Retrospective Examination of Habitat Use by Blueback Herring (Alosa aestivalis) Using Otolith Microchemical Methods

Molly Payne Wynne
University of Southern Maine

Follow this and additional works at: http://digitalcommons.usm.maine.edu/etd
Part of the Biology Commons

Recommended Citation
Wynne, Molly Payne, "Retrospective Examination of Habitat Use by Blueback Herring (Alosa aestivalis) Using Otolith Microchemical Methods" (2014). All Theses & Dissertations. 193.
http://digitalcommons.usm.maine.edu/etd/193
Retrospective Examination of Habitat Use by Blueback Herring

(*Alosa aestivalis*) Using Otolith Microchemical Methods

Molly L. Payne Wynne

A Thesis
Submitted in Partial Fulfillment of the
Requirements for the Degree of Master of Science in Biology
At the
University of Southern Maine

Advisor:
Dr. Karen A. Wilson
University of Southern Maine, Department of Environmental Science

Committee Members:
Dr. Christine R. Maher\(^1\), Dr. Theresa A. Theodose\(^1\),
Dr. Karin E. Limburg\(^2\)

\(^1\)University of Southern Maine, Department of Biological Science
\(^2\)State University of New York College of Environmental Science and Forestry,
Department of Environmental and Forest Biology
THE UNIVERSITY OF SOUTHERN MAINE
DEPARTMENT OF BIOLOGICAL SCIENCES

We hereby recommend that the thesis of Molly L. Payne
entitled: Retrospective Examination of Habitat Use by
Blueback Herring (Alosa aestivalis) Using Microchemical Methods
be accepted as partial fulfillment of the requirements for the degree of

Master of Science in Biology

Signatures
Author: Molly L. Payne Date: 2/24/2014
Advisory Committee:
(Graduate Advisor) Date: 2/24/2014
Date: 2/26/2014
Date: 2/26/2014
Date: 4/14/2014

Chair of the Department of Biological Sciences:
Date: 2/26/2014

Dean of the College of Arts and Sciences
Date: 3/4/14
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acknowledgements</td>
<td>II</td>
</tr>
<tr>
<td>Abstract</td>
<td>III</td>
</tr>
<tr>
<td>List of Tables</td>
<td>VI</td>
</tr>
<tr>
<td>List of Figures</td>
<td>V</td>
</tr>
<tr>
<td>Introduction</td>
<td>1</td>
</tr>
<tr>
<td>Methods</td>
<td>7</td>
</tr>
<tr>
<td>Results</td>
<td>15</td>
</tr>
<tr>
<td>Discussion</td>
<td>19</td>
</tr>
<tr>
<td>Conclusions</td>
<td>24</td>
</tr>
<tr>
<td>References</td>
<td>25</td>
</tr>
<tr>
<td>Tables</td>
<td>34</td>
</tr>
<tr>
<td>Figures</td>
<td>39</td>
</tr>
<tr>
<td>Appendix A: Otoliths</td>
<td>52</td>
</tr>
<tr>
<td>Appendix B: Chemical Analysis</td>
<td>54</td>
</tr>
<tr>
<td>Appendix C: Otolith Microchemical Signatures</td>
<td>56</td>
</tr>
<tr>
<td>Appendix D: Back-calculation Methods</td>
<td>59</td>
</tr>
</tbody>
</table>
ACKNOWLEDGMENTS

First and foremost, I would like to thank my advisor, Dr. Karen Wilson, for her guidance, enthusiasm, and encouragement from the beginning. Her support has allowed me to grow incredibly as a scientist and I feel so fortunate to have had this opportunity to pursue my graduate education under her mentorship.

Secondly, I owe innumerable thanks to Dr. Karin Limburg. Her unwavering support since my days as a college freshman has opened a multitude of doors and continues to inspire and amaze me. Without her, my passion for fisheries would not be what it is today.

I would also like to thank my committee members Dr. Chris Maher, and Dr. Terry Theodose for their support, thought provoking suggestions and guidance throughout the process.

I certainly must thank all who have provided technical, logistical and moral support at USM and SUNY-ESF; especially Sara Tuner, who inspired me to have my own undergraduate lab assistant (which in fact I never had), and my fellow graduate students.

Thank you to my parents and sister, Hannah, who have done everything in their power to allow me to follow my dreams.

Last but certainly not least, to my husband, Ryan, who inspires me beyond words.
ABSTRACT

Life histories of anadromous fish are difficult to examine due to constraints on direct observation. However, an understanding of patterns of habitat use is critical to management efforts since recruitment to spawning age is vital to population sustainability. Blueback herring (*Alosa aestivalis*) are anadromous fish that utilize a variety of freshwater, estuary and nearshore marine habitats. Rapid declines in their abundance and a subsequent petition for listing under the US Endangered Species Act in 2012 have ushered in an immediate need for life history information. This study focused on five river systems and seven spawning runs along the coast of Maine, USA. Systems with greater areas of freshwater and/or estuary were predicted to support young fish for a greater portion of the life cycle based on advantages provided by low to no salinity nursery habitat. I used otolith microchemistry and ambient water concentrations of Ca, Ba and Sr within fresh, estuarine and marine waters to identify habitat use in 131 returning adult fish collected during the spawning runs of 2010 and 2012. Several measures of low salinity habitat were used in analyses, including area of freshwater, estuary area and distance to sea from capture point. Total area of available habitat ranged from 213 to 6053 ha of freshwater and 204 to 3395 ha of estuary. Ambient concentrations of Sr:Ca ratios in each habitat were strongly correlated with salinity and were therefore used as the primary marker of habitat use in otoliths. A rapid increase in otolith Sr:Ca was interpreted as migration into seawater from freshwater. Migration histories varied among individuals, with some fish migrating into seawater well before the end of their first year, and others exhibiting freshwater and estuary habitat residency for a large portion of their first year. In addition, duration of low salinity habitat use was correlated with extent of freshwater habitat. One spawning run (Winnegance Lake) showed evidence of alternative life history strategies, and several spawning runs had distinct migration patterns. These results illustrate the importance of freshwater areas and estuaries for juvenile habitat, and have significant implications for riverine and estuary management. Anthropogenic impacts such as diminished water
quality and dredging on lower reaches of rivers and adjacent estuaries will have direct implications on survival and productivity of blueback herring.
LIST OF TABLES

Table 1. Sample site locations, estuary size estimates and fish data within the five study rivers. Estuary sizes were calculated based on area of “mixing” zone (salinity=0.5-25 ppt) as defined by NOAA Salinity Zone Data. Distance to sea from each collection location was measured as a direct route to the seaward edge of the mixing zone. One standard deviation for mean total length (TL, in mm) and mean wet weight (wet wt., in g), and mean age (in years) is shown in parentheses..........................................................................................................................34

Table 2. Mean correlation (Pearson's r) of individual elemental ratios and corresponding standard deviation (SD) and coefficient of variation (CV) within runs. Only CV values from Winnegance Sr:Ca and Hadley Lake Ba:Ca (in bold) indicate a low degree of coherence of signatures within those runs..........................................................35

Table 3. Results of pairwise comparisons using Tukey's test for differences in the number of regime shifts of Sr:Ca, Ba:Ca and Mn:Ca signatures per run. Significant differences (p< 0.05) are in bold...............................................................36

Table 4. Means (+ SD) by location for variables used in multiple discriminant analysis. Length at year 1 and egress are back-calculated estimations. Regime shifts are from one randomly selected side of the otolith transect (from core to edge)..........................................................................................................................37

Table 5. Classification and jackknifed classification matrix results of discriminant analysis based on estimated length at year 1 and egress, and number of regime shifts (Sr:Ca, Ba:Ca, Mn:Ca). "% correct" represents the percentage of individuals successfully classified to their collection location..................................................................38
LIST OF FIGURES

Page

Figure 1. Locations of study river systems along the coast of Maine. Black stars indicate approximate river mouth location........................................................................................................39

Figure 2. Maps of each study river; A) Kennebec, B) St. George, C) Orland, D) Patten, E) East Machias. Fish harvest/sample locations are indicated by stars. A stream connects Patten Pond (D) to the sea........................................................................................................40

Figure 3. Line drawing of an intact blueback herring otolith with horizontal lines depicting the placement of the transverse section which was used in otolith analysis........................................................................................................................................................................41

Figure 4. An otolith (200x magnification) with visible trench resulting from laser ablation. Vertical arrows indicate the core (year 0; center) and the first annulus (right; ventral). The individual was sampled at Benton Falls on the Kennebec River.................................................................................................................................................................41

Figure 5. Low salinity habitat use index (LSHU) calculation for one individual from the core to the dorsal edge of the otolith (sampled from East Machias River). Otolith Sr:Ca is represented by the solid line. The dotted line is the distance along the laser transect that is considered low salinity residency (Sr:Ca < 2; 221 µm). The dashed line represents the measured distance from the core to the first annulus (534 µm). The calculated LSHU value is 0.41 indicating the individual moved into sea water well before formation of the first annulus.................................................................42

Figure 6: Second order polynomial function used to fit data for length back-calculation of sample individuals ($R^2 =0.97$). Otolith width and total length data from YOY and yearling blueback herring from ME and NY, and larval American shad from NY........................................................................................................................................................................................................43
Figure 7. Concentrate-salinity plots depicting average Sr:Ca, Ba:Ca, and Mn:Ca versus salinity. Points are average concentrate ratios and salinity at each water sample location from two or three replicates. Relationships were fit to logarithmic functions. Standard deviation from the mean was ≤ 0.025 for all points.

Figure 8. Typical inverse relationships of Sr:Ca and Ba:Ca otolith microchemistry. The three example individuals are from Hadley Lake (A), Winnegance Lake (B), and Patten Pond (C). Also shown are Mn:Ca signatures from each individual. The primary axis of each graph depicts Sr:Ca values; the secondary, Ba:Ca and Mn:Ca values. The y axis indicates the distance from the core along the otolith sample transect in microns. Note the scale of the y axis' differs between graphs.

Figure 9. LSHU index value (i.e., portion of time spent in low salinity habitat in the first year) versus estuary size, freshwater area, and distance to sea) for each study river. Linear regression analysis ($R^2=0.07$) did not indicate a significant relationship. The center vertical line marks the median, the length of each box shows the range within which the central 50% of the values fall, with the box edges at the first and third quartiles. Whiskers show the range of observed values that fall within the inner quartile range. Pearson's correlation coefficient ($r$) between LSHU and each variable is $r = -0.16$, $P = 0.07$ for estuary size, freshwater area, $r = 0.368$, $P < 0.001$, and distance to sea, $r = 0.244$, $P < 0.001$.

Figure 10. Mean lengths at capture (total length), year 1, and egress. Lengths at year 1 and egress are estimates base on back-calculation methods. Error bars depict one standard deviation from the mean. Solid data points indicate significantly different means ($p ≤ 0.001$); mean total length in Patten Pond was significantly longer than all other runs except for Dresden Mills, mean length at year one in Hadley Lake was significantly less than all other runs with the exception of
Orland Dam and Seven Tree Pond; mean length at egress in Winnegance was significantly less than all other runs.

**Figure 11.** Estimated length at egress versus the maximum Mn:Ca regime value by location. Pearson's correlation coefficient = 0.437, P<0.001.

**Figure 12.** Average Sr:Ca signatures for transects spanning the entire otolith. Winnegance Lake and Patten Pond are visually distinct from the other runs. Solid vertical lines denote average distance to the first annulus on either side of the core (508 µm), dashed vertical line indicates the core.

**Figure 13.** Contrasting migration strategies in two individuals from Winnegance Lake. The individual represented by the dotted line showed prolonged low salinity habitat use (LSHU= 0.72), whereas the individual depicted by the solid line emigrated almost immediately into the estuary (LSHU= 0.02).
INTRODUCTION

Anadromous fish face numerous challenges, not the least of which is the use of dramatically different habitats throughout their lifetimes. Anadromy may have evolved to aid reproductive success and survival through the use of advantageous habitats (Gross 1987, Gross et al. 1988). Recent work suggests significant within species variation in the extent to which different runs within a species, and individuals within runs, use these freshwater and estuarine habitats (Limburg 1998, Secor 1999, Limburg 2001, Secor et al. 2009, Schindler 2012). This variation may lead to resilience in these species (Schindler 2012). In this study, variation in habitat use patterns during the first year in blueback herring (*Alosa aestivalis*) was investigated. The information was gathered by applying a retrospective approach utilizing elemental composition of otoliths from returning adults.

Blueback herring (family Clupeidae) are anadromous, schooling fishes believed to spend most of their lives at sea. They and alewife (*A. pseudoharengus*) are known collectively as "river herring" and both are valued economically, historically, and culturally along the eastern seaboard (Fay et al. 1983, Yako et al. 2002, Greene et al. 2009). The range of blueback herring overlaps that of alewife and extends north to Cape Breton, Nova Scotia and the Miramichi River, New Brunswick and south to the St. Johns River, Florida (Loesch 1987, Greene et al. 2009). Both blueback herring and alewife play important ecological roles as key links in food web interactions (Fay et al. 1983, Greene et al. 2009, Jones et al. 2010) and as nutrient vectors bringing marine derived nutrients into coastal freshwater systems (Garman and Macko 1998, MacAvoy et al. 2000, West et al. 2010).

Historic populations of river herring are thought to have immensely outnumbered those present in coastal systems today (Saunders et al. 2006, Limburg and Waldman 2009, Hall et al. 2011, 2012). Until recently, these declines were largely overlooked. In 2006 blueback herring and alewife were listed as species of concern by the National Marine Fisheries Service (Greene et al. 2009). Their decline in abundance
has been attributed to anthropogenic factors, such as dam construction, overfishing, watershed pollution, and urbanization (Saunders et al. 2006, Limburg and Waldman 2009, Hall et al. 2011). Harvest restrictions have been implemented in all eastern seaboard states since 2012 and the fishery remains closed in Connecticut, Massachusetts, Virginia, and Rhode Island (NMFS). In 2012, a review by the National Oceanographic and Atmospheric Administration (NOAA) deemed that the listing of river herring as threatened under the United States Endangered Species Act (ESA) was unwarranted. This review cited insufficient data as the reason for the decision, emphasizing the need for more information about river herring life histories. A greater understanding of where and when river herring use specific habitats could therefore influence effective management and potentially facilitate future restoration actions.

**Life History**

Variation in habitat use by young river herring suggests that a combination of habitats and life history strategies may be critical for juvenile survival. Current knowledge of juvenile river herring in freshwater, estuarine and marine waters tends to be fairly ambiguous, as prior studies reveal inconsistent timing of migration and habitat use (Milstein 1981, Limburg 1998, Turner & Limburg 2012, Gahagan et al. 2012). This is particularly true regarding blueback herring in the northern portion of its range, as the data collected is not extensive. Divergent migratory contingents have been observed in many fish species, which have substantial implications for management (e.g. defining stocks; Secor 1999). Differences in migratory patterns within a population may be the culmination of individual energy allocation decisions in early life stages (Secor 1999). Divergent migration strategies in blueback herring may be based on juvenile residency time in particular habitat types, and this diversity in migration strategy may stabilize populations (Schindler et al. 2010).

Although blueback herring and alewife are often regarded and managed as a single species, morphology, timing of migration events, and spawning habitat
preference differs between the two (Bigelow and Schroeder 1953, MacLellan et al. 1981, Greene et al. 2009). Throughout their sympatric range, spawning characteristics of the two species are separated spatially and temporally and are cued by changes in temperature (Loesch and Lund 1977, Schmidt et al. 1988, Greene et al. 2009). The spawning peaks of blueback herring usually follow those of alewife by 2-4 weeks (Fay et al. 1983, Greene et al. 2009). Blueback herring are broadcast spawners with a variety of spawning habitat preferences (Walsh et al. 2005, Greene et al. 2009). They typically spawn in lotic habitat (Loesch 1987, Greene et al. 2009), but vary preferred spawning substrate and distance up-river to spawn where they are sympatric with alewife (Bigelow and Schroeder 1953, Loesch and Lund 1977, Walsh et al. 2005).

By definition, blueback herring are considered "estuary dependent," i.e., they use estuaries during a portion of their life history (Able 2005). Young fish utilize this “safer” and more productive nursery habitat before migration seaward (Gross et al. 1988, Limburg 2001). However, the reported extent of freshwater and estuary use varies dramatically from system to system and from year to year. Typically, young-of-year (YOY) remain in natal habitat for all or part of the first growing season (Greene et al. 2009). Movement downriver may occur in as few as 1-3 months after hatch (Bigelow and Schroeder 1953, Yako et al. 2002). No evidence of coordinated seaward migration across systems exists; migration likely depends on several factors including temperature, flow rate, and size of the individual (Kosa and Mather 2001, Iafrate and Oliveira 2008, Limburg 2001). In addition, varied habitat use, such as persistence in nearshore marine habitat (< 8 km from shore; Milstein 1981), overwintering in estuaries (Milstein 1981, Limburg 1998) and migration back into freshwater from more saline habitats (Limburg 1998, Turner & Limburg 2012, Gahagan et al. 2012), demonstrates the existence of numerous migration patterns.

The elusive nature of early life stages makes pinpointing habitat use patterns and migration strategies a research priority for these species. Tagging, both natural and applied, is an established method used to examine fish movement (Gillanders et al.
2003, Walther and Limburg 2012). For river herring, natural tags (chemical, genetic) are advantageous over applied tags because they are permanent, present from early life stages throughout the lifetime of the fish, and unaffected by the fragility of individuals at younger life stages (Gahagan 2012).

**Otoliths**

The use of otolith microstructure and chemistry as a natural tag has become increasingly valuable to understanding life histories and movements of teleost fishes (Campana and Neilson 1985, Secor and Rooker 2000, Campana and Thorrold 2001, Begg et al. 2005, Elsdon et al. 2008). Otoliths are the small (< 1 cm in adult alosines) inner ear stones that reflect the physical and chemical environment over an individual’s lifetime (Appendix A). Otoliths are ideally suited for aging, growth analysis, and examination of micro-chemical composition, because the structure is metabolically inert relative to other structures such as bones or scales (Campana 1999). Otoliths grow in incremental bands similar to tree rings, and deposition of material marks various scales of growth (daily, seasonal, annual) over the lifetime of the fish. Additionally, the chemistry of otoliths essentially serves as an “elemental fingerprint” (Campana 2005); otoliths permanently retain certain elements and isotopes from the surrounding environment as well as from endogenous processes (Campana 1999). Elemental composition is primarily derived from the water in which the fish resided (Campana and Neilson 1985, Thorrold et al. 1998) although minimally influenced by physiological variables such as stress, temperature, and age (Secor 1992), and environmental variables such as salinity and exposure time (Elsdon and Gillanders 2005b). Coupled with growth increments, concentrations of elements in the otolith can be correlated to reconstruct habitat use patterns along a relative time scale and provide unprecedented tools for examining life histories (Campana and Thorrold 2001).

Previous studies of otolith microchemistry have examined concentrations of strontium (Sr), barium (Ba), calcium (Ca), manganese (Mn), Sodium (Na), magnesium
(Mg), potassium (K), lithium (Li), uranium (U), Iron (Fe), nickel (Ni), copper (Cu), and zinc (Zn) (Campana and Thorrold 2001). However, certain elements are more useful than others when differentiating specific habitats. In many cases, chemical composition within otoliths has been used to gain information about migration of fishes across freshwater, estuary and marine environments (Campana and Neilson 1985, Limburg 1998, Secor and Rooker 2000, Campana and Thorrold 2001, Begg et al. 2005, Elsdon et al. 2008). Because of the high correlation of Sr and Sr:Ca to water salinity (Bath et al. 2000, Secor and Rooker 2000). Sr:Ca ratios have been used successfully to discriminate among migratory phases of many anadromous fish species including alosines (Limburg 2001, Bradbury et al. 2008, Yang et al. 2011).

On the eastern coast of North America, large scale differences in magnitude of Sr:Ca among freshwater, estuarine and marine environments exist (Limburg 1995). Although Sr concentrations tend to be relatively constant in open marine waters, levels in freshwater systems may vary greatly due to underlying geologic conditions such as the presence of significant marine clay deposits (Odum 1957). Freshwater systems with increased levels of strontium due to these geologic and soil conditions prevent the use of Sr:Ca to distinguish between freshwater and marine habitat use. Barium (Ba) and manganese (Mn) in otoliths also have been used as environmental indicators (Ba; Bath et al. 2000, McCulloch et al. 2005, Elsdon and Gillanders 2005a, Tabouret et al. 2010 and Mn; Gillanders 2002, Limburg et al. 2011). Fresh water often contains substantially more Ba than seawater due to an abundance of Ba in soils and therefore groundwater inputs (Shaw et al. 1998). Ba therefore can be used to distinguish low or no salinity habitats when these geologic conditions exist. Mn is among the elements most sensitive to deoxygenation and therefore may indicate residency in habitats of hypoxic conditions (Limburg et al. In revision). With appropriate instrumentation, Ba and Mn can be examined simultaneously with Sr and Ca and supplement information gained from Sr:Ca ratios.
**Research Objectives**

The objective of this study was to determine if there was variation in freshwater and estuarine habitat use patterns of blueback herring during the first year of growth. Habitat use was retrospectively examined through elemental concentrations of Sr, Ba and Mn, in ratio to Ca, in blueback herring otoliths across each individual life span from hatch to capture as an adult. Specifically, I predicted:

- Variation in habitat use and migration exists among the different river systems due to differences in habitat availability. Juveniles with access to large areas of freshwater or estuarine habitat would remain there for a greater portion of the juvenile stage than juveniles without access, because of the adaptive advantages provided by nursery habitat (Gross et al. 1988, Beck et al. 2001, Limburg 2001). In other words, I predicted that area of fresh water, estuary size, and distance from freshwater capture point to sea would be positively related to duration of residency in these areas.

- Fish growth rate would relate to the patterns of habitat use and migration. Juveniles that remained in freshwater or estuarine habitats would experience greater growth because of advantages conferred by freshwater and estuarine residency (Gross et al. 1988, Beck et al. 2001, Limburg 2001), and growth would be positively correlated with extent of fresh water, estuary and distance from fresh water to sea.

- Patterns of habitat use and growth would be similar within runs, but differ among runs, reflecting differences in extent of freshwater habitat, estuary habitat, and distance to sea. Individuals of unknown origin should be able to be assigned to a run based on a series of otolith derived parameters.
METHODS

Site selection

Five coastal river systems in the state of Maine, USA, were selected for this study based on total available freshwater/estuarine habitat as well as presence of blueback herring. Presence of river herring harvest operations was also a consideration. Datasets from the National Wetlands Inventory (NWI), National Oceanic and Atmospheric Administration (NOAA), and the Maine Department of Marine Resources (MEDMR) informed site selection. Study rivers and sampling locations are described as follows in geographic order from west to east (Figure 1):

Kennebec River — The Kennebec is the second largest drainage basin in Maine and contains a wide variety of habitats utilized by many anadromous species (Figure 2, Table 1). Importantly, a large (approximately 1900 ha) freshwater tidal habitat known as Merrymeeting Bay exists where the Kennebec converges with the Androscoggin River, 27 km upriver from the ocean. Unlike the other study rivers, there are three distinct river herring harvest locations within the Kennebec River system:

Benton Falls, the furthest upriver sampling location, lies approximately 105 km from sea and is the site of a hydroelectric dam. Sea-run fish are assisted over the falls via fish lift. Individuals were collected within the fish lift apparatus. Several impoundments and Sabasticook Lake are located upriver of the collection point.

Dresden Mills is a harvest point on the Eastern River, a free flowing freshwater tidal tributary of the Kennebec. It is located approximately 7 km upriver from Merrymeeting Bay. A harvesting operation is located on a small creek less than 0.5 km from the main stem of the Eastern River. Individuals were collected at the harvest point.
Winnegance Pond is a 55-ha freshwater impoundment located in the lower reaches of the Kennebec approximately 14 km down river of Merrymeeting Bay. A fishway allows river herring to enter and leave the headwater lake, which is immediately adjacent to salt marsh. Water outside the impoundment measures approximately 8ppt salinity. Individuals were collected from within the lake impoundment.

St. George River — The St. George River lies within the central coastal watershed (Figure 2, Table 1). All artificial barriers have been removed from the main stem. Individuals were sampled from Seven Tree Pond which lies approximately 30 km from sea. The pond is roughly 170 ha and is the second of four lakes along the main stem of the river.

Orland River — The Orland River is a tributary to the lower Penobscot River, Maine's largest watershed (13792 km$^2$), which empties into Penobscot Bay (Figure 2, Table 1). Individuals were collected at the Orland Dam harvest location, situated within the tidal zone of the river. A fish ladder allows river herring passage over the dam where 508 ha of spawning habitat are located in Alamooskook Lake.

Patten Pond — Patten Pond is an approximately 280 ha headwater pond located in the eastern coastal watershed in close proximity to the Union River (Figure 2, Table 1). It empties via Patten Stream directly into Patten Bay and Union River Bay. Individuals were collected within the pond.

East Machias River — The East Machias is a relatively un-developed river in the eastern coastal watershed drainage (Figure 2, Table 1). The main stem empties into the Machias River/ Machias Bay. Samples were collected in Hadley Lake, an approximately 635-ha freshwater lake located on the main stem and the presumed lowest spawning habitat in the watershed.
Habitat Parameter Estimates

Area of freshwater habitat (ha) was determined based on data from the National Wetlands Inventory (U.S. Fish and Wildlife Service 2013; Table 1). The size of each estuary was approximated as the total area of the mixing zone (0.5 – 25 ppt salinity) as defined by NOAA (Table 1) and derived from NOAA's Coastal Assessment Framework. Distance to sea was considered the direct distance from the fish collection point to the edge of the near-ocean edge of the mixing zone (Table 1).

Elemental Composition of Ambient Water

Water samples were collected to quantify elemental ratios in ambient water in each system, as well as to establish that a gradient of elements exists from freshwater to marine habitats. Samples were collected at freshwater and estuarine locations associated with each river system in fall 2013. Because of the known overall consistency of elemental ratios in the marine system (Limburg 1995), one seawater location was sampled. Salinity values were measured in the field at each sample location using a calibrated refractometer; however, salinity values vary at any tidal location given the amount of freshwater discharge and tidal stage at the time of sample collection. Three replicate samples from each location were collected from shore in opaque, acid washed (2% HCl), polyethylene bottles. Sample bottles were rinsed with collected water prior to filling. All samples were filtered in the field using Whatman 0.45 µm glass fiber filters and fixed with trace metal grade nitric acid (2% of sample). Samples were stored at 4 °C until analysis several days later.

Samples were analyzed at the Sawyer Environmental Chemistry Research Laboratory at the University of Maine to quantify concentrations of Ba, Ca, Mg, Mn, Pb and Sr using the EPA Standard Operating Procedure for the Analysis of Metals in Waters Method 200.7 (EPA 1994; instrument details in Appendix B). For each water sample location, 2-3 replicates were examined and the mean of those replicates was calculated for further analysis. Thus, elemental concentration of the water chemistry for study
sites was reported as mean concentration of each element per location. Concentrate-salinity plots (*sensu* Walther and Limburg 2012) fit with logarithmic models were used to identify the relationship between elemental ratios and salinity.

**Otolith Collection and Preparation**

Otoliths from returning adults in the St. George, Orland, Union and East Machias Rivers were collected in 2009 and 2010 (Cronin-Fine et al. 2013, Labbe 2013). Additional adult specimens were collected during the spring spawning migration period in 2012 at river herring harvest locations in Dresden Mills, Winnegance Lake and Benton Falls. Fish were caught by seine or cast net or acquired from harvesters. Total length, wet weight, and sex were collected from each individual when possible (Table 1). Saggital otoliths were extracted (Appendix A) and cleaned via ultrasonication in a 50% bleach-water solution to remove organic material from the otolith material. Cleaned otoliths were dried in a laminar flow hood in centrifuge tubes and embedded in Epofix™ epoxy resin. For ease of polishing, samples were sectioned in the transverse plane (Figure 3) using a Buehler diamond-blade low speed Isomet saw that was lubricated using de-ionized water. Sectioned samples were fixed to glass slides using Crystalbond™ mounting adhesive and a series of 30 – 0.5µm aluminum oxide polishing papers was used to expose a smooth, flat surface at the core. Prior to microchemical analysis 95% ethanol was used to clean the otolith surface.

**Otolith Microchemistry**

Otolith microchemistry was examined using Laser Ablation Inductively Coupled Plasma-Mass Spectrometry (LA-ICP-MS; instrument details in Appendix B) at the State University of New York College of Environmental Science and Forestry (SUNY-ESF). This method was selected over other microchemical analytical methods because of its relatively non-destructive nature for the otolith, allowing for aging and other analyses after LA. On the surface of the otolith the laser ablated the sample from end to end, using a 35µm spot size at a speed of 3 µm per second along a transect drawn to follow
the apex of growth rings and to pass through the core (Figure 4). Because of the nature of otolith composition, one side of the core was presumed nearly identical in composition to the other; thus sampling from edge to edge allowed for replicate analysis of otolith material. The ablated material was entrained in argon and helium gas for analysis using mass spectrometry. Reference standards included a National Institute of Standards and Technology (NIST) 610 glass and a known concentration pellet made from pulverized fish otoliths (Limburg et al. 2011). These standards also were sampled along a straight transect approximately every 6 otolith samples.

Otolith elemental concentrations were corrected based on results from the otolith pellet standard. Extreme anomalies were attributed to cracks in the sample and removed from analysis. Photographs of each otolith before and after laser ablation were taken using a Nikon digital camera with NIS Elements D 3.2 software at optimal resolution and visual clarity. Fiji Image-J image analysis software was used to measure growth parameters along transect lines pre and post laser ablation. Values of elemental concentrations in otolith signatures were examined as a ratio to the paired calcium concentration. Location of the core and other growth features on each otolith transect were visually identified based on the photographs and corresponding symmetry of the elemental concentration data.

Habitat Use Patterns

Otolith microchemistry revealed habitat use patterns and transitions that were visually identified by rapid changes in ratio levels along the otolith transect (Secor 1992, Secor and Rooker 2000, Limburg 2001, Gahagan 2012). Using a regime shift detection algorithm (Rodionov 2004, Turner and Limburg 2012), significant changes in Sr:Ca, Ba:Ca and Mn:Ca were quantified using a sequential F-test based on variance of the dataset and set parameters (P ≤ 0.05, cut-off length =10) to evaluate number and magnitude of habitat transition factors. The algorithm excluded outliers using Huber's Weight
Parameter (Rodionov 2004). Regime shift factors were detected in ratios from the core to the edge of one randomly selected side (dorsal or ventral) of each otolith.

Duration of use of freshwater and estuary habitat was measured from the core to a rapid increase in Sr:Ca and quantified by a zero to low salinity habitat use index (LSHU) factor. LSHU was measured based on visual identification of a Sr:Ca shift. Sr:Ca signatures of approximately ≤ 2 were considered indicative of zero to low salinity habitat use, although these values were strictly freshwater signatures for most individuals (Appendix C, Figure III). The LSHU factor was calculated for each individual based on raw Sr:Ca values from both sides of the core by dividing distance from the core spent in low salinity conditions (i.e., Sr:Ca approximately ≤ 2; Appendix C) by total distance from the core to the first annulus (Figure 5). The LSHU factor was reported as the mean value from dorsal and ventral sides. The greater the LSHU factor, the longer the residency in zero to low salinity (i.e., sustained low Sr:Ca); LSHU < 1 indicated that an individual moved into seawater before the end of the first year of growth, LSHU = 1 indicated migration at the first annulus, and values > 1 indicated an individual overwintered in zero to low salinity.

**Otolith and Somatic Growth**

Because analysis of daily growth to the first year was unreliable given the orientation and thickness of the samples, alternative methods were implemented to examine growth characteristics. All fish were aged based on visible annual growth rings (annuli) along the otolith transect. Because otolith growth is correlated with somatic growth (Campana and Neilson 1984, Campana 1999, Limburg 2001), the length of fish at Age-1 and at egress from low salinity were estimated using back calculation methods. Otolith width (OW; measured from edge to edge following the axis of maximum growth) and total length (TL) data from young of year (Y0Y; N = 9) and yearling (N = 9) blueback herring from the Penobscot Estuary, ME and the Hudson River, NY, as well as larval American shad (N = 10; Hudson River, NY) were fit with a second order polynomial
function (Appendix D). In the absence of larval blueback herring samples, shad were used under the assumption that blueback herring and shad larvae have similar sized otoliths at the same total length and that differences in otolith morphology are negligible in circular larval otoliths. The OW (µm) to TL (mm) relationship was defined by the following equation ($R^2 = 0.97$, Figure 6):

$$\text{TL} = 0.00004 (\text{OW}^2) + 0.0436 (\text{OW}) + 12.972$$

Measurements of otolith width at the first annulus and at egress of sample individuals were then used to estimate fish length at these events. Length at egress was used as a means to estimate growth before significant migration, as well as an indicator of duration of low salinity habitat use. Length at year one served as a proxy for growth during the first year.

**Statistical Analysis**

Statistical analyses were performed using SYSTAT version 12 (Systat 2007) and Microsoft Excel. Data sets were tested for normality using a Shapiro-Wilk test; freshwater and estuary area, and distance to sea data were log transformed to meet assumptions of normality. All means are reported as $\pm 1 \text{ SD}$.

To determine if access to large areas of freshwater or estuary influences habitat use patterns during the first year, LSHU values and estimated lengths at egress were examined with each habitat parameter (freshwater area, estuary area, distance to sea) using Pearson's correlation analysis. Significance was determined using Bonferroni probabilities ($P \leq 0.05$).

Growth advantages inferred by habitat types were investigated by incorporating results from the aforementioned analysis using length at egress. Among runs, differences in estimated length parameters were assessed using one-way analysis of variance (ANOVA; $P \leq 0.05$), followed by pair wise comparisons using Tukey's Test.
Pearson's correlation with Bonferroni probability was used to examine the relationship between estimated length at year 1 and each habitat parameter.

To determine if habitat use patterns are unique to each river system I considered similarities and differences among runs in terms of habitat use patterns and growth. Coherence in migration patterns in otolith Sr, Ba and Mn ratios within spawning runs was assessed by Pearson's correlation analysis followed by examination of the coefficient of variation (CV) of Pearson's coefficient for each run; CV was used as a uniform way to compare the degree of variation of elemental signatures among runs. Significant differences in factors among runs were assessed using ANOVA (P ≤ 0.05) followed by Tukey’s Test.

Lastly, discriminant function analysis (DFA; tolerance = 0.001) was used to determine if differences among otolith derived parameters (estimated length at year 1 and egress, number of regime shifts in Sr:Ca, Ba:Ca and Mn:Ca signatures) were sufficient to allow assignment of individuals of unknown origin to distinct runs. Results were reported as simple and jackknifed classifications for each run as well as the total of all runs.
RESULTS

Water Chemistry

Elemental concentrations in water indicate a gradient of Sr:Ca, Ba:Ca and Mn:Ca ratios exist from freshwater to marine end members in the study systems (Figure 7). Sr:Ca ratios were highly correlated with salinity (Pearson's correlation coefficient = 0.828, \( P \leq 0.001 \); Figure 7 A) and therefore supported the use of these ratios as a primary indicator of habitat salinity in otoliths. The mean values for Ba:Ca \( \times 100 \) (0.05 ± 0.02) and Mn:Ca \( \times 100 \) (0.33 ± 0.30) in freshwater habitats and near-zero levels in estuary and marine habitats (Ba:Ca \( \times 100 = 0.001 \pm 0 \); Mn:Ca \( \times 100 = 0.003 \pm 0.003 \)) support their use as secondary indicators of habitat (Figure 7 B and C).

Low Salinity Habitat Use

Sustained rapid increases in Sr:Ca were interpreted as migration to seawater and Sr:Ca ratios were the inverse of Ba:Ca ratios (Figure 8), confirming that shifts in elemental signatures in fact indicate habitat shifts. LSHU across individuals from all runs ranged from 0.13 to 0.63 (Table 5). Mean LSHU was not significantly correlated with estuary size (Pearson's correlation coefficient = –0.16, \( P = 0.07 \); Figure 9). Because the mean LSHU of individuals collected in Winnegance Lake was significantly lower than the LSHU from all other sampling locations (\( F =14.20, \ P <0.001 \)) and the majority of Winnegance fish showed a low LSHU even though the run abuts a large, albeit upstream, estuary, Winnegance individuals were removed from the analysis. In this case there was a positive yet still weak correlation (Pearson's correlation coefficient = 0.072, \( P < 0.001 \)) between LSHU and estuary size. LSHU was significantly positively correlated with freshwater area (Pearson's correlation coefficient = 0.368, \( P < 0.001 \)) and distance to sea (Pearson's correlation coefficient = 0.244, \( P < 0.001 \); Figure 9).
Otolith and Somatic Growth

Similarities in mean estimated age among runs indicate that the majority of samples were collected from the same cohort (Table 1). Total length at capture ranged from 209 to 289 mm, with the longest fish collected at Patten Pond ($F = 15.96$, $P < 0.001$; Figure 10). Estimated length at year 1 ranged from 33.2 to 133.9 mm, and length at egress varied from 13.0 to 96.3 mm. Hadley Lake individuals were significantly smaller in length at age year 1 ($F = 8.97$, $P < 0.001$) than individuals from all other runs with the exception of Orland Dam and Seven Tree Pond. Winnegance fish were significantly smaller than individuals from all other runs at egress ($F = 9.26$, $P < 0.006$ Figure 10).

Estimated length at year 1 was not significantly correlated with any of the habitat parameters. However, estimated length at egress was positively correlated with freshwater area (Pearson's coefficient $= 0.328$, $P < 0.001$) and distance to sea (Pearson's coefficient $= 0.287$, $P = 0.001$), meaning that fish that were larger at egress were sampled from systems with greater freshwater habitat and distance to sea. Length at egress was positively correlated with LSHU (Pearson's coefficient $= 0.935$, $P < 0.001$), indicating that residency in low salinity habitat infers a growth advantage at egress. Length at egress was also positively correlated with maximum Mn:Ca regime values (Pearson's coefficient $= 0.437$, $P \leq 0.001$; Figure 11) which suggests habitats with higher Mn:Ca signatures yield larger fish at egress.

Distinguishing Runs

On average all runs were strikingly similar in habitat use patterns, with the exception of Winnegance and Patten. Similar LSHU and estimated length at egress were supplemented by visually similar timing and magnitude of migration peaks in Sr:Ca, followed by a drop in Sr:Ca values as the fish aged (Figure 12). Migration patterns within runs exhibited low overall variation (i.e., a high coherence, Table 2), although three individuals from Winnegance Lake showed evidence of alternative migration strategies as juveniles (Figure 13). This variation in the Sr:Ca of individuals from Winnegance Lake
accounts for the higher coefficient of variation in Winnegance compared to all other runs (Table 2). All but one individual (from Benton Falls; migrated at the first annulus, LSHU value = 1.00) migrated to sea before the end of the first year of growth. On average, fish from Winnegance Lake spent the least amount of time in low salinity habitat as evidenced by the rapid increase in Sr:Ca close to the core (mean LSHU= 0.132 ± 0.256 ). In Patten Pond, the overall habitat use pattern is similar to that of other runs (Figure 12) until migration, when sustained elevated Sr:Ca signatures similar to brief peaks in fish from other runs suggest extended time spent in full salinity marine conditions.

Mean LSHU values of individuals collected in Winnegance Lake was significantly lower than the means from all other sampling locations (F = 14.20, P < 0.0001). None of the other runs had significantly different LSHU values. Among runs, fish showed significant differences in growth inferred from total length and estimated length at year one and at egress (P ≤ 0.05; Figure 10).

Fish in most spawning runs showed a similar number of habitat shifts (regime shifts) in the time between hatching and their first annulus, with the exception of those from Dresden Mills. The number of regime shifts in Sr:Ca signatures was significantly higher in fish sampled at Dresden Mills than all other runs with the exception of Benton Falls and Winnegance (F = 6.88, P <0.001, Table 3). The number of Ba:Ca regime shifts in Dresden fish was significantly higher from fish sampled at Hadley (P = 0.009), Orland (P = 0.015) and Seven Tree Pond (P = 0.008, Table 3). Fish sampled at Benton Falls had a greater number of Mn:Ca regime shifts compared to fish from Hadley Lake (P = 0.009), Orland Dam (P=0.015) and Seven Tree Pond (P = 0.008, Table 3).

Discriminant function analysis using estimated length at year 1 and egress, the number of regime shifts in Sr:Ca, Ba:Ca and Mn:Ca ratios as predictors of sample run (mean values per run in Table 4) revealed individuals of unknown origin could be assigned successfully to a particular run 47% of the time (or 40% by Jackknifed
classification). Winnegance had the greatest percent classification score at 80% of the time (Jackknifed classification 70%, Table 5). These results support Winnegance being distinctly different from other runs.
DISCUSSION

Investigating the migration histories of individuals can provide insight into the life histories of anadromous populations as a whole. Previous studies have uncovered discrete patterns in habitat use among individual groups within populations, described as migration strategies, alternative life histories or migratory "contingents" (Limburg 1998, Secor 1999, Elsdon and Gillanders 2005). These alternative life histories exist within the constraints of anadromy; however, timing of migration and habitat use may vary greatly among individuals or groups. Resilience in anadromous fish populations may be supported by this variation in life histories (Schindler 2010). One way to examine variation in life histories is through otolith microchemistry, which has been implemented to examine differences in habitat use by blueback herring in many systems throughout their range (Milstein 1981, Limburg 1998, Turner & Limburg 2012, Gahagan et al. 2012). However this is the first study to examine juvenile life histories through otolith microchemistry in Maine rivers.

Habitat Use and Migration Patterns

By examining otoliths of adult blueback herring, rather than juveniles post egress from freshwater habitats, I was able to determine migration strategies of individuals that survived successfully into reproductive ages. The Sr:Ca and Ba:Ca ratios observed in this study reflected habitat salinity transitions similar to those reported by others (Limburg 1995, 1998; Elsdon and Gillanders 2005a; Turner and Limburg 2012) indicating that these otolith microchemical results do in fact illustrate shifts from freshwater to marine habitats.

There was no evidence to support overwintering in freshwater or low salinity habitat which has been observed in other East Coast systems (the Hudson River, Limburg 1998; the Peconic River, Turner & Limburg 2012). These other systems differ in several respects to the rivers in this study; for example, the Hudson River drainage area is approximately 12000 km² greater than the Kennebec, affording much greater expanses of freshwater habitat and longer travel distances to the ocean.
As predicted, overall, freshwater/low salinity residency (i.e., LSHU) was positively correlated with the area of freshwater habitat available, as well with distance to sea. In contrast to larger systems along the east coast, Maine’s smallest coastal streams have almost no freshwater or estuarine habitat. In this study, fish spent the least amount of time in freshwater or low salinity habitats in these small systems, indicating that habitat factors play an important role in dictating variation in migration patterns. Along with the reduced amount of freshwater habitat available relative to all other runs (213 ha and 326 ha, respectively), Winnegance Lake and Patten Pond have limited down river estuary habitat (500 ha and 204 ha, respectively), and short distances to the ocean (18 km and 22 km to marine waters respectively). The near lack of residency time in freshwater/low salinity habitats (i.e., low LSHU, 0.13 ± 0.26) in Winnegance Lake was consistent with the fact that the lake is a dammed arm of a salt marsh with a sharp contrast in habitat salinity that must be experienced by fish leaving the lake and immediately entering the estuary. Neither Winnegance nor Patten fish appear to seek out estuaries or other areas of low salinity after moving into marine waters.

Variation of emigration timing within runs at an individual level may indicate alternative migration strategies. For the most part, individual timing of emigration within runs was analogous. The similar range of CV values for Sr:Ca ratios (0.39 to 0.63) within all runs with the exception of Winnegance, indicates that the majority of individuals within each run exhibited similar migratory patterns. The higher degree of variation in Winnegance is directly attributed to three individuals that exhibited migration histories in clear contrast to the general pattern of the run (Figure 13). This difference in migration patterns could indicate two possibilities: 1) Winnegance fish exhibit discrete alternative life histories of extended versus minimal freshwater/low salinity habitat use, or 2) these three individuals strayed into Winnegance and originated from another run. Because each individual differed in age, an annual effect on one cohort can be ruled out as the cause of the disparity in migration patterns. After
close examination of elemental ratios in these individuals, their habitat use patterns appear most similar to those of Benton Falls individuals and support a case for straying as cause for variation in migration patterns observed within runs. It is likely that Winnegance is prone to straying more than other runs simply because it is the lowest available spawning habitat in the Kennebec River with several runs upriver. Other elemental analysis, notably \(^{87/86}\text{Sr}\) and \(^{\delta^{18}}\text{O}\) also could be implemented as a means to examine natal origin (Kennedy et al. 2002, Campana 2005) and may help distinguish between straying and alternative life histories.

Differences in habitat parameters were exemplified in contrasting habitat use patterns. Five out of seven runs had similar patterns of habitat use, but the two systems with limited freshwater and estuary habitat area (Winnegance and Patten Pond) demonstrated unique habitat use patterns. These results support the existence of three migration strategies with varying degrees of freshwater/low salinity habitat use. Preserving a diversity of habitats will work to preserve variation in migration strategies. Maintaining this variation in life histories will support diversity and long-term sustainability of blueback herring populations (i.e. the "Portfolio Effect"; Schindler et al. 2010).

**Growth**

The advantages of freshwater and estuary environments for many aquatic species have been well studied, and they include functions as nursery and critical habitat for juvenile stages (Beck et al. 2001, Able 2005). Likewise, my work illustrates a positive relationship between these habitat types and blueback herring growth and survival. The positive correlation between length at egress and time spent in low salinity (\(r=0.94\)) suggests a growth advantage associated with low salinity habitats, however these measures are likely to be correlated as length at egress is derived from the LSHU parameter. The correlation between high Mn:Ca regimes and larger sizes at egress may also reveal a more specific advantage for individuals using wetland habitat (i.e., in or
near hypoxic conditions). Previous studies suggest Mn (Mn:Ca) signatures in otoliths are correlated with hypoxic conditions (Mohan et al. 2012, Limburg et al. accepted pending minor revision). In coastal pond estuaries of Maine, wetland habitats provide the reducing conditions that support Mn in the reduced, dissolved form Mn$^{2+}$, which is more readily available for uptake (Mitsch and Gosselink 2007). Because elevated concentrations of Mn:Ca in otoliths in this study occurred primarily in peaks on either side of the core and not directly at the core region, the results likely indicate Mn signatures as a consequence of habitat. If true, this result supports the preservation and restoration of wetland habitat for the success of blueback herring. Restoration of wetland habitat has shown increased use of the habitat type by other species (Bottom et al. 2005). This study further emphasizes the importance of protecting and restoring wetland habitats, and maintaining wetlands as a conservation priority.

**Distinguishing Runs**

The discriminant function analysis was used to see if runs or individuals fell within unique groups, which would allow future researchers to use otolith chemistry as a proxy for habitat use and migration patterns. Several runs did stand out from one another in terms of growth and otolith derived variables. The significant differences between Winnegance Lake fish and fish from other systems that occurred across the majority of otolith parameters clearly indicate an alternative life history of elevated salinity habitat use in this system. The high percentage of correct classification via discriminant function analysis of unknown individuals to Winnegance is promising. The addition of more variables to the model could increase the ability to classify individuals to runs based on otolith chemical parameters.

The majority of runs resulted in low correct classification of unknowns, but their similarities could indicate several possible conditions. Because all of the fish examined were ultimately successful recruits, the similar habitat use patterns observed among runs suggests a dominant pattern in successful life history strategies. Alternatively, since
blueback herring are known to spawn in lotic habitat (Loesch and Lund 1977, Greene et al. 2009) and larval fish are reared in these conditions, patterns in elemental signatures across all runs would be similar, unless elemental signatures were environmentally constrained and thus unique (e.g., headwater habitats of Winnegance Lake and Patten Pond; Figure 2). Since little is known about homing or straying rates for river herring, blueback herring may stray considerably (Messieh 1977, Gahagan 2012). Finally, although restorative stocking in Maine is focused on alewives, presumably blueback herring are some portion of this effort, inferring a homogenizing effect on runs (Cronin-Fine et al. 2012, Labbe 2012). These possibilities alone or in combination could influence the ability to detect differences in runs based on otolith parameters.

Unique otolith signatures in freshwater nursery habitat could be used to identify natal origin of blueback herring, an approach which has been attempted in studies of other systems (e.g., in Connecticut; Gahagan et al. 2012). The high degree of variation observed in otolith Ba:Ca and Mn:Ca signatures of freshwater habitat, as well as variation in water chemistry of freshwater end members, has the potential for differentiation of freshwater habitats at a finer scale. Additionally, the transition from low to high salinity (i.e., the slope) varied among individuals, with some fish exhibiting a gradual/step-wise elevation of Sr:Ca and others an immediate steep elevation to the maximum. However, the transition was not quantified in this study. In previous studies this slope in elemental concentrations over habitat transitions, has been hypothesized to show the rate of transition (Gahagan 2012, Turner and Limburg 2012) and has the potential to reveal more information about characteristics of migration within and among runs. The freshwater variation and transition characteristics are promising for future work delineating stocks of blueback herring.
CONCLUSIONS

A greater understanding of habitat use patterns in blueback herring has implications for management and conservation of freshwater and estuary habitat for the species. As populations across the eastern seaboard struggle for sustainability, gaining information about life histories and migration strategies is critical. Knowledge about young life stages is crucial for designating nursery and critical habitats. In this study, individuals were observed using freshwater and estuarine habitat for up to one year after hatching in Maine river systems. Although freshwater or low salinity residency was not as prolonged as individuals found in some systems (Limburg 1998), it was similar to those found in similar size river systems (Gahagan 2012). The majority of individuals appear to rely on freshwater and estuarine habitat regardless of the size of the area available to them, suggesting that they would use more habitat if it were available. Therefore freshwater and estuarine habitats may be critical to blueback herring survival during a vulnerable period in the life cycle (Able 2005). Depletion or destruction of habitat between spawning areas and the sea could have staggering consequences for restoration of populations to their historical levels. This research supports management actions that ensure the existence of a diversity of habitats, thus allowing for blueback herring populations to sustain variation in life history which may be pivotal to the resilience of the species.
REFERENCES


**TABLES**

**Table 1.** Sample site locations, estuary size estimates and blueback herring data within the five study rivers. Estuary sizes were calculated based on area of “mixing” zone (salinity= 0.5-25 ppt) as defined by NOAA Salinity Zone Data. Distance to sea from each collection location was measured as a direct route to the seaward edge of the mixing zone. One standard deviation for mean total length (TL, in mm) and mean wet weight (wet wt., in g), and mean age (in years) is shown in parenthesis.

<table>
<thead>
<tr>
<th>River</th>
<th>Kennebec</th>
<th>St. George</th>
<th>Orland</th>
<th>Patten</th>
<th>East Machias</th>
</tr>
</thead>
<tbody>
<tr>
<td>Watershed Area (km²)</td>
<td>15200</td>
<td>580</td>
<td>444</td>
<td>1458</td>
<td>1291</td>
</tr>
<tr>
<td>Sample Sites</td>
<td>Benton Falls</td>
<td>Dresden Mills</td>
<td>Winnegance</td>
<td>Seven Tree Pond</td>
<td>Orland Dam</td>
</tr>
<tr>
<td>Coordinates (N/W)</td>
<td>44°34’48”</td>
<td>44°5’1”</td>
<td>43°52’30”</td>
<td>44°12’7”</td>
<td>44°34’16”</td>
</tr>
<tr>
<td>Freshwater Area (ha)</td>
<td>6053</td>
<td>3511</td>
<td>213</td>
<td>513</td>
<td>508</td>
</tr>
<tr>
<td>Estuary Size (ha)</td>
<td>2578</td>
<td>2578</td>
<td>2578</td>
<td>653</td>
<td>3395</td>
</tr>
<tr>
<td>Distance to Sea (km)</td>
<td>106</td>
<td>52</td>
<td>18</td>
<td>30</td>
<td>14</td>
</tr>
<tr>
<td>N (Fish)</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>18</td>
</tr>
<tr>
<td>Mean TL (mm)</td>
<td>242 (20.7)</td>
<td>257 (11.7)</td>
<td>245 (13.5)</td>
<td>242 (11.3)</td>
<td>232 (7.4)</td>
</tr>
<tr>
<td>Mean Wet Wt. (g)</td>
<td>117 (34.9)</td>
<td>134 (24.8)</td>
<td>107 (20.1)</td>
<td>119 (22.2)</td>
<td>110 (14.4)</td>
</tr>
<tr>
<td>Mean Age (years)</td>
<td>3.4 (1.2)</td>
<td>3.6 (0.8)</td>
<td>3.3 (1.1)</td>
<td>3.6 (0.8)</td>
<td>3.5 (0.6)</td>
</tr>
</tbody>
</table>
Table 2. Mean correlation (Pearson’s r) of individual elemental ratios and corresponding standard deviation (SD) and coefficient of variation (CV) within runs. Only CV values from Winnegance Sr:Ca and Hadley Lake Ba:Ca (in bold) indicate a low degree of coherence of signatures within those runs.

<table>
<thead>
<tr>
<th>Location</th>
<th>Sr:Ca</th>
<th></th>
<th></th>
<th>Ba:Ca</th>
<th></th>
<th></th>
<th>Mn:Ca</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean r</td>
<td>SD</td>
<td>CV</td>
<td>Mean r</td>
<td>SD</td>
<td>CV</td>
<td>Mean r</td>
<td>SD</td>
<td>CV</td>
</tr>
<tr>
<td>Benton</td>
<td>0.44</td>
<td>0.25</td>
<td>0.56</td>
<td>0.55</td>
<td>0.21</td>
<td>0.38</td>
<td>0.54</td>
<td>0.25</td>
<td>0.47</td>
</tr>
<tr>
<td>Dresden</td>
<td>0.70</td>
<td>0.32</td>
<td>0.46</td>
<td>0.60</td>
<td>0.19</td>
<td>0.31</td>
<td>0.52</td>
<td>0.20</td>
<td>0.38</td>
</tr>
<tr>
<td>Winnegance</td>
<td>0.21</td>
<td>0.30</td>
<td>1.41</td>
<td>0.56</td>
<td>0.17</td>
<td>0.30</td>
<td>0.37</td>
<td>0.20</td>
<td>0.54</td>
</tr>
<tr>
<td>Seven Tree</td>
<td>0.43</td>
<td>0.22</td>
<td>0.50</td>
<td>0.48</td>
<td>0.24</td>
<td>0.50</td>
<td>0.37</td>
<td>0.32</td>
<td>0.86</td>
</tr>
<tr>
<td>Orland</td>
<td>0.58</td>
<td>0.23</td>
<td>0.39</td>
<td>0.73</td>
<td>0.10</td>
<td>0.14</td>
<td>0.51</td>
<td>0.23</td>
<td>0.46</td>
</tr>
<tr>
<td>Patten Pond</td>
<td>0.49</td>
<td>0.22</td>
<td>0.44</td>
<td>0.71</td>
<td>0.12</td>
<td>0.17</td>
<td>0.69</td>
<td>0.14</td>
<td>0.21</td>
</tr>
<tr>
<td>Hadley Lake</td>
<td>0.44</td>
<td>0.28</td>
<td>0.63</td>
<td>0.55</td>
<td>0.22</td>
<td>0.40</td>
<td>0.34</td>
<td>0.37</td>
<td>1.11</td>
</tr>
</tbody>
</table>
Table 3. Results of pairwise comparisons using Tukey's test for differences in the number of regime shifts of Sr:Ca, Ba:Ca and Mn:Ca signatures per run. Significant differences ($P \leq 0.05$) are bolded.

<table>
<thead>
<tr>
<th>Location A</th>
<th>Location B</th>
<th>Sr:Ca Difference</th>
<th>Sr:Ca p-value</th>
<th>Ba:Ca Difference</th>
<th>Ba:Ca p-value</th>
<th>Mn:Ca Difference</th>
<th>Mn:Ca p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benton Falls</td>
<td>Dresden Mills</td>
<td>-0.85</td>
<td>0.025</td>
<td>-0.2</td>
<td>0.996</td>
<td>0.15</td>
<td>0.998</td>
</tr>
<tr>
<td>Benton Falls</td>
<td>Hadley Lake</td>
<td>0.754</td>
<td>0.16</td>
<td>1.031</td>
<td>0.068</td>
<td>1.119</td>
<td>0.009</td>
</tr>
<tr>
<td>Benton Falls</td>
<td>Orland Dam</td>
<td>0.4</td>
<td>0.749</td>
<td>0.4</td>
<td>0.878</td>
<td>0.95</td>
<td>0.015</td>
</tr>
<tr>
<td>Benton Falls</td>
<td>Patten Pond</td>
<td>0.267</td>
<td>0.961</td>
<td>0.189</td>
<td>0.998</td>
<td>0.461</td>
<td>0.7</td>
</tr>
<tr>
<td>Benton Falls</td>
<td>Seven Tree</td>
<td>0.4</td>
<td>0.749</td>
<td>0.9</td>
<td>0.077</td>
<td>1</td>
<td>0.008</td>
</tr>
<tr>
<td>Benton Falls</td>
<td>Winnegance</td>
<td>-0.2</td>
<td>0.99</td>
<td>0.45</td>
<td>0.805</td>
<td>0.6</td>
<td>0.351</td>
</tr>
<tr>
<td>Dresden Mills</td>
<td>Hadley Lake</td>
<td>1.604</td>
<td>0</td>
<td>1.231</td>
<td>0.012</td>
<td>0.969</td>
<td>0.041</td>
</tr>
<tr>
<td>Dresden Mills</td>
<td>Orland Dam</td>
<td>1.25</td>
<td>0</td>
<td>0.6</td>
<td>0.507</td>
<td>0.8</td>
<td>0.075</td>
</tr>
<tr>
<td>Dresden Mills</td>
<td>Patten Pond</td>
<td>1.117</td>
<td>0.001</td>
<td>0.389</td>
<td>0.904</td>
<td>0.311</td>
<td>0.939</td>
</tr>
<tr>
<td>Dresden Mills</td>
<td>Seven Tree</td>
<td>1.25</td>
<td>0</td>
<td>1.1</td>
<td>0.012</td>
<td>0.85</td>
<td>0.046</td>
</tr>
<tr>
<td>Dresden Mills</td>
<td>Winnegance</td>
<td>0.65</td>
<td>0.188</td>
<td>0.65</td>
<td>0.404</td>
<td>0.45</td>
<td>0.697</td>
</tr>
<tr>
<td>Hadley Lake</td>
<td>Orland Dam</td>
<td>-0.354</td>
<td>0.905</td>
<td>-0.631</td>
<td>0.591</td>
<td>-0.169</td>
<td>0.998</td>
</tr>
<tr>
<td>Hadley Lake</td>
<td>Patten Pond</td>
<td>-0.487</td>
<td>0.696</td>
<td>-0.842</td>
<td>0.259</td>
<td>-0.658</td>
<td>0.412</td>
</tr>
<tr>
<td>Hadley Lake</td>
<td>Seven Tree</td>
<td>-0.354</td>
<td>0.905</td>
<td>-0.131</td>
<td>1</td>
<td>-0.119</td>
<td>1</td>
</tr>
<tr>
<td>Hadley Lake</td>
<td>Winnegance</td>
<td>-0.954</td>
<td>0.026</td>
<td>-0.581</td>
<td>0.683</td>
<td>-0.519</td>
<td>0.672</td>
</tr>
<tr>
<td>Orland Dam</td>
<td>Patten Pond</td>
<td>-0.133</td>
<td>0.999</td>
<td>-0.211</td>
<td>0.996</td>
<td>-0.489</td>
<td>0.638</td>
</tr>
<tr>
<td>Orland Dam</td>
<td>Seven Tree</td>
<td>0</td>
<td>1</td>
<td>0.5</td>
<td>0.714</td>
<td>0.05</td>
<td>1</td>
</tr>
<tr>
<td>Orland Dam</td>
<td>Winnegance</td>
<td>-0.6</td>
<td>0.275</td>
<td>0.05</td>
<td>1</td>
<td>-0.35</td>
<td>0.884</td>
</tr>
<tr>
<td>Patten Pond</td>
<td>Seven Tree</td>
<td>0.133</td>
<td>0.999</td>
<td>0.711</td>
<td>0.325</td>
<td>0.539</td>
<td>0.522</td>
</tr>
<tr>
<td>Patten Pond</td>
<td>Winnegance</td>
<td>-0.467</td>
<td>0.619</td>
<td>0.281</td>
<td>0.986</td>
<td>0.139</td>
<td>0.999</td>
</tr>
<tr>
<td>Seven Tree</td>
<td>Winnegance</td>
<td>-0.6</td>
<td>0.275</td>
<td>-0.45</td>
<td>0.805</td>
<td>-0.4</td>
<td>0.801</td>
</tr>
<tr>
<td>Est. Length at Year 1 (mm)</td>
<td>Benton Falls</td>
<td>Dresden Mills</td>
<td>Winnegance</td>
<td>Seven Tree Pond</td>
<td>Orland Dam</td>
<td>Patten Pond</td>
<td>Hadley Lake</td>
</tr>
<tr>
<td>---------------------------</td>
<td>--------------</td>
<td>---------------</td>
<td>------------</td>
<td>----------------</td>
<td>------------</td>
<td>------------</td>
<td>------------</td>
</tr>
<tr>
<td></td>
<td>97.89</td>
<td>101.81</td>
<td>105.67</td>
<td>92.51</td>
<td>93.03</td>
<td>111.95</td>
<td>79.15</td>
</tr>
<tr>
<td></td>
<td>(12.41)</td>
<td>(14.98)</td>
<td>(10.20)</td>
<td>(23.87)</td>
<td>(7.12)</td>
<td>(5.95)</td>
<td>(15.95)</td>
</tr>
<tr>
<td>Est. Length at Egress (mm)</td>
<td>56.30</td>
<td>42.58</td>
<td>21.74</td>
<td>39.94</td>
<td>45.57</td>
<td>51.62</td>
<td>46.25</td>
</tr>
<tr>
<td></td>
<td>(20.31)</td>
<td>(10.33)</td>
<td>(18.99)</td>
<td>(17.73)</td>
<td>(10.12)</td>
<td>(17.74)</td>
<td>(13.16)</td>
</tr>
<tr>
<td>No. Ba:Ca Regime Shifts</td>
<td>3.80</td>
<td>4.00</td>
<td>3.35</td>
<td>2.90</td>
<td>3.40</td>
<td>3.61</td>
<td>2.77</td>
</tr>
<tr>
<td></td>
<td>(1.11)</td>
<td>(1.17)</td>
<td>(0.99)</td>
<td>(0.91)</td>
<td>(0.68)</td>
<td>(1.09)</td>
<td>(1.17)</td>
</tr>
<tr>
<td>No. Sr:Ca Regime Shifts</td>
<td>3.60</td>
<td>4.45</td>
<td>3.80</td>
<td>3.20</td>
<td>3.20</td>
<td>3.33</td>
<td>2.85</td>
</tr>
<tr>
<td></td>
<td>(0.94)</td>
<td>(0.83)</td>
<td>(0.83)</td>
<td>(0.77)</td>
<td>(0.52)</td>
<td>(1.03)</td>
<td>(0.99)</td>
</tr>
<tr>
<td>No. Mn:Ca Regime Shifts</td>
<td>4.35</td>
<td>4.20</td>
<td>3.75</td>
<td>3.35</td>
<td>3.40</td>
<td>3.89</td>
<td>3.23</td>
</tr>
<tr>
<td></td>
<td>(0.93)</td>
<td>(0.77)</td>
<td>(1.29)</td>
<td>(0.59)</td>
<td>(0.68)</td>
<td>(0.76)</td>
<td>(1.17)</td>
</tr>
</tbody>
</table>

Table 4. Mean (SD) by location for variables used in multiple discriminant analysis. Length at year 1 and egress are back-calculated estimations. Regime shifts are from one randomly selected side of the otolith transect (from core to edge).
Table 5. Classification and jackknifed classification matrix results of discriminant analysis based on estimated length at year 1 and egress, and number of regime shifts (Sr:Ca, Ba:Ca, Mn:Ca). "% correct" represents the percentage of individuals successfully classified to their collection location.

<table>
<thead>
<tr>
<th>Classification</th>
<th>Benton Falls</th>
<th>Dresden Mills</th>
<th>Hadley Lake</th>
<th>Orland Dam</th>
<th>Patten Pond</th>
<th>Seven Tree Pond</th>
<th>Winnegance</th>
<th>% correct</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benton Falls</td>
<td>10</td>
<td>4</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>Dresden Mills</td>
<td>5</td>
<td>9</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>45</td>
</tr>
<tr>
<td>Hadley Lake</td>
<td>2</td>
<td>0</td>
<td>8</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>62</td>
</tr>
<tr>
<td>Orland Dam</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>8</td>
<td>1</td>
<td>5</td>
<td>0</td>
<td>40</td>
</tr>
<tr>
<td>Patten Pond</td>
<td>4</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>7</td>
<td>2</td>
<td>1</td>
<td>39</td>
</tr>
<tr>
<td>Seven Tree P.</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>Winnegance</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>16</td>
<td>80</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>20</td>
<td>14</td>
<td>18</td>
<td>18</td>
<td>17</td>
<td>19</td>
<td>47</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Jackknifed Classification</th>
<th>Benton Falls</th>
<th>Dresden Mills</th>
<th>Hadley Lake</th>
<th>Orland Dam</th>
<th>Patten Pond</th>
<th>Seven Tree Pond</th>
<th>Winnegance</th>
<th>% correct</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benton Falls</td>
<td>8</td>
<td>5</td>
<td>0</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>40</td>
</tr>
<tr>
<td>Dresden Mills</td>
<td>5</td>
<td>7</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>35</td>
</tr>
<tr>
<td>Hadley Lake</td>
<td>2</td>
<td>0</td>
<td>7</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>54</td>
</tr>
<tr>
<td>Orland Dam</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>7</td>
<td>1</td>
<td>5</td>
<td>0</td>
<td>35</td>
</tr>
<tr>
<td>Patten Pond</td>
<td>5</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>6</td>
<td>2</td>
<td>1</td>
<td>33</td>
</tr>
<tr>
<td>Seven Tree P.</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>Winnegance</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>14</td>
<td>70</td>
</tr>
<tr>
<td>Total</td>
<td>24</td>
<td>21</td>
<td>15</td>
<td>17</td>
<td>18</td>
<td>17</td>
<td>19</td>
<td>40</td>
</tr>
</tbody>
</table>
Figure 1. Locations of study river systems along the coast of Maine. Black stars indicate approximate river mouth location.
Figure 2. Maps of each study river; A) Kennebec, B) St. George, C) Orland, D) Patten, E) East Machias. Fish harvest/sample locations are indicated by stars. A stream connects Patten Pond (D) to sea.
**Figure 3.** Line drawing of an intact blueback herring otolith with horizontal lines depicting the placement of the transverse section which was used in otolith analysis.

**Figure 4.** An otolith (200x magnification) with visible trench resulting from laser ablation. Vertical arrows indicate the core (year 0; center) and the first annulus (right; ventral). The individual was sampled at Benton Falls on the Kennebec River.
Figure 5. Low salinity habitat use index (LSHU) calculation for one individual from the core to the dorsal edge of the otolith (sampled from East Machias River). Otolith Sr:Ca is represented by the solid line. The dotted line is the distance along the laser transect that is considered low salinity residency (Sr:Ca < 2; 221 µm). The dashed line represents the measured distance from the core to the first annulus (534 µm). The calculated LSHU value is 0.41 indicating the individual moved into sea water well before formation of the first annulus.
Figure 6. Second order polynomial function used to fit data for length back-calculation of sample individuals ($R^2 = 0.97$). Otolith width and total length data from YOY and yearling blueback herring from ME and NY, and larval American shad from NY.
Figure 7. Concentrate-salinity plots depicting average Sr:Ca, Ba:Ca, and Mn:Ca versus salinity. Points are average concentrate ratios and salinity at each water sample location from two or three replicates. Relationships were fit to logarithmic functions. Standard deviation from the mean was ≤ 0.025 for all points.
Figure 8. Typical inverse relationships of Sr:Ca and Ba:Ca otolith microchemistry. The three example individuals are from Hadley Lake (A), Winnegance Lake (B), and Patten Pond (C). Also shown are Mn:Ca signatures from each individual. The primary axis of each graph depicts Sr:Ca values; the secondary, Ba:Ca and Mn:Ca values. The y axis indicates the distance from the core along the otolith sample transect in microns. Note the scale of the y axis differs between graphs.
Figure 9. LSHU index value (i.e., portion of time spent in low salinity habitat in the first year) versus estuary size, freshwater area, and distance to sea) for each study river. The center vertical line marks the median, the length of each box shows the range within which the central 50% of the values fall, with the box edges at the first and third
quartiles. Whiskers show the range of observed values that fall within the inner quartile range. Pearson's correlation coefficient (r) between LSHU and each variable is $r = -0.16$, $P = 0.07$ for estuary size, freshwater area, $r = 0.368$, $P < 0.001$, and distance to sea, $r = 0.244$, $P < 0.001$. 
Figure 10. Mean lengths at capture (total length), year 1, and egress. Lengths at year 1 and egress are estimates based on back-calculation methods. Error bars depict one standard deviation from the mean. Solid data points indicate significantly different means ($p \leq 0.001$); mean total length in Patten Pond was significantly longer than all other runs except for Dresden Mills, mean length at year one in Hadley Lake was significantly less than all other runs with the exception of Orland Dam and Seven Tree Pond; mean length at egress in Winnegance was significantly less than all other runs.
Figure 11. Estimated length at egress versus the maximum Mn:Ca regime value by location. Pearson's correlation coefficient = 0.437, $P \leq 0.001$. 
Figure 12. Average Sr:Ca signatures for transects spanning the entire otolith. Winnegance Lake and Patten Pond are visually distinct from the other runs. Solid vertical lines denote average distance to the first annulus on either side of the core (508 \( \mu \text{m} \)) and the dashed vertical line indicates the core.
Figure 13. Contrasting migration strategies in two individuals from Winnegance Lake. The individual represented by the dotted line showed prolonged low salinity habitat use (LSHU= 0.72), whereas the individual depicted by the solid line emigrated almost immediately into the estuary (LSHU= 0.02).
APPENDIX A

Otoliths

Fish possess three pairs of otoliths: the asterisci, lapilli, and sagittae, which are used for balance and hearing. Sagittae (sagittal otoliths) are most commonly used in research due to their larger overall size, thus easier removal and manipulation.

Otoliths are comprised mostly of calcium carbonate (CaCO$_3$) mainly as aragonite, but they also contain organic matrix and trace elements (Campana 1999). Age and growth estimations of individuals can be quantified by counting and measuring distance between growth bands. Calcium and trace element deposits within these growth increments are derived primarily from the water in which the fish resides (Campana and Neilson 1985, Thorrold et al. 1998) and result from ion substitution for Ca$^{2+}$ or coprecipitation of an alternative form of carbonate (Campana 1999). Incorporation of elemental concentrations also may be influenced by stress, temperature, and age (Secor 1992). Dissolved elements pass over the gills, enter the bloodstream, and diffuse into the endolymph where they are incorporated into the otolith structure (Campana, 1999). During the first months of life material is accrued at daily increments producing daily growth bands (Pannella 1971). As the fish ages annual bands known as annuli form.

Extraction of otoliths in river herring is completed by making an incision just before the formation of scales begin on the head (Figure A-1 A). Using small forceps the brain matter is removed and the otoliths can be carefully extracted (Figure A-1 B).

Figure A-1. Otolith extraction technique for river herring; A) illustrates the location of the incision made to open the brain cavity and B) illustrates the extraction of the otolith. Photograph credit: Government of Canada (2013).
References


APPENDIX B

Chemical Analysis

Analysis of water samples was performed using Inductively Coupled Plasma Atomic Emission Spectrometry at the Sawyer Environmental Chemistry Research Laboratory at the University of Maine in Orono, Maine (Table B-1). Elemental concentration data were reported as average parts per million (ppm) based on 3 internal replicates, n sample replicates and a software calculated calibration curve. Detection limits of the equipment were in the range of parts per billion for each element analyzed.

Otolith samples were analyzed using Laser Ablation paired with Inductively Coupled Plasma Mass Spectrometry (LA-ICPMS) at the State University of New York College of Environmental Science and Forestry (SUNY-ESF) in Syracuse, New York (Table B-1). The LA-ICPMS technique is performed by ablating along a transect of each prepared sample with a pulsed laser beam and then analyzing composition of the resulting vapor. Nominal concentrations (based on a series of previous analyses) for each element were divided by the mean counts per second (CPS) per element to establish a correction factor. Over the course of a sampling day the counts per second (CPS) of the sampled material may "drift" up or down based on equipment conditions, specifically ambient room temperature and duration of laser ablation. Generally, analysis of the standard produced stable results over time (i.e., negligible drift); therefore, the correction factor was calculated based on a "grand mean" of all standard transects. If the coefficient of variation (CV) over time was greater than 0.1 ($R^2 > 0.5$), a more detailed approach was used to determine the correction factor by accounting for instrument drift over time. However, this rarely occurred.
Table B-1. Instrumentation and operating conditions of the LA-ICP-MS (otolith samples) and ICP-AES (water samples).

<table>
<thead>
<tr>
<th><strong>Laser ablation</strong></th>
<th><strong>SUNY-ESF, Syracuse, NY</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Instrumentation</td>
<td>UP-193 laser ablation system-</td>
</tr>
<tr>
<td></td>
<td>Electro Scientific Industries</td>
</tr>
<tr>
<td>Flow rate Helium</td>
<td>800 ml/min</td>
</tr>
<tr>
<td>Power</td>
<td>70%</td>
</tr>
<tr>
<td>Spot size</td>
<td>35 μm</td>
</tr>
<tr>
<td>Scan speed</td>
<td>3 μm /second</td>
</tr>
<tr>
<td>Replicate Rate</td>
<td>10 Hz</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>ICP-MS</strong></th>
<th><strong>SUNY-ESF, Syracuse, NY</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Instrumentation</td>
<td>PerkinElmer DRC-e Inductively Coupled Plasma Mass Spectrometer (ICPMS)</td>
</tr>
<tr>
<td>Dwell Time per AMU</td>
<td>50 ms</td>
</tr>
<tr>
<td>RF Power</td>
<td>1500W</td>
</tr>
<tr>
<td>Nebulizer gas flow (He)</td>
<td>1.08 L/min</td>
</tr>
<tr>
<td>Internal standard (m/z)</td>
<td>43Ca</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>ICP-AES</strong></th>
<th><strong>University of Maine, Orono, ME</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Instrumentation</td>
<td>Perkin-Elmer Model 3300XL</td>
</tr>
<tr>
<td></td>
<td>Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES)</td>
</tr>
</tbody>
</table>
Figure C-1. Sr:Ca signatures (black line) detected along a full edge-to-edge LA transect transposed over a photograph of the sampled otolith. The resulting trench from LA is visible in the photograph. Vertical arrows indicate the core (dotted, center) and the first annulus formation (dashed, right and left). The individual was sampled from Dresden Mills (Kennebec River) and shows evidence of migration into sea waters before the first year (calculated LSHU value =0.49).
Figure C-2. Sr:Ca signatures from individuals collected at A) Benton Falls, B) Winnegance Lake, C) Seven Tree Pond, and D) Orland illustrating variation in migration patterns and habitat use. Solid vertical lines depict the first annulus, dotted lines represent calculated...
regime shifts, and dashed lines indicate the portion of the transect used to calculate LSHU value.
APPENDIX D

Back Calculation Methods

A piecewise approach was used to obtain reasonable length estimates for individuals at egress and at year one due to their small size at these events. The entire data set of otolith width (OW) to total length (TL), which includes the OW and TL at capture of individuals from each of the study river systems, as well as data from young of year (YOY; N = 9) and yearling (N = 9) blueback herring from the Penobscot Estuary, ME and the Hudson River, NY, and larval American shad (N = 10; Hudson River, NY) is shown below (Figure IV). Data were fit with a second order polynomial function using Statistica software.

Figure IV: Back calculation model containing the entire dataset of fish sizes. In this case Distance Weighted Least Squares is the best fit model ($R^2 = 0.95$) which does not allow for straightforward length estimates.