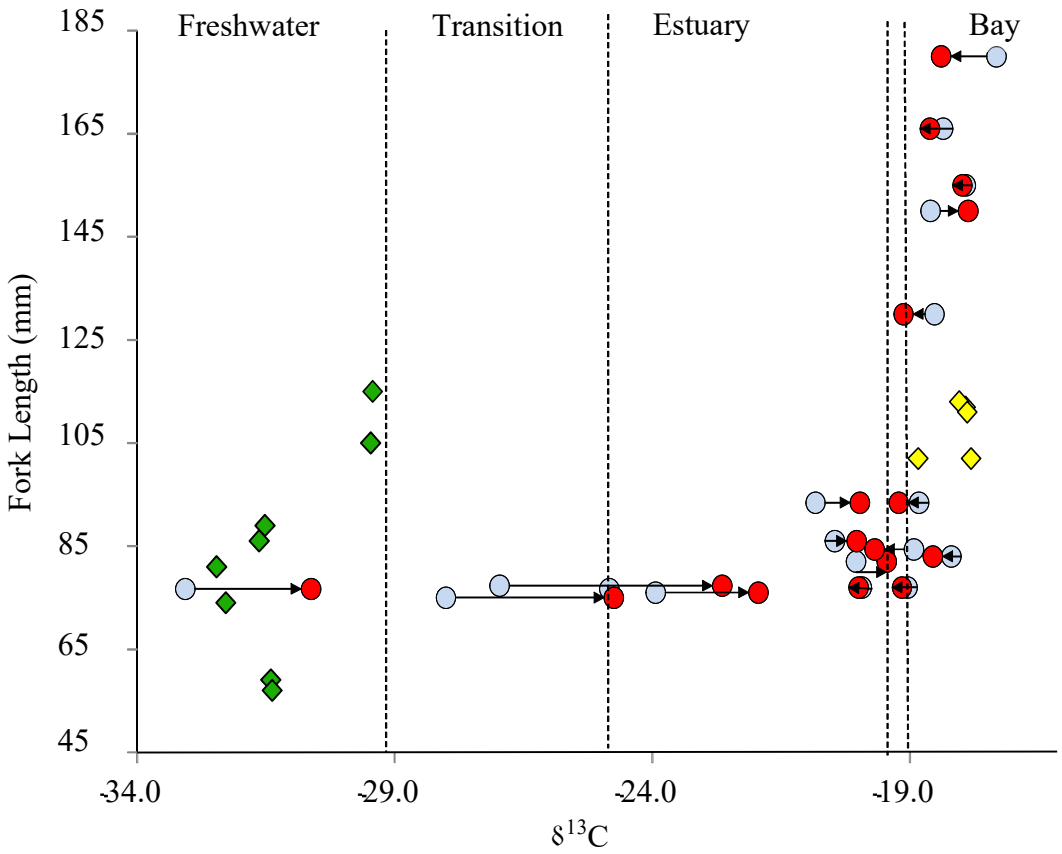


Figure 5. Relationship between ^{13}C of tissue and total length of juvenile alewife caught in the Penobscot Estuary in fall. Each individual fish's muscle tissue is shown as a light blue circle connected by its liver tissue (red circle) to show direction of the diet shift. Individual green diamonds represent young of year alewife collected from freshwater lakes before exiting to the Penobscot River system, and individual yellow diamonds represent juvenile alewife collected from the Penobscot Bay that were identified as bay residents. Habitat isotopic ranges and transition areas are shown on each graph separated by vertical black dotted lines.

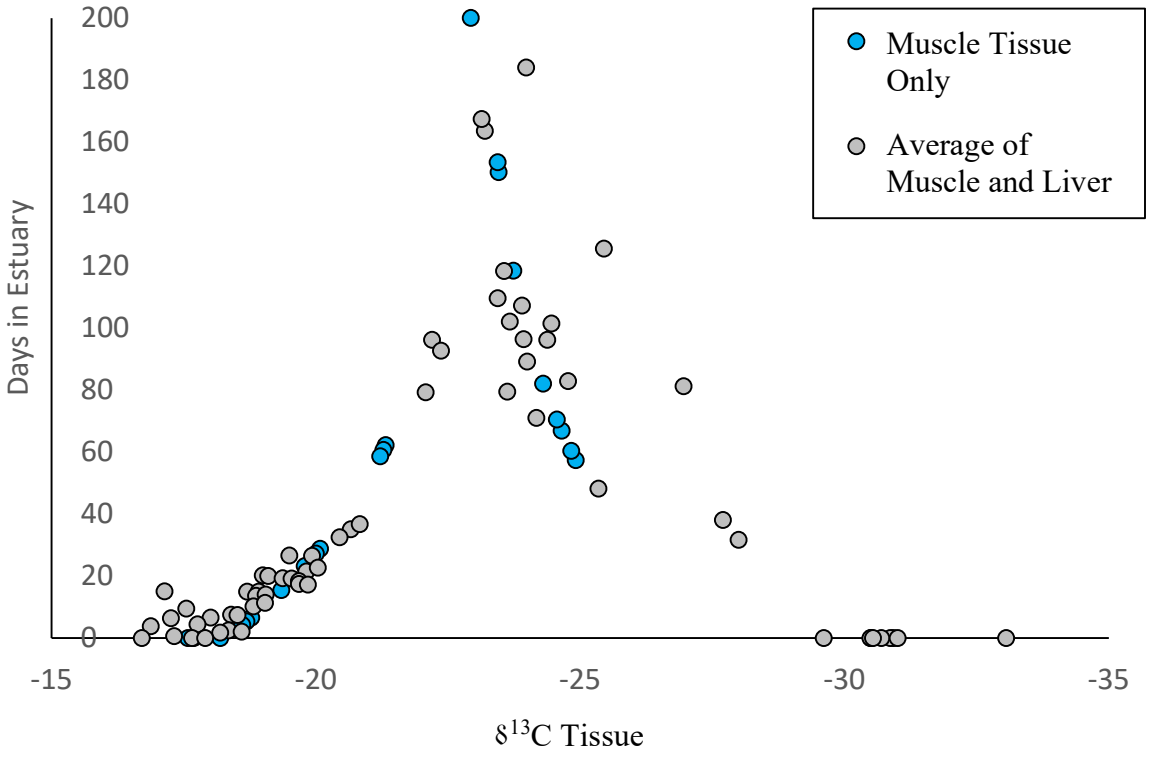


Figure 6. Relationship between estimated residence time and ^{13}C value of tissue from individual fish using an average of both muscle and liver (gray) or muscle tissue only (blue) for 22 fish without liver ^{13}C values. Estimated days in the estuary based on one tissue type follows a similar pattern as calculating residence time using two tissue types.

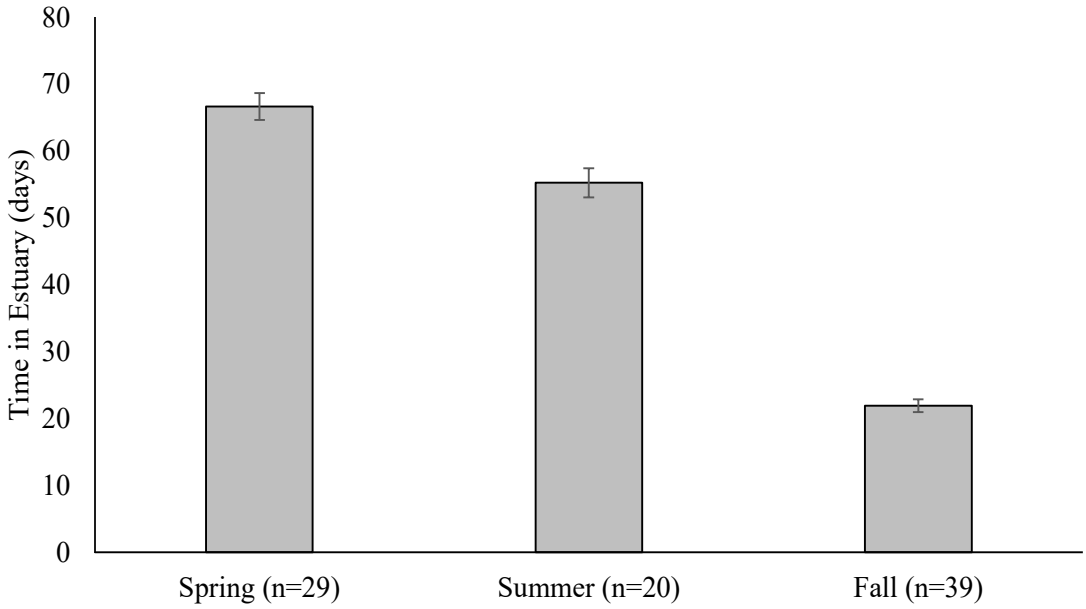


Figure 7. Mean ± SE estimated time spent (days) in the estuary for juvenile alewife collected from the Penobscot River estuary in spring, summer and fall 2013 and 2014.

Table 1. Lakes, location, size and depth where young of year alewife were collected during fall 2014. The number in parentheses indicates number of fish sampled.

Lake	Latitude	Longitude	Size (hectares)	Maximum depth (m)
Pierce Pond (3)	44.48231	-68.71937	44.5	3.6
Chemo Pond (2)	44.82314	-68.57067	494.9	7.3
Fields Pond (2)	44.72971	-68.73577	209.6	9.4
South Branch Lake (3)	45.38943	-68.67553	801.7	8.5

Table 2. Mean ± SD ^{13}C values of invertebrates and fish collected in three habitats. The number in parenthesis is the number of individual tissue values used to calculate the expected ranges.

Species	**Lake**	**Estuary**		**Bay**
		Upper	**Lower**	
Barnacle		-19.35 ± 0.17 (n = 3)	-18.66 ± 0.02 (n = 2)	
Crab		-18.01 ± 0.23 (n = 2)	-16.39 ± 0.44 (n = 4)	-15.03 ± 1.21 (n = 3) *
Mussel	-30.23 ± 1.32 (n = 5)	-18.98 ± 0.20 (n = 5)	-18.48 ± 0.38 (n = 7)	-18.17 ± 1.00 (n = 3) *
Mysid		-26.71 ± 0.38 (n = 3)	-21.31 ± 0.68 (n = 9)	
Periwinkle		-14.74 ± 1.35 (n = 3)	-15.28 ± 0.67 (n = 3)	
Alewife	-31.03 ± 1.18 (n = 9)			-18.37 ± 0.60 (n = 6)
Zooplankton		-25.79 ± 2.05 (n = 4)	-20.76 ± 0.51 (n = 4)	
^{13}C Habitat Range	**≤ -32.21 (n = 6)**	**-25.58 to -20.23 (n = 20)**		**≥ -18.97 (n = 6)**

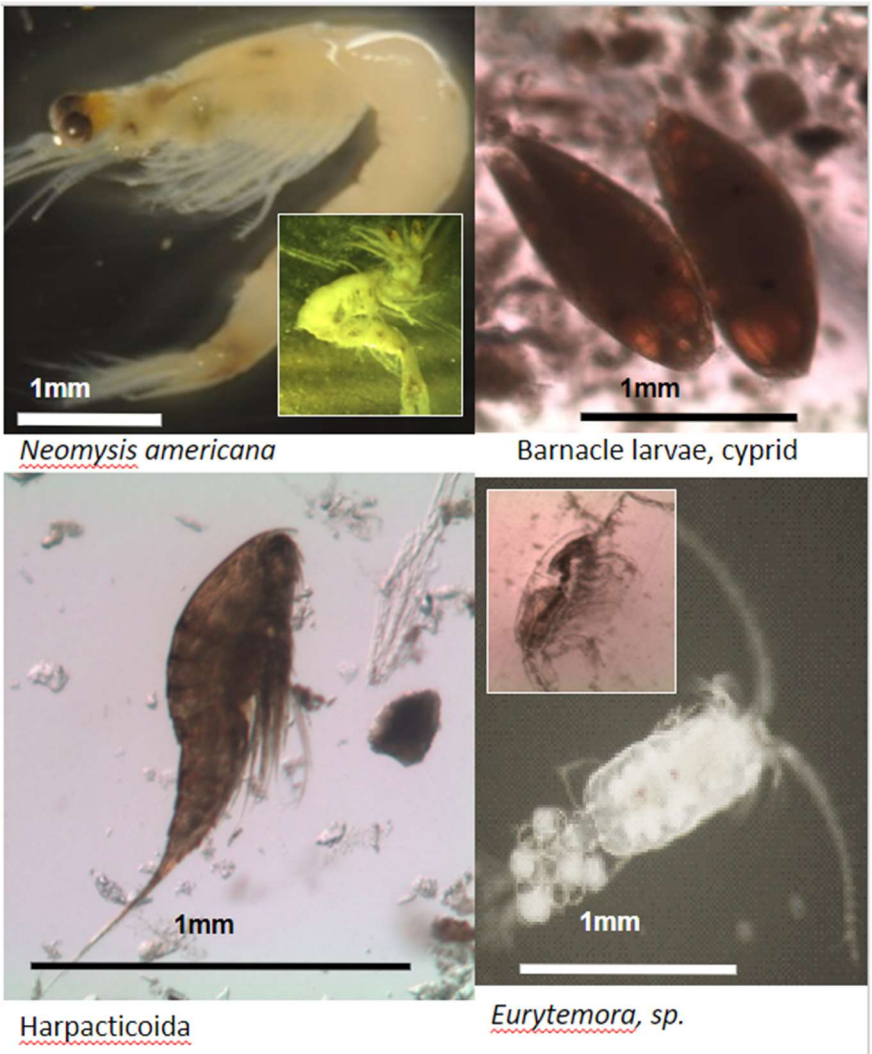
* Mussels and crabs from the Bay from Wilson, unpublished data (2009) were used only for comparison purposes and were not used in designation of bay habitat.

Table 3**.** Classification of juvenile alewife by ^{13}C values based on which habitat their muscle and liver tissues fell into (freshwater habitat: FW, estuary: EST and Marine: BAY). All juvenile alewife analyzed were caught in the estuary, so a fish with a bay liver and bay muscle ^{13}C value is still considered a transient from the bay, because it was caught in the estuary.

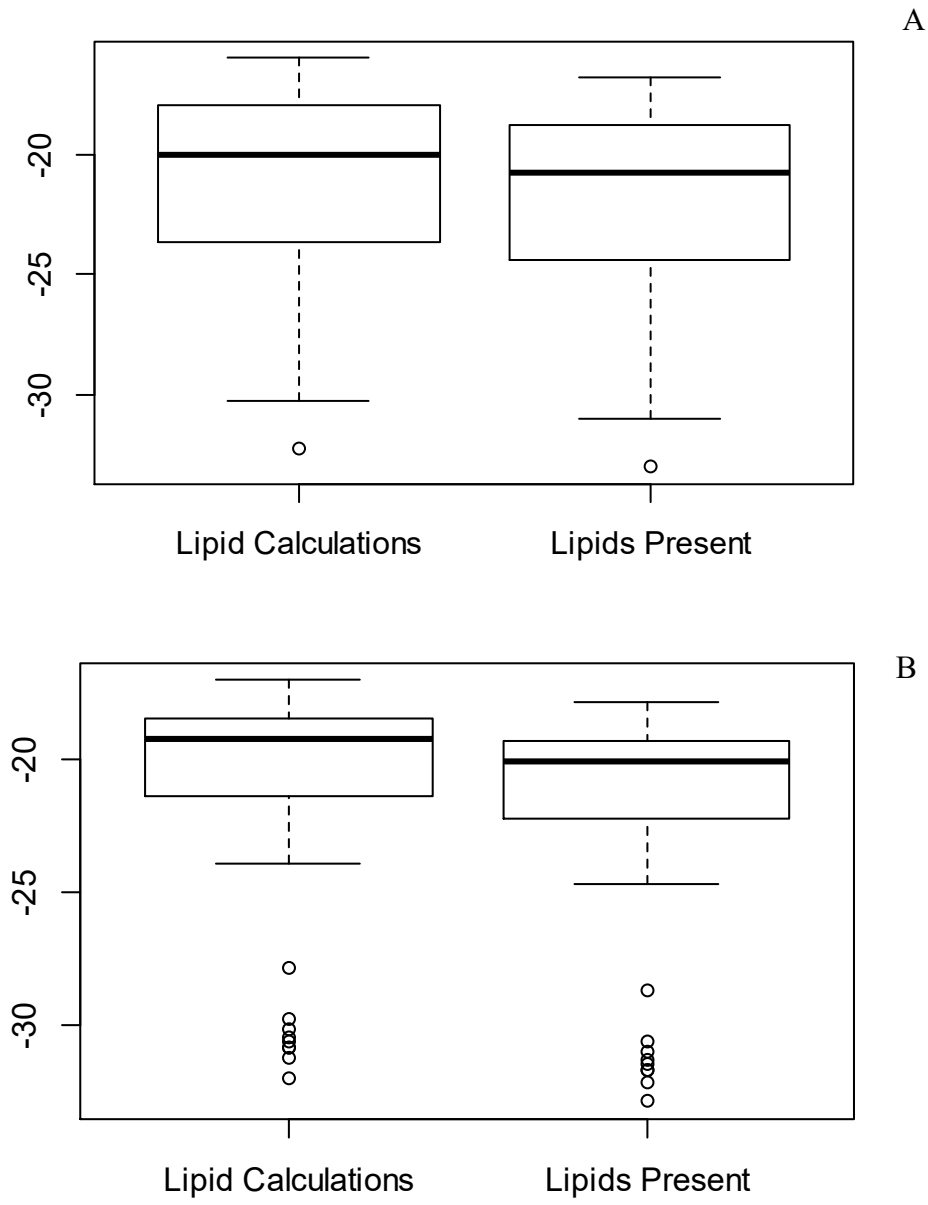
Capture location	**Liver ^{13}C habitat designation**	**Muscle ^{13}C habitat designation**	**Final designation**
EST	EST	EST	EST Resident
EST	EST	BAY	BAY Transient
EST	EST	FW	FW Transient
EST	BAY	EST	BAY Transient
EST	BAY	BAY	BAY Transient
EST	FW	FW	FW Transient
EST	N/A	EST	EST Resident
EST	N/A	BAY	BAY Transient
EST	N/A	FW	FW Transient

Appendix A-Supplemental Material

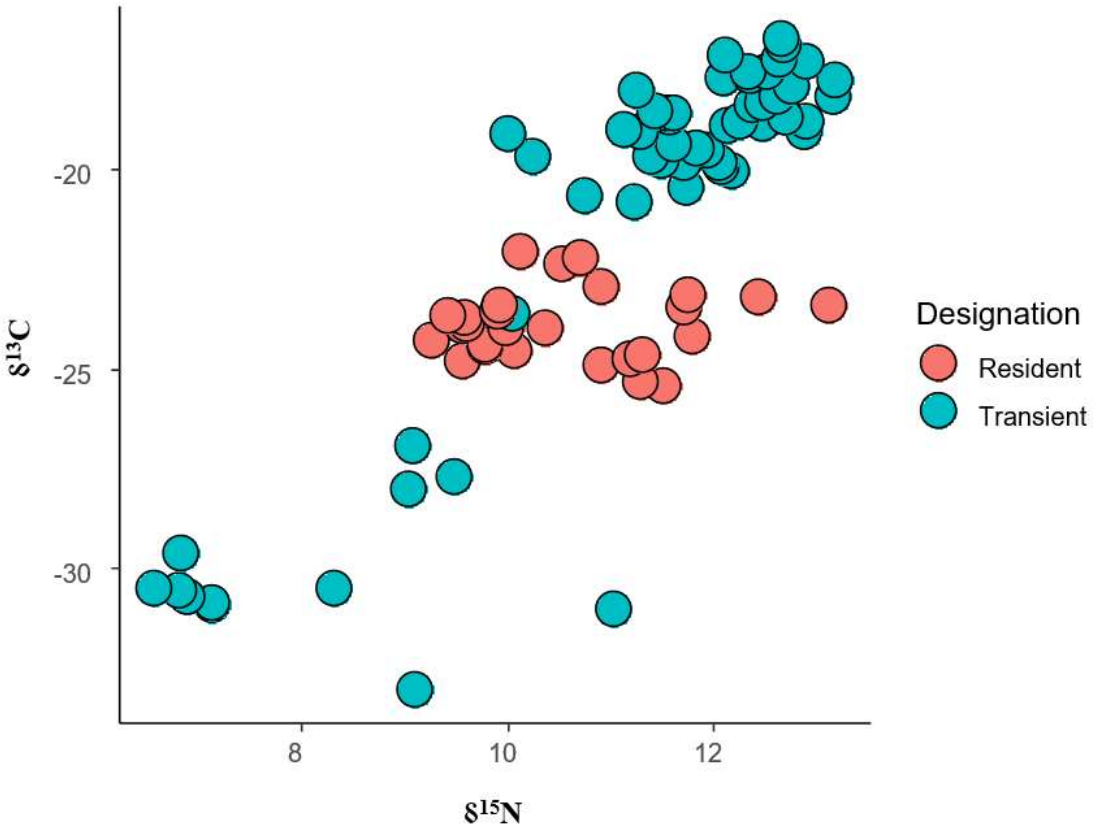
S1. NOAA Fisheries Maine Field Station surveying, sampling, sorting, weighing and measuring the fish community in the Penobscot River estuary.



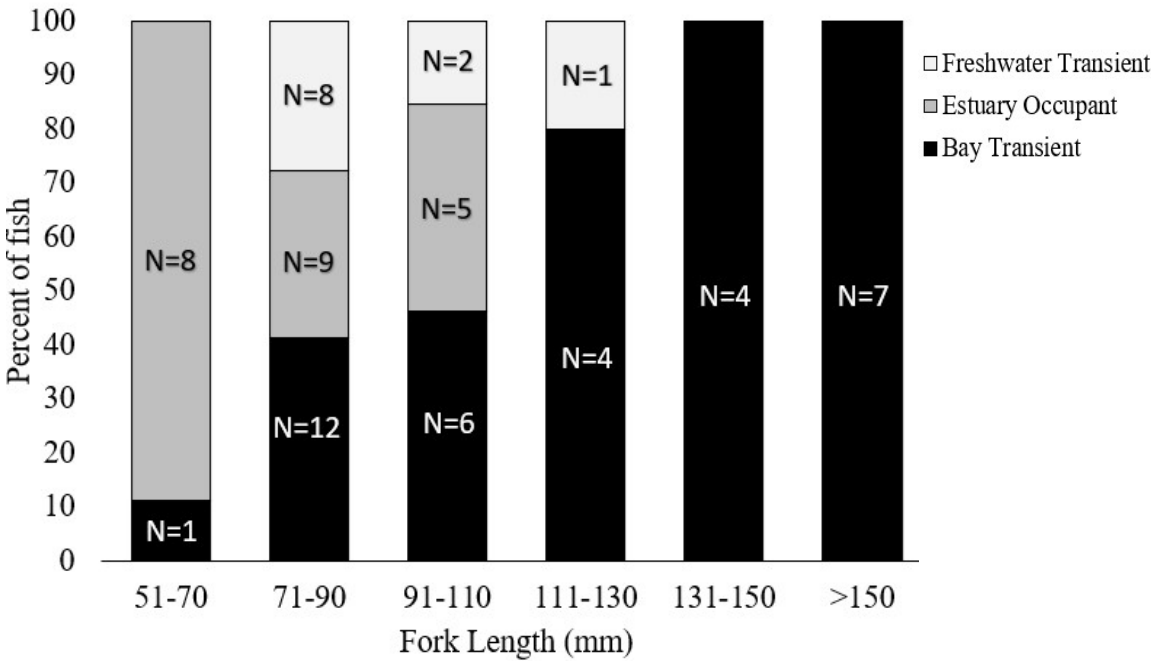
S2. Common prey items identified within juvenile alewife stomachs. The two photos in the upper left and bottom right are photos of zooplankton from the water column (before digestion) taken by Dr. Rachel Lasley-Rasher. The small inserts within these photos are photos of the same organism identified within stomachs. Photos of barnacle larvae and Harpacticoida also represent prey found within stomachs.



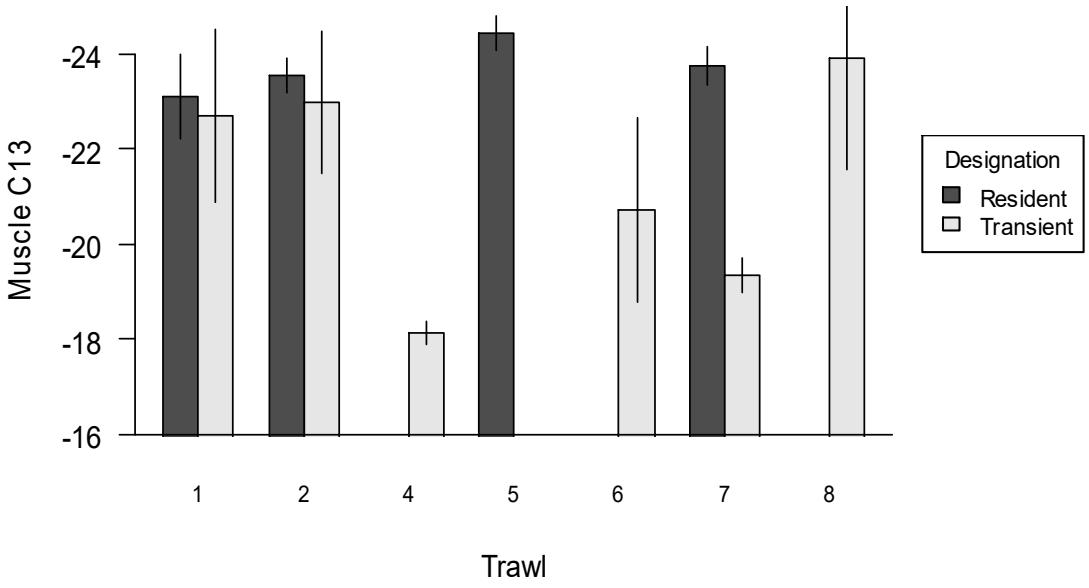
S3. Lipid corrected samples and samples with lipids present for A) ^{13}C of muscle tissue and B) ^{13}C of liver tissue. Lipid corrected samples were enriched in both muscle and liver tissue. Medians are indicated by thick black horizontal lines within the boxes, and 2nd and 3rd quartiles are represented by boxes, with the 1st (top) and 4th (bottom) quartiles marked by lines extended from the boxes.



S4. Relationship between ^{13}C and ^{15}N values for 88 muscle tissue (circles) of juvenile alewife collected from the Penobscot estuary in May, July and September 2013 (n = 76) and October 2014 (n = 12) coded by estuarine residents and transients (fresh water or bay). Transients from fresh water fall on the bottom half of the graph, whereas transients from the bay fall within the top half of the graph.



S5. Juvenile alewife caught in the Penobscot estuary coded by the percentage of fish in each designation by fish size. Sample size is shown within bars.



S6. Mean ± SE ^{13}C values of muscle tissue for estuarine residents and transients (regardless of habitat) for each collection site.

Appendix B-Final Approval

THE UNIVERSITY OF SOUTHERN MAINE
DEPARTMENT OF BIOLOGICAL SCIENCES

Date: 10 Nov 2021

We hereby recommend that the thesis entitled:

Juvenile alewife *(Alosa pseudoharengus)* feeding habits, movement and residency in a northern temperate estuary

Be accepted as partial fulfillment of the requirements for the degree of

Master of Science in Biology

Signatures

Author: _____ Date: 10 Nov 2021

Advisory Committee:

_____ Date: 12 Nov 2021

(Graduate Advisor)

_____ _____Date: 11 Nov 2021

_____ _____ Date: 11 Nov 2021

_____ _____Date: 12 Nov 2021

Chair of the Department of Biological Sciences:

_____ Date: 24 Nov 2021

Dean of the College of Science, Technology and Health:

_____ Date: 16 Dec 2021