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CLAIRE L. ENTERLINE

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UNDERSTANDING SPAWNING BEHAVIOR AND HABITAT USE BY ANADROMOUS RAINBOW SMELT (*OSMERUS MORDAX*) USING PASSIVE INTEGRATED TRANSPONDER SYSTEMS AND TELEMETRY

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B. A., Boston University, 2006

Submitted to the University of New Hampshire

In Partial Fulfillment of

The Requirements for the Degree of

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In

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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>iv</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>vi</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>viii</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>xi</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>MONITORING WITHIN-SEASON SPAWNING BEHAVIOR USING PASSIVE INTEGRATED TRANSPONDER SYSTEMS</td>
<td>8</td>
</tr>
<tr>
<td>MOVEMENTS AND HABITAT USE BY RAINBOW SMELT WITHIN AN EMBAYMENT DURING AND FOLLOWING THE SPAWNING PERIOD</td>
<td>52</td>
</tr>
<tr>
<td>CONCLUSIONS</td>
<td>101</td>
</tr>
<tr>
<td>LIST OF REFERENCES</td>
<td>104</td>
</tr>
<tr>
<td>APPENDIX A – IACUC APPROVAL</td>
<td>113</td>
</tr>
</tbody>
</table>
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# LIST OF TABLES

Table 1.1. Length distribution of smelt used for two PIT tag mortality and retention studies. Smelt were collected from study locations (Mill Creek, Freeport, Maine and the Fore River, Braintree, Massachusetts). Laboratory studies began while smelt were still in spawning condition ................................................................. 14

Table 1.2. The number of tagged smelt at two spawning sites in the Gulf of Maine by gender, age and year tagged ................................................................................................................ 25

Table 1.3. Mortality at five different time periods compared among laboratory trials … 31

Table 1.4. Mean mortality due to tagging during three laboratory studies for female and male smelt ±1SE with two-tailed t-test p-values comparing mortality between genders ................................................................................................................ 32

Table 1.5. Mean mortality due tagging during three laboratory studies for three length categories ±1SE with one-way ANOVA p-values for comparisons among length categories for five time periods and cumulatively ........................................................................ 32

Table 1.6. The average number (and range) of within-season spawning movements is shown for each study year and location. Females were observed returning up to 5 times, while males returned up to 10 times within a single spawning season .............................................................................................................................. 35

Table 1.7. Mean repeat spawn events by male and female smelt from two study sites and three sampling years .............................................................................................................................. 37

Table 1.8. The number of nighttime movements to the spawning grounds made with the tide and against the tide is shown by year where calculated. Percentage of all movements is first shown followed by the number of movements (n) .................................................................................................................................................. 40

Table 2.1. Length distribution of smelt used for hydroacoustic transmitter mortality and retention studies ........................................................................................................................................ 57

Table 2.2. Number of smelt tagged with VEMCO transmitters in 2011 and 2012. Smelt were tagged at two locations with VEMCO V5 or V6 transmitters. Tagging by age was random and favored the dominant age caught (age-2) .............................................................................................................................. 64
Table 2.3. Retention of three hydroacoustic transmitters by adult rainbow smelt in two replicate tanks.

Table 2.4. The number of mortalities by transmitter type is shown with the total number tagged (n), and mean mortality by tag type. Smelt in tank 1 tagged with Lotek JSAT were the only group to also receive a Floy tag.

Table 2.5. Cumulative mortality between two replicate tanks (±1SE) is shown for control fish and tagged fish at 6 periods. Comparisons between control and tagged fish were made using Dunnett's test, p-values for each comparison are shown.

Table 2.6. Mean length (TL mm) of mortalities by tag type and for the control group.

Table 2.7. The mean movement time between receiver locations (±1SE) is shown with the corresponding distance between locations and the calculated travel time.

Table 2.8. The average number of days that smelt were detected at each site is shown for smelt tagged and released at two locations, the Oyster and Squamscott rivers, NH (±1SE). Where no SE is given, only one smelt was observed at that location.

Table 2.9. Temporal variation among the sites is compared using four variables. Cluster assignments (Ward's method) based on these variables found that spawning rivers, Adams Point, and locations leading to coastal waters grouped separately.

Table 2.10. Distance (km) between receiver location and smelt spawning grounds at the head-of-tide of each river.
LIST OF FIGURES

Figure 1.1. Fyke nets were set above the head-of-tide facing downstream on coastal streams to capture spawning smelt.................................................................12

Figure 1.2. HDX RFID systems were placed in Mill Creek, Freeport, Maine. Smelt were captured and released upstream of the antenna array (a). Each set of antennas was made of two side-by-side antennas to span the width of the river channel, fixed to A-frames anchored in the middle of the channel. Dashed lines represent antenna loops (b)...........19

Figure 1.3. HDX RFID systems were placed at the head-of-tide of the Fore River, Braintree, Massachusetts. Antennas were placed downstream of the fyke net used to capture smelt (a). Antennas in the same span were fixed to A-frames anchored in the middle of the river channel. Dashed lines represent antenna loops (b)...........................20

Figure 1.4. Length at age for all smelt tagged with Passive Integrated Transponders (PIT) at two sites, the Fore River in Braintree, Massachusetts (solid line, data combined 2010-2012), and Mill Creek, Freeport, Maine (dashed line, data combined 2009-2010)...........22

Figure 1.5. Example of RFID detection data and identification of missed detections. The top panel (a) shows the actual detections of a single smelt (note that the smelt is observed to “miss” the downstream antenna on multiple occasions). The bottom panel (b) shows the assumed missed detections (X). Efficiency values were calculated as the number of actual detections to the number of assumed total detections (sum of actual and assumed missed)..................................................................................................................................28

Figure 1.6. Mean mortality ±1SE for (a) smelt tagged with 23mm PIT tags and control smelt within a laboratory setting for five periods and cumulatively, (b) male and female tagged smelt, and (c) compared among length categories for tagged smelt. Significant differences are indicated (*)...................................................................................................33

Figure 1.7. The average number of repeat spawn events (±1SE) for male and female smelt at two study sites over three years.................................................................36

Figure 1.8. The average number of repeat spawning events (±1SE) for (a) all tagged smelt, (b) females, and (c) males.........................................................................................38

Figure 1.9. The mean number of repeat spawn events (log normalized) compared to the study week in which smelt were tagged.................................................................39
Figure 1.10. The average frequency of PIT tag detections recorded by the RFID system at Mast Landing, Freeport, ME 2010-2011 (±1SE) at each daily hour ..................................................41

Figure 2.1. Deployment locations of 14 (2011, panel a) and 15 (2012, panel b) VEMCO VR2W-180kHz hydroacoustic receivers set to monitor the movement of smelt tagged with acoustic transmitters between riverine, estuary and marine habitats in the Great Bay and Piscataqua River estuary system, New Hampshire ........................................................................60

Figure 2.2. Comparison of cumulative mortality (± 1SE) of adult smelt over fifteen weeks after six periods for four tag types ........................................................................................72

Figure 2.3. Examples of three movements patterns represented by: (a) three individual smelt tagged with hydroacoustic transmitters whose last detections were recorded in one of the spawning rivers; (b) three smelt last detected at Adams Point; and (c) three smelt last detected at the Piscataqua River mouth ........................................................................75

Figure 2.4. Relationship between the movement time between receivers (hrs, ±1SE) and the distance between receivers (km) ....................................................................................77

Figure 2.5. The mean number of daily detections for each smelt (± 1SE) was highest at the Bellamy, Oyster, and Squamscott rivers, and Adams Point among smelt tagged/released from the Squamscott River ........................................................................79

Figure 2.6. The mean number of days (± 1SE) spent at each receiver site by smelt tagged/released at the Oyster River was highest at the Adams Point receiver and lowest at receivers located on the pathway to coastal waters ........................................................................81

Figure 2.7. The mean number of days (±SE) spent at each location by smelt released from both the Oyster and Squamscott rivers was highest at Adams Point and the Bellamy River and lowest at receivers placed on the pathway to coastal waters ........................................83

Figure 2.8. Biplot showing cluster assignments (Ward’s method) with PCA results. The first cluster’s placement was associated with later detection dates (symbol = •; Adams Point). The second cluster (symbol = +; General Sullivan Bridge, Little Harbor, and the Piscataqua Mouth) had later median dates and later dates with the highest number of detections. The third cluster (symbol = 0; Bellamy, Lamprey, Oyster, Piscataqua, and Squamscott rivers) had earlier first, median, and last detection dates, and the date with the highest number of detections was also earlier ......................................................................85

Figure 2.9. The mean (a) latest detection dates ±1SE and (b) median detection dates ±1SE differed among three clusters of receiver sites: Cluster 1 = Adams Point; Cluster 2 = Gen. Sull. Bridge, Little Harbor, and the Piscataqua mouth; Cluster 3 = Bellamy, Lamprey, Oyster, Piscataqua, and Squamscott rivers (one-way ANOVA).................................87
Figure 2.10. The number of individual tagged smelt detected at each receiver location daily varied between the sites and study years. Cells are colored by density of fish detected daily: light gray = few smelt; black = higher numbers.

Figure 2.11. The 2011-2012 mean number of tagged smelt detected daily. A higher number were observed before mid-April at the spawning rivers, in mid to late April at Adams Point, in late April and through May and at pathways to coastal waters. Cells are colored by density of fish detected daily: light gray = few smelt; black = higher numbers.
ABSTRACT

UNDERSTANDING SPAWNING BEHAVIOR AND HABITAT USE BY ANADROMOUS RAINBOW SMELT (OSMERUS MORDAX) USING PASSIVE INTEGRATED TRANSPONDER SYSTEMS AND TELEMETRY

by

Claire L. Enterline

University of New Hampshire, December 2013

Rainbow smelt (Osmerus mordax) are anadromous fish that spawn in coastal streams and rivers and provide an important prey base to many marine predators. Populations range-wide have experienced severe decline possibly due to habitat degradation and loss of spawning habitat. This study quantified within-season repeat spawning behavior by rainbow smelt at two spawning sites in the Gulf of Maine, and described movements and habitat use during and following the spawning season in the Great Bay and Piscataqua River estuary complex in New Hampshire. Repeat spawning behavior was found to be a predominantly male behavior, consistent with past studies. The rate of repeat spawning by males was found to be consistent among years and between two study sites. Regarding larger scale movements, smelt of both genders visited multiple rivers within an embayment during the spawning season, and further, made use of a tidal estuary system after spawning activity ceased.
INTRODUCTION

Rainbow smelt (*Osmerus mordax*) are small anadromous fish that live in nearshore coastal waters and spawn in the spring in coastal rivers immediately above the head of tide in freshwater (Buckley 1989, Kendall 1926, Murawski *et al.* 1980). Landlocked populations of smelt also occur naturally in lakes in the Northeast U. S. (Hoover 1936, Rupp 1959) and Canada (Bernatchez 1997, Sirois 2012), and have been introduced to many freshwater systems, including the Great Lakes (Warner *et al.* 2009). Anadromous smelt serve as an important prey species for commercially and culturally valuable species, such as Atlantic cod, Atlantic salmon, lake trout, Atlantic gray seals, and striped bass (Clayton *et al.* 1978, O’Gorman *et al.* 1987, Kircheis and Stanley 1981, Kirn 1986, Stewart *et al.* 1981). Historically, the range of rainbow smelt extended from Chesapeake Bay to Labrador (Buckley 1989, Kendall 1926), but over the last century the range has contracted northward.

Populations in Long Island Sound and Narragansett Bay may be seriously impacted, perhaps to the point of extirpation (Keller *et al.* 1999, Oviatt *et al.* 2003). Trawl surveys in Long Island Sound performed during 1984-1994 found at total of 31 smelt (Gottschall *et al.* 2000) while none were found in surveys performed in Connecticut in 2004 (Fried 2006). The factors contributing to declines in rainbow smelt populations are not well understood, but may include degradation of spawning habitat (Enterline *et al.* 2012a, Murawski and Cole 1978, Wyatt *et al.* 2010), stream obstructions (Chase and

Life History

Smelt are small-bodied and short-lived, seldom exceeding 25 cm in length or five years of age, in the Gulf of Maine region (Murawski and Cole 1978, Lawton et al. 1990). By age two, smelt are fully mature and undergo spawning migrations (Kendall 1926, Buckley 1989). Life history appears to be influenced by latitude. Few age-1 smelt participate in Canadian smelt runs (McKenzie 1964), but higher numbers are found in Massachusetts, New Hampshire, and southern Maine migrations (Murawski and Cole 1978, Lawton et al. 1990, Enterline et al. 2012a). Studies in Massachusetts found that the majority of these age-1 fish were spawning males (Murawski and Cole 1978, Lawton et al. 1990). Recent spawning surveys found that runs in the U. S. Gulf of Maine were dominated by age-2 smelt, with few older fish in Massachusetts, New Hampshire, and southern Maine. Older ages were better represented in midcoast and eastern Maine (Enterline et al. 2012a).

Spawning Behavior

A gender bias has been described for multiple spawning populations, where males were more frequently encountered on the spawning grounds. Mark and recapture studies
documented that individual males returned to spawning grounds multiple times within a single season, while females were less likely to return (Murawski et al. 1980, Rupp 1968). Multiple males have been observed attending a single female during spawning (Langlois 1935, Hoover 1936, Clayton 1976), a behavior described in other anadromous and non-anadromous species, e.g. pink salmon (*Oncorhynchus gorbuscha*; Nikolsky 1963); winter flounder (*Pseudopleuronectes americanus*; Stoner et al. 1999), Atlantic cod (*Gadus morhua*; Hutchings et al. 1999), and California grunion (*Leuresthes tenuis*; Byrne and Avise 2009).

Spawning site fidelity may be low in certain rainbow smelt populations. Several studies documented smelt straying between several tributaries within the same river system or estuary (Marcotte and Trembly 1948, Murawski et al. 1980). In contrast, where geospatially and temporally distinct runs occur within the same river or estuary, tagging studies found that the rate of recaptures at sites other than the release areas were low (McKenzie 1964, Coulson et al. 2006). In the St. Lawrence River, up to five spawning populations have been identified, based on genetic and morphological distinctions (Bradbury et al. 2006, Coulson and Bentzen 2009). For these divergent populations to be maintained, the rate of straying between spawning sites must remain consistently low through time.

*Larval and Juvenile Life History*

Spawning females deposit demersal (sinking), adhesive eggs that attach to the substrate and hatch in 7-21 days, depending on water temperature (Kendall 1926). Upon hatching, larvae are immediately transported downstream into the tidal zone, at which
point the larvae begin feeding on zooplankton. Downstream larval dispersion is primarily passive and fish respond to river flow and coastal circulation patterns, however larvae also actively swim vertically in the water column (Bradbury et al. 2006b) to maintain their position in zooplankton rich water (Laprise and Dodson 1989, Dauvin and Dodson 1990, Sirois and Dodson 2000). This active swimming behavior can be overwhelmed by passive transport in some areas. Thus, coastal circulation patterns may be responsible for maintaining regional genetic stock structure (Kovach et al. 2012).

Juvenile smelt remain in the estuary, bay, or sheltered coastal areas throughout the summer and sometimes early fall. Annual, fishery-independent seine surveys reported the capture of juvenile smelt from June to November in Great Bay, New Hampshire with peak abundance during August. Captures occurred from July to October in Maine, with evenly distributed abundances in the Kennebec River and Merrymeeting Bay estuary complex between August, September, and October (Enterline et al. 2012a).

Adult Annual Migrations

Habitat use in marine waters is largely unknown but can be inferred through interviews with coastal fishermen and from state trawl surveys. Adult smelt appear to migrate in search of optimum water temperatures, moving offshore during the summer months to greater depths with cooler water (Buckley 1989). Based on low catches by fishermen in freshwater and larger catches in brackish and saltwater in May, the presumed end of the spawning run, it has been assumed that adults return to estuaries and coastal waters immediately after spawning (Bigelow and Schroeder 1953). Recent trawl surveys have found small schools of smelt as far from the coast as 60 km and in depths
up to 77 m (S. Sherman, Maine Department of Marine Resources, pers. comm.). Spring trawl surveys find smelt further from the coast and in deeper water (spring avg. depth = 29.7 m) than during fall trawl surveys (fall avg. depth = 19.9 m), however, the average spring catch is smaller than that in the fall (spring average catch 2001-2012 = 31, fall average catch 2000-2011 = 129). This is likely because adult smelt are within coastal streams and rivers as part of the spawning event during the spring period. The smelt that are caught further offshore in the spring are smaller, with lengths associated with age-1 which were likely too young for recruitment into the spawning migration (Enterline et al. 2012a).

As offshore water temperatures drop in the fall, smelt likely move towards the coast, eventually migrating into the upper estuaries where they overwinter (McKenzie 1964, Clayton 1976, Buckley 1989). Anecdotal reports from recreational hook-and-line ice-fishermen describe smelt moving into tidal rivers with the nighttime flood tide and out with the ebb tide, with some moving as far up as the head of tide each night. These foraging movements are the basis for robust recreational fisheries in the fall and winter at many locations in the Gulf of Maine.

Understanding Habitat Use and Behavior Patterns

Although the general annual life cycle of smelt has been well described, information about important habitats other than spawning grounds is lacking. For example, post-spawning adults are assumed to move to coastal waters immediately after the spawning period (McKenzie 1964, Buckley 1989), but their residence time within estuaries prior to out-migration has not been documented. Understanding habitat use is
imperative to effective management. Degradation of important habitat can have significant impacts and lead to population declines (Langton et al. 1996, Bigford 2013). Habitat may be considered essential if species are found in the area for long time periods (e.g. overwintering), or if essential phases in the life cycle occur there (e.g. spawning, post-spawn feeding, larval rearing, etc.). Identifying these areas and describing their temporal use is necessary for species management.

Furthermore, understanding behavior and movement patterns is necessary to interpret patterns in survey and catch data and make conclusions about the status of a population. Spawning runs displaying a substantially higher proportion of males may be indicative of a stressed population because the limiting factor for population growth is often the abundance of eggs. Recent surveys performed at various rainbow smelt spawning sites spanning the U. S. Gulf of Maine reported sex ratios ranging between 1.5 (male to female) at Schoppee Brook, Maine and 9.5 at the Parker River, Massachusetts (Enterline et al. 2012a). Fairly even sex ratios have been reported during sampling efforts targeting smelt during non-breeding seasons. For example, Murawski et al. (1980) found that age 2+ females comprised only 11.4% of the sampled population during one spring spawning survey compared to 47.4% of the winter commercial fishery catch within the same year. Repeat spawning behavior by males has been documented for a number of spawning smelt populations (e.g. Marcotte and Trembly 1948, Murawski et al. 1980). It is possible then, that within-season repeat spawning behavior may be masking underlying skewed sex ratios within stressed populations if the rate of repeat spawning behavior does not differ among spawning runs.
The present study quantified within-season repeat spawning behavior by rainbow
smelt at two spawning sites in the Gulf of Maine, Mill Creek, Freeport, Maine and the
Fore River, Braintree, Massachusetts, and described smelt movements and habitat use
during and following the spawning season in the Great Bay and Piscataqua River estuary
complex, New Hampshire. Behavior and movements were quantified using Passive
Integrated Transponder (PIT) tags in concert with radio frequency identification (RFID)
systems, and hydroacoustic telemetry transponders and receivers. The specific objectives
were to: 1) observe differences in spawning frequency between male and female smelt; 2)
determine whether frequency of spawning was age-dependent; 3) describe tidal and diel
influences on spawning movements; 4) monitor movement between riverine, estuary and
marine habitats during and following the spawning season; 5) assess relative use of each
habitat area; and 6) determine spawning site fidelity within a spawning season.
CHAPTER I

MONITORING WITHIN-SEASON SPAWNING BEHAVIOR USING PASSIVE INTEGRATED TRANSPONDER SYSTEMS

Introduction

Rainbow smelt (Osmerus mordax) are a small anadromous fish that live in nearshore coastal waters and spawn in the spring in freshwater coastal rivers immediately above the head of tide (Kendall 1926, Buckley 1989, Murawski et al. 1980). The spawning run occurs annually in the springtime but differs in timing, beginning in early March and continuing through mid-May in Massachusetts (Murawski and Cole 1978, Chase 2006) and beginning progressively later moving northeast (McKenzie 1958, Enterline et al. 2012a). Similar to other anadromous species, the onset of spawning may be influenced by many factors, including changes in discharge (Abou-Seedo and Potter 1979, Paragamian and Wakkinen 2011), habitat competition with other anadromous spawning species (Janetski et al. 2011), and water temperature fluctuations (Loesch and Lund 1977, Paragamian and Wakkinen 2011).

During the spawning run, highly skewed sex ratios have been observed in which a greater number of males than females were present on the spawning grounds (Marcotte and Tremblay 1948, Murawski et al. 1980). Multiple males have also been observed attending a single female during spawning (Langlois 1935, Hoover 1936, Clayton 1976,
Lischka and Magnuson 2006), a behavior described in other anadromous and non-anadromous species, e.g. pink salmon (*Oncorhynchus gorbuscha*; Nikolsky 1963); winter flounder (*Pseudopleuronectes americanus*; Stoner *et al.* 1999), Atlantic cod (*Gadus morhua*; Hutchings *et al.* 1999), and California grunion (*Leuresthes tenuis*; Byrne and Avise 2009). Fertilization from several males has been shown to increase fertilization success of rainbow smelt eggs (Purchase *et al.* 2007), likely because milt quality differs among males, and because females broadcast spawn in sections of streams with relatively high velocities (Hulbert 1974, Burness *et al.* 2004). Male smelt have been observed on the spawning grounds multiple times within the same season (Marcotte and Tremblly 1948, Rupp 1968, Murawski *et al.* 1980), and ovulate during one spawning event (Marcotte and Tremblay 1948).

Fairly even sex ratios were found during sampling events that targeted smelt during non-breeding seasons. Murawski *et al.* (1980) found that age 2+ females comprised only 11.4% of the sampled population during one spring spawning survey compared to 47.4% of the winter commercial fishery catch within the same year. Results from 2008 fyke net surveys at a spawning site on the Harraseeket River, Maine found females comprised only 14.6% of the catch (Enterline *et al.* 2012a), whereas an almost even sex ratio (46.2% female) was found from a fall near-shore trawl survey conducted in the embayment area below this site (S. Sherman, Maine Department of Marine Resources, pers. comm.).

While repeat spawning behavior has been described, it has not been quantified. Differences in the frequency of repeat spawning between gender and age could bias
mortality estimates if the behavior is consistently associated with a certain gender and age group. Murawski and Cole (1978) estimated a higher mortality rate for males than females in the Parker River, Massachusetts using a frequency-at-age model based on spawning survey catches. This higher mortality rate for males may be influenced by a higher rate of within-season repeat spawning by males compared to females. Further, if the rate of repeat spawning varies by age, age cohort mortality calculations will be biased. Quantifying the rate of repeat spawning by age and gender will allow the frequency at age to be corrected if necessary and allow accurate morality estimates calculated. This study tests the hypothesis that male anadromous smelt make more frequent visits to the spawning grounds compared to females, and aims to quantify this behavior for each gender and by age.

Various internal and external fish tagging methods have emerged in recent years, and greater success has been observed with larger fish, which are less prone to tagging injury and disturbances in behavior. When tagging smaller species, such as rainbow smelt (~16 cm), external tags can disrupt equilibrium and cause the fish to be more visible to predators. Further, vital organs can easily be punctured or disturbed when placing tags internally. Small fish, such as smelt, can be marked using visible implant elastomer (VIE) tags with high retention and little to no mortality (Olsen and Vollestad 2001, Bryondocx et al. 2002, Griffiths 2002, Brennan et al. 2007), but due to variable temporal recruitment during the spawning run, smelt would need to be tagged and recaptured every day of the spawning run for the results to be statistically significant (G. Nelson, Massachusetts Division of Marine Fisheries, pers. comm.). Passive integrated
transponder (PIT) tags can be placed internally in small fish with little mortality and tag loss (Bryondocx *et al.* 2002) and be detected twenty-four hours a day by in-stream radio frequency identification (RFID) systems. The use of PIT tags is advantageous because the ability to continuously monitor PIT tags allows for tagging to occur periodically and provides a high probability of ‘recapture’ by the RFID system, thus reducing effort while increasing the likelihood of accurate results.

This study quantified smelt movement during the spawning season using 23 mm Passive Integrated Transponder (PIT) tags that were recorded by RFID systems at two spawning sites in the Gulf of Maine: Mill Creek, Freeport, Maine and the Fore River, Braintree, Massachusetts. The specific objectives were to: 1) observe differences in spawning frequency between male and female smelt; 2) determine whether spawning frequency is age dependent; and 3) determine if movements to the spawning ground were influenced by the tidal cycle or time of day.

**Methods**

*Laboratory Studies*

Smelt were collected as part of the regionally standardized rainbow smelt spawning assessment survey conducted by the Massachusetts (MA) Division of Marine Fisheries (DMF) and Maine (ME) Department of Marine Resources (DMR). Smelt were collected at two spawning locations, Mill Creek, Freeport, ME (May 19, 2009) and the Fore River, Braintree, MA (April 22, 2011 and March 22, 2012) using large fyke nets that were placed immediately downstream of the spawning grounds (fyke nets: mouth
4'x4' box; 11' approximate length from mouth to cod end; three chambers; first throat tapered to 5"; second throat tapered to 3"; 2.5' diameter hoops; wings 4'x4' box on each side; Memphis Net and Twine, Memphis, TN; Figure 1.1). The fyke nets were set at mid-channel in the intertidal zone below the downstream limit of smelt egg deposition. Nets were set three days each week, hauled on each of these three days during the morning low tide after overnight sets. The fyke net opening faced downstream to intercept the spawning movements of smelt that occur at night during the flood tide. Fyke net catches were assumed to be representative of the size and gender composition of the spawning run.

Tag retention and handling mortality was examined for each study population. A laboratory retention and mortality study was completed at the ME DMR West Boothbay Harbor Lab during May 19 – July 21, 2009. A total of 54 smelt were collected for the study from Mill Creek, Freeport, ME; of these, 29 control smelt were placed into tanks with minimal handling and 27 smelt were tagged and placed into the same tank. Smelt

Figure 1.1. Fyke nets were set above the head-of-tide facing downstream on coastal streams to capture spawning smelt.
were measured to the nearest millimeter (TL) and gender was determined by the presence (males) or absence (females) of nuptial tubercles on their ventral surfaces (Warfel et al. 1943) or by the expression of milt with slight ventral massage. Smelt were tagged internally using 23mm PIT tags (23 x 3.8 mm, 0.6 g air weight, OregonRFID, Portland, Oregon). PIT tags were sterilized with commercially available isopropyl (99% isopropyl rubbing alcohol) and gently inserted into a small incision (<1 cm) made with a hooked scalpel in the peritoneal cavity directly posterior to the pectoral fins. A small amount of antibacterial ointment (Neosporin®) was used to cover the wound. Each smelt receiving a PIT tag was also tagged with a visible implant elastomer mark (Northwest Marine Technology, Inc., Shaw Island, WA) in the operculum for the purpose of visual identification upon recapture. Smelt were then placed into a 460 gallon polyethylene round tank (70” x 30”, Pentair Aquatic Eco-systems, Inc., Apopka, FL) with flow-through sea water. The average sea surface temperature at the West Boothbay Harbor Marine Lab during the study period was 13.8°C (range: 9.8°C on May 19 – 18.2°C on July 2). The smelt used for this study were comprised largely of smaller fish because smelt were collected late in the run (Table 1.1). Daily mortalities were removed and gender, length, and PIT tag number recorded. The holding tank was monitored daily for any expelled tags. During the first week at the ME DMR laboratory, a large (20+ lb) lobster was placed in the tank with the study smelt because of failure of Maine State Aquarium holding tanks (housed at the same facility). Aquarium staff also performed a tank cleaning during this first study week.
The second retention and mortality study was performed at the MA DMF Annisquam River Marine Fisheries Field Station in Gloucester, MA during April 22 – July 7, 2011 by MA DMF staff Scott Elzey, Matthew Ayer, and Katie L’Heureux. Smelt (n = 180) in spawning condition were taken from the Fore River, Braintree, MA as part of the annual fyke net survey. Control and tagged fish were selected according to gender and length (approximately 30 from each category: M ≤ 149 mm, F ≤ 149 mm, M 150 mm-209 mm, F 150 mm-209 mm, M ≥ 210 mm, F ≥ 210 mm; Table 1.1). Smelt were

<table>
<thead>
<tr>
<th>Length</th>
<th>ME DMR Study</th>
<th>MA DMF Study No. 1</th>
<th>MA DMF Study No. 2</th>
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<td>No. Control</td>
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<td>6</td>
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<tr>
<td>All Lengths</td>
<td>29 27</td>
<td>90 90</td>
<td>60 63</td>
</tr>
</tbody>
</table>

Table 1.1. Length distribution of smelt collected from study locations (Mill Creek, Freeport, Maine and the Fore River, Braintree, Massachusetts) used for two PIT tag mortality and retention studies. Laboratory studies began while smelt were still in spawning condition.
measured, gender determined and tagged as described above. Smelt were then placed into a 200 gallon polyethylene round tank (48” x 30”, Pentair Aquatic Eco-systems, Inc., Apopka, FL) with water supplied from the Gloucester public water supply (tap) and was de-chlorinated by heating the water to steaming point so the chlorine gas evaporated. The water was brought to 20 ppt using Instant Ocean Synthetic Sea Salt (Blacksburg, VA). Water temperature was held constant at 15°C by an in-line chiller (Aqua Logic Delta Star® model DS-5 chiller, San Diego, CA). Mortalities were removed daily and gender, length, and PIT tag number recorded. The holding tank was monitored daily for any expelled tags.

A third laboratory study was performed at the MA DMF Annisquam River Marine Fisheries Field Station in Gloucester, MA during March 22 – May 24, 2012 by MA DMF staff named above. Smelt (n = 123) in spawning condition were taken from the Fore River, Braintree, MA as part of the annual fyke net survey. Control and tagged fish were selected according to gender and length (approximately 15 from each category: M ≤149 mm, F ≤149 mm, M ≥210 mm, F ≥210 mm; approximately 30 from each category: M 150 mm-209 mm, F 150 mm-209 mm, Table 1.1). Smelt were measured, gender determined and tagged as described above with the following exception. During the first study performed at the MA DMF laboratory, water quality declined precipitously during the first night following capture and tagging because most smelt released their gametes. Learning from this mistake, smelt were held overnight before being tagged during the second study at the MA DMF laboratory, and this problem did not reoccur. Smelt were held under the same conditions as described above for the Gloucester facility. Mortalities
were removed daily and gender, length, and PIT tag number recorded. The holding tank was monitored daily for any expelled tags.

Field Studies

Movement to and from the spawning grounds was assessed directly downstream of rainbow smelt spawning grounds at Mill Creek, Freeport, ME in each of three years (2009, 2010, 2011), and the Fore River, Braintree, MA in two years (2011 and 2012). Mill Creek is located at the head of tide of the Harraseeket River in Freeport, ME and drains into Casco Bay. Historically and currently, this site supports annual spawning comparable with the larger spawning runs in the state. The average water velocity during the spawning season is 0.468 m/s (Enterline et al. 2012a). The surrounding watershed (20.7 km²) remains relatively undeveloped (3.0% impervious surface cover; 59 people/km²) and is primarily forested (75.3%), as are most other watersheds supporting smelt spawning in the state (state means = 4.1% impervious surface cover; 67.6% forest cover; 65.7 people/km², Mills and Enterline 2012 in Wood et al. 2012). The Mill Creek spawning grounds are located in land protected for wildlife, but the spawning run also supports a recreational smelt dip-net fishery during the spawning run each spring. This unaltered stream flows over a gravel bed at the spawning grounds and opens downstream to an intact salt marsh.

The Fore River is a coastal river and estuary area with its mouth at Boston Harbor in Quincy, MA. The river has traditionally supported a strong rainbow smelt spawning run, with the spawning grounds located directly above the head-of-tide in Braintree, Massachusetts. The river continues to support one of the strongest documented spawning
runs in the state (Chase and Childs 2001, Chase 2006). Average spring velocity is 0.623 m²/s, and average spring discharge is 1.92 m³/s. The surrounding watershed (74.7 km²) is primarily developed land (47.3% of watershed area) although forested area is the secondary land cover (28.1%). The watershed is densely populated (831 person/km²), with a high percentage of impervious surface coverage (27.4%; Enterline et al. 2012a).

Half duplex (HDX) RFID systems were placed in Mill Creek at the head-of-tide of the Harraseeket River for the duration of the rainbow smelt spawning season (March–June) in 2009, 2010, and 2011. Two sets of antennas were placed in the river approximately ten meters apart in order to obtain information on the directionality of fish movement. Antennas were placed downstream of the fyke net used to capture smelt so that any returning tagged fish would trigger the antennas before being caught in the net (Figure 1.2a). Each set of antennas was made of two side-by-side antennas (each approximately 1.5 m high x 4.2 m wide) to span the width of the stream channel (~8.5 m). Antennas in the same span were fixed to 1.75 m A-frames anchored in the middle of the creek channel (Figure 1.2b). Antennas were powered continuously for the entire sample period using two 125 watt solar panels (Sunwize Technologies, Kingston, NY) connected to four deep-cycle 12V batteries (OPTIMA® BLUETOP®, Milwaukee, WI) in 2010 and 2011. In 2009, batteries were charged off-site and replaced daily. Antennas were made of double wrapped 4-gauge welding cable (Maine Oxy, Auburn, ME) connected to a multiplexer circuit board which combined the input into the reader control module (OregonRFID, Portland, OR). All reader components, batteries, and electrical circuits were housed in weatherproof Pelican® cases (San Antonio, TX). Detection
effectiveness was tested daily using a 23mm PIT tag placed by hand at multiple points within the detection range of each antenna. Antennas were tuned as needed to maximize the detection range. The normal detection range for each antenna reached the entire inner portion of the antenna loop and up to 1.5 m on both the upstream and downstream sides of each antenna.

A second HDX RFID system was installed at the head-of-tide of the Fore River for the duration of the spawning season (March – June) in 2011 and 2012 by MA DMF staff Scott Elzey, Matthew Ayer, Katie L’Heureux, Christopher Wood, and Kimberly Trull. These staff members also performed smelt tagging and ensured accurate data recording at the site. Antennas were made of 4-gauge welding cable (Maine Oxy, Auburn, ME) connected to a 4-reader system (OregonRFID, Portland, OR) running on 14V DC power supply plugged into AC power. Two sets of antennas were placed in the river approximately ten meters apart in order to obtain information on the directionality of fish movement. Antennas were placed downstream of the fyke net used to capture smelt (Figure 1.3a). Each set of antennas was formed by two antennas to span the entire river width (each antenna appx. 1.75 m high x 6.6 m wide, channel = 13.7 m). Antennas in the same span were fixed to 2 m high A-frames anchored in the middle of the river channel. Instead of forming a single loop, each antenna was wrapped to form three loops within its cross-sectional area in order to decrease the detection area within each antenna loop (Figure 1.3b). This design also minimized electrical interference. All reader components and electrical circuits were housed in weatherproof Pelican® cases (San Antonio, TX). Detection effectiveness was tested daily using a 23mm PIT tag placed by
Figure 1.2. HDX RFID systems were placed in Mill Creek, Freeport, Maine. Smelt were captured and released upstream of the antenna array (a). Each set of antennas was made of two side-by-side antennas to span the width of the river channel, fixed to A-frames anchored in the middle of the channel. Dashed lines represent antenna loops (b).
Figure 1.3. HDX RFID systems were placed at the head-of-tide of the Fore River, Braintree, Massachusetts. Antennas were placed downstream of the fyke net used to capture smelt (a). Antennas in the same span were fixed to A-frames anchored in the middle of the river channel. Dashed lines represent antenna loops (b).
hand at multiple points within the detection range of each antenna. Antennas were tuned as needed to maximize the detection range. The normal detection range for each antenna reached the entire inner portion of the antenna loop and up to 1.5 m on both the upstream and downstream sides of each antenna.

Smelt were collected as part of the regionally standardized survey performed by MA DMF and the ME DMR during annual spring spawning runs (March to May) using large fyke nets placed directly below the spawning grounds as described above. All non-target species were counted and a subset (30) measured (TL mm), and released. All smelt caught as part of the fyke net survey were counted, sexed and a subset (100 males and 100 females) were measured to the nearest millimeter (TL). Gender was determined as described above. Scale samples were taken from the dorsal area of the fish directly below the dorsal fin for a subset of all measured fish to create site-specific age-at-length keys. All smelt that were not tagged were released live with no additional handling. Smelt were tagged as described above. In 2011 at the Mill Creek site, 3M™ Vetbond™ Tissue Adhesive (n-butyl cyanoacrylate, St. Paul, MN) was used to seal the wound by placing the tip of the applicator bottle to the wound and allowing the adhesive to emerge from the bottle while drawing the applicator tip along the length of the wound. No more than 60 smelt were tagged each week for ten weeks. Tagging was based on gender and age class as determined by length (10 individuals from each category: M ≤169mm, F ≤169mm, M 170mm-209mm, F 170mm-209mm, M ≥210mm, F ≥210mm). While we aimed to tag equal numbers of fish within each of these bins each week, the actual number of fish tagged was also dependent on fyke net catches. In each year at both study
sites, the male to female tagging ratio was higher earlier in the run, varying between 2.2 - 4.3:1 (M:F). As the run progressed, the male to female tagging ratio evened out, varying between 0.9 - 1.5:1.

Each smelt receiving a PIT tag was also tagged with a visible implant elastomer mark (Northwest Marine Technology, Inc., Shaw Island, WA) in the operculum for the purpose of visual identification upon recapture. All tagged smelt were placed in a recovery tank with aerated river water held at stream temperature for at least ten minutes before being released above the capture site. Tagged smelt were monitored using in-stream continuously running RFID systems.

Scale samples were taken from all tagged fish to confirm age in all sampling years except 2009 (Figure 1.4). The first sampling year (2009) was conducted as pilot study and handling of tagged fish was kept to a minimum until laboratory retention and mortality studies could be performed. When collected, scales are covered by a partly-transparent mucous membrane that can obscure annuli and lead to erroneous age assignments, particularly for higher ages. To remove the mucous membrane, scales were

![Figure 1.4. Length at age for all smelt tagged with Passive Integrated Transponders (PIT) at two sites, the Fore River in Braintree, MA (solid line, data combined 2011-2012), and Mill Creek, Freeport, ME (dashed line, data combined 2010-2011).](image-url)
first placed in small brass screen baskets and then immersed in a solution of pancreatin (800 mg per 500 mL of water), or placed in plastic centrifuge tubes filled with pancreatin solution (800 mg per 500 mL of water) and agitated using a sonicator. To determine ages, scales were viewed using the image analysis program Image-Pro (V6.2, MediaCybernetics, Warrendale, PA) which drove a digital video camera mounted atop a dissecting microscope with transmitted lighting (Olympus, Center Valley, PA or Kramer Scientific, LLC, Amesbury, MA). Two individuals aged each scale with no prior information about the length or gender of the fish. If there was a discrepancy, age was assigned by a third more experienced reader or by consensus (Enterline et al. 2012b).

Data Analysis

Hourly tide data covering the entire sampling periods were obtained using WXTide32 (1.6.2, 2007). Tide data from the Fore River at Weymouth, MA was offset 15 minutes on the ebb tide and twenty minutes on the flood tide consistent with field observations. Tide data from the Harraseeket River, South Freeport, ME was offset thirty minutes on the ebb tide and forty minutes on flood tide consistent with field observations. Tag detection and tidal data were compared in Microsoft Excel (Windows XP 2003, Microsoft Corporation, Redmond, WA) and R statistical software (Version 2.15.1, R Foundation for Statistical Computing, Vienna, Austria).

Statistical analyses were performed using JMP Pro 10.0.0 (Copyright © 2012 SAS Institute Inc., Cary, NC) and SYSTAT 13.1 (SYSTAT 2009, Chicago, IL). Laboratory retention and mortality studies were analyzed using one-way ANOVA (or two-tailed t-tests when only two categories) to compare the proportion of mortalities that
occurred in control and tagged groups. Linear regression was used to analyze mortality by length. The three laboratory mortality and retention studies were considered replicates in order to compare the mortality rates occurring between tagged and control fish, male and females, and length categories. The null hypothesis was that there was no difference in mortality between tagged and control fish, and that mortality among tagged fish was not different between genders.

When comparing the mortality rates, data in each study were combined into five time periods so that they could be compared between the studies: period-1 = week 1; period-2= week 2; period-3 = weeks 3-4; period-4 = weeks 5-6; period-5 = weeks 7-10. Cumulative mortality over all time periods was also calculated and compared between tagged and control fish. The mortality rate for each time period ($p_i$; control or tagged fish) was calculated as the number of mortalities occurring within each time period group ($m_i$) over the total number of fish within that group alive at the beginning of each time period ($t_i$):

$$p_i = \frac{m_i}{t_i}$$

Mortality rates were non-normal and so were transformed before performing statistical analyses by taking the arcsine square root of the proportion of the number of mortalities ($m_i$) to the number fish alive ($t_i$) using correction values for the proportions to improve the normal fit of the resulting data (Anscombe 1948):

$$p' = \arcsin \sqrt{\frac{X + 3/8}{n + 3/4}}$$
Data from field studies were analyzed for Mill Creek, ME 2010-2011 and the Fore River, MA 2011-2012. Data from tagging completed at Mill Creek, ME in 2009 were excluded. This initial pilot year experiment was conducted to test field equipment, tagging methods, and ability of the RFID system to provide information about repeat spawning. Tagging did not begin in 2009 until after the peak of the run and selection of smelt for tagging did not follow length and gender protocols. As a result, the majority of smelt tagged in 2009 were age-2 males (Table 1.2). Further, the timer used to control the power source in 2009 was inadvertently set to turn the system off from midnight to 3am daily for the first week of the study. All analyses performed, including detection proportions and efficiency calculations, exclude data collected in 2009.

The performance of each RFID system was assessed by examining the proportion of tagged smelt that were detected, as well as the efficiency of the RFID systems in detecting and recording all of the movements by those fish that were detected. In theory, the RFID system should have detected each fish that received a PIT tag because: 1) all smelt were released upstream of the antenna arrays; and 2) the antenna arrays encompassed the entire stream cross-sectional area and the detection range of each

| Age Group | Fore River, Braintree, MA | | | | Mill Creek, Freeport, ME |
|-----------|--------------------------|-----------------|-----------------|--------------------------|
|           | 2011 | 2012 | 2009 | 2010 | 2011 |
| Female    |       |       |       |       |       |
| 1         | 20   | 40   | 17   | 22   |
| 2         | 68   | 72   | 39   | 73   |
| 3+        | 5    | 8    | 9    | 10   |
| Male      |       |       |       |       |       |
| 1         | 58   | 40   | 27   | 44   |
| 2         | 88   | 78   | 81   | 72   |
| 3+        | 8    | 22   | 14   | 26   | 1    |
| Grand Total | 247  | 260  | 143  | 112  | 222  |

Table 1.2. The number of tagged smelt at two spawning sites in the Gulf of Maine by gender, age and year tagged.
antenna filled the area within each. Therefore, the fish that were not detected either died upstream (but were never observed to descend the stream), or did in fact descend through the antenna arrays but were never detected. While half-duplex RFID systems have high detection rates when tuned properly, they are limited by their read rate (14 scans per second). If large numbers of tagged fish swam through an antenna simultaneously, the RFID would not have detected all movements. This was likely to occur during spawning runs where smelt, as schooling fish, migrated into the spawning grounds in large aggregations. This situation was also likely to occur when smelt were released as a group above the antenna arrays after being tagged, when many were likely to descend the stream together immediately after being released. The proportion of tagged smelt detected by the readers was examined using Chi Square analysis and the Pearson p-value statistic. Smelt that were not detected were excluded from all subsequent analyses (including efficiency analysis).

The efficiency of the RFID readers at each site and within each year was calculated for each detected smelt based on the number of actual antenna detections and the number of detections assumed to be missed by either an antenna or the reader. For example, if a fish were observed moving upstream (detected by first a downstream antenna and then an upstream antenna) and after a period of time was then observed by only an upstream antenna, a downstream detection was assumed to be “missed” because it was not likely for smelt to remain within the 10 m distance between the arrays. Similarly, if a fish was observed to move upstream one night, was not detected descending, but was again observed moving upstream the following evening, two
detections were assumed to be "missed": one at each upstream and downstream antenna during the un-detected downstream movement (Figure 1.5).

The resulting efficiency value (a percentage) is the proportion of actual detections over the number of assumed total detections (sum of assumed missed and actual detections). Efficiency values were non-normal and so were transformed by taking the arcsine square root of the proportion of actual to detections \((X)\) to the number of assumed total detections \((n)\) using correction values for the proportions to improve the normal fit of the resulting data (Anscombe 1948):

\[
p' = \arcsin \sqrt{\frac{X + 3/8}{n + 3/4}}
\]

Differences between average efficiency values were compared using one-way ANOVA on these transformed data. When differences were found using ANOVA, Each Pair Student's t tests were used to determine which pairs were significantly different.

Repeat spawn movements were identified by assessing the patterns, timing and frequency of upstream and downstream movements made by each tagged fish. Directional movement was identified by the sequence of detections on upstream and downstream antennas. A tagged fish was considered to have moved upstream when detections were made first on downstream antennas, and next on upstream antennas, and then no detections recorded for a period of time. Similarly, a downstream movement was identified when detections were recorded on an upstream antenna, then on a downstream antenna, followed by a period of no detections. A spawning movement was assigned when a single fish made an upstream movement, followed by a period of no detections for more than one hour, and then made a downstream movement. Continuous detections
Figure 1.5. Example of RFID detection data and identification of missed detections. The top panel (a) shows the actual detections of a single smelt (note that the smelt is observed to “miss” the downstream antenna on multiple occasions). The bottom panel (b) shows the assumed missed detections (X). Efficiency values were calculated as the number of actual detections to the number of assumed total detections (sum of actual and assumed missed).
by the antenna array were assumed to be passive movements and not spawning
migrations because no spawning habitat was within the antenna array area. In many
cases, spawning movements were easily identified as a series of 5-10 detections on the
downstream antennas followed immediately (within 2 minutes) by 5-10 detections on the
upstream antenna during night time hours (7 – 11pm), followed by a period of no
detections, and finally 5-10 detections on the upstream and then downstream antennas in
the early morning hours (3 – 6am). In no cases were tagged fish continuously detected
by antenna arrays at either site for longer than one hour. When continuous detections
occurred, the movement coincided with the incoming or high tide.

The average number of repeat spawning events was assessed first using standard
least square models, and second using one-way analysis of variance (ANOVA) tests if
significant differences were found within least square models. Data were first
normalized using a logarithmic transformation, where:

$$x' = \log_{10}(x + 1)$$

using the x+1 correction factor because some values were equal to zero (Bartlett 1947).
Although the sample size between age and gender groups were not equal, because larger
variances were associated with larger samples sizes the probability of a Type I error
(unnecessarily rejecting the null hypothesis) is less likely (Kohr and Games 1974) and so
the use of the ANOVA is not inappropriate (Zar 1999).

The difference between genders was evaluated using year and site within the same
model. The average number of repeat spawning events by age was evaluated for age-1,
age-2, and age-3+, where all smelt over age-3 (up to age-5 was encountered) were
grouped into the last category. Older ages are encountered infrequently in smelt runs, and sample sizes for ages 3, 4, and 5 were very small as separate groups. The difference between age groups was evaluated for each gender separately (as gender was found to be significant). Least square models were used to evaluate differences among age groups by site, combining all years' data at each site because there were not enough data points within the age-3+ group to evaluate differences by year. Where the least square models found significant differences within a variable (e.g. gender or age group), a one-way ANOVA and subsequent Each Pair Student's t test was performed to determine significant differences between pairs. For all tests, statistical significance was considered when $p < 0.05$. 
Results

Laboratory Studies

Mean mortality rates were compared between smelt tagged with a 23 mm PIT tag and control fish during three trials. The mortality rate measurements varied among the three studies for both control and tagged fish. Mortality rates at each time period were comparable between the study conducted at the ME DMR laboratory and the first trial performed at the MA DMF laboratory, however, mortality was considerably lower for both tagged and control fish during the second study performed at the MA DMF laboratory (Table 1.3).

While the mortality rate (number of mortalities over the number alive at the beginning of each period) was significantly higher for tagged fish during period-2 ($p = 0.043$), there was no significant difference in mean mortality between control and tagged fish during the other periods (period-3 $p = 0.645$; period-4 $p = 0.742$; period-5 $p = 0.393$; Table 1.3, Figure 1.6). During period-1, mortality was comparatively higher for tagged fish. While the difference was not significantly different at the $\alpha = 0.05$ level ($p = $...
0.056; Table 1.3, Figure 1.6), the difference may be biologically important. Comparing cumulative mean mortality, there was no significant difference observed between control and tagged fish (p = 0.444; two-tailed t-test; Table 1.3, Figure 1.6a).

Among tagged fish, mean mortality did not differ significantly between male and female smelt for any time period (two-tailed t-test; Table 1.4, Figure 1.6b). Mean mortality did not significantly differ among length categories for any single time period or cumulatively (one-way ANOVA; Table 1.5, Figure 1.6c). Although cumulative mortality seemed to decline with increasing fish length, the trend was not significant (adjusted $R^2 = 0.31$, p = 0.151, linear regression).

<table>
<thead>
<tr>
<th></th>
<th>Female</th>
<th>Male</th>
<th>Standard Error</th>
<th>p-value</th>
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</thead>
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<td>30.7%</td>
<td>± 0.24</td>
<td>0.483</td>
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<td>± 0.07</td>
<td>0.39</td>
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<td>0.0%</td>
<td>± 0.16</td>
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<td>15.8%</td>
<td>± 0.17</td>
<td>0.975</td>
</tr>
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<td>Period-5</td>
<td>17.2%</td>
<td>26.2%</td>
<td>± 0.15</td>
<td>0.635</td>
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<tr>
<td>Cumulative</td>
<td>68.9%</td>
<td>56.4%</td>
<td>± 0.28</td>
<td>0.607</td>
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</table>

Table 1.4. Mean mortality due to tagging during three laboratory studies for female and male smelt ±1SE with two-tailed t-test p-values comparing mortality between genders.

<table>
<thead>
<tr>
<th>Length</th>
<th>Period-1</th>
<th>Period-2</th>
<th>Period-3</th>
<th>Period-4</th>
<th>Period-5</th>
<th>Cumulative</th>
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<tbody>
<tr>
<td>&lt; 15 CM</td>
<td>57.2%</td>
<td>26.7%</td>
<td>16.7%</td>
<td>33.3%</td>
<td>35.7%</td>
<td>80.8%</td>
</tr>
<tr>
<td>15 - 20 CM</td>
<td>36.0%</td>
<td>5.9%</td>
<td>3.3%</td>
<td>11.1%</td>
<td>25.2%</td>
<td>59.3%</td>
</tr>
<tr>
<td>&gt; 20 CM</td>
<td>27.2%</td>
<td>8.1%</td>
<td>0.0%</td>
<td>9.8%</td>
<td>12.5%</td>
<td>47.0%</td>
</tr>
</tbody>
</table>

Table 1.5. Mean mortality due tagging during three laboratory studies for three length categories ±1SE with one-way ANOVA p-values for comparisons among length categories for five time periods and cumulatively.
Figure 1.6. Mean mortality ±1SE for (a) smelt tagged with 23mm PIT tags and control smelt within a laboratory setting for five periods and cumulatively, (b) male and female tagged smelt, and (c) compared among length categories for tagged smelt. Significant differences are indicated (*).
Of the 236 smelt tagged, only 2 tags were documented as not being retained, both in the first study performed at the MA DMF. It is possible that a small number of tags were shed during the study performed at the ME DMR, but these went unrecorded when department personnel cleaned the tanks. Considering the MA study only, the two lost tags represent a 2.2% loss of all fish tagged (90).

Field Studies

The performance of each RFID system was assessed by examining the proportion of tagged smelt that were detected and the efficiency of the RFID systems in detecting and recording all of the movements by those fish that were detected. Not all smelt that received PIT tags were detected by the systems, however 100% detection of tagged fish was not expected because limited read rates by the half-duplex system as explained in the Methods section. The proportion of tagged smelt detected by the system differed significantly between the two sites: Mill Creek, Freeport, ME, and the Fore River in Braintree, MA (ME = 82.0% detected, MA = 88.6% detected, p = 0.008), but did not differ significantly by year within each site (MA: 2011 = 88.3%, 2012 = 88.9%, p = 0.836; ME: 2010 = 85.7%, 2011 = 80.2%, p = 0.214). It was important to establish whether the proportion of detected fish differed by gender and age group because subsequent analyses assessed the difference between the numbers of repeat spawning events for these variables. Males were detected significantly more than females (M = 89.0%, F = 81.9 %, p = 0.003), but detection rates did not vary significantly between age groups (age-1 = 85.1%, age-2 = 87.6%, age-3 = 80.8%, p = 0.187; Chi Square test of proportions, Pearson statistic for all preceding tests of detection proportions).
The efficiency of the RFID readers was calculated for each detected smelt based on the ratio of the number of detections made to the number of detections assumed to be missed by the reader. Efficiency of the RFID systems did not differ significantly between sites (ME = 82.9% of movements detected, MA = 80.1%, \( p = 0.181 \)). The efficiency did not differ significantly at either site between years (MA 2011 = 82.9%, 2012 = 77.3%, \( p = 0.110 \); ME 2010 = 82.0%, 2011 = 83.4%, \( p = 0.418 \)). It was important to establish whether the proportion of detected fish differed for gender and age group because subsequent analyses assessed the difference between numbers of repeat spawning events for these variables. Efficiency values did not differ by gender (M = 81.5%, F = 80.5%, \( p = 0.171 \)), or among age groups (age-1 = 85.6%, age-2 = 80.4%, age-3 = 72.0%, \( p = 0.055 \), one-way ANOVA).

The difference in the number of repeat spawn events was examined by gender to determine if males visited the spawning grounds more frequently than females. Females were observed returning up to 5 times, while males returned up to 10 times within a single spawning season (Table 1.6). Further, a higher proportion of males made one or more repeat spawn trips (M = 80.2%, F = 62.1%). The difference between genders was even greater when comparing the proportions that made two or more repeat spawn trips (M = 52.2%, F = 16.7%). This second comparison may be more reflective of normal

<table>
<thead>
<tr>
<th></th>
<th>Fore River, MA</th>
<th>Mill Creek, ME</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2011</td>
<td>2012</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>Avg. No. Returns (Range)</td>
<td>0.86  (0-4)</td>
<td>2.38  (0-10)</td>
</tr>
</tbody>
</table>

Table 1.6. The average number (and range) of within-season spawning movements is shown for each study year and location. Females were observed returning up to 5 times, while males returned up to 10 times within a single spawning season.
spawning behavior. Of the females that returned only one time, most (74.8%) made this return the night following being tagged. Considering all fish that returned one or more times, the majority of both females (76.8%) and males (78.5%) made their first return the night following tagging. The first return may be a result of the capture/tagging interrupting the normal spawning behavior.

The average number of repeat spawn events was significantly higher for males than females at both sites (M = 2.07, F = 0.84, p < 0.0001), but a significant difference also occurred between study years (p = 0.018, standard least squares). Further analysis showed that the average number of repeat spawn events did not differ between any years for males (2010ME = 2.15, 2011MA = 2.38, 2011ME = 1.72, 2012MA = 2.02, p = 0.250, one-way ANOVA). For females, the average number of repeat spawn events was higher during 2012 at the MA site compared to both sampling years in Maine, but was not different from the 2011 MA sampling year (2010ME = 0.74, 2011ME = 0.58, 2011MA = 0.86, 2012MA = 1.18, p = 0.003, one-way ANOVA, Each Pair Students t; Figure 1.7).

![Graph showing the average number of repeat spawn events for male and female smelt at two study sites over three years.](image)

Figure 1.7. The average number of repeat spawn events (±1SE) for male and female smelt at two study sites over three years.
The average number of repeat spawn events did not vary significantly by age group (age-1 = 1.58, age-2 = 1.56, age-3+ = 1.81, p = 0.425) and there was no significant interaction between age and site (p = 0.119, standard least squares; Figure 1.8a). The frequency of repeat spawning was also analyzed by age for each gender. Data were analyzed for all years combined, and combining older ages into a single age-3+ group because few older age smelt (age-3 and up) are encountered during the spawning run at the two study sites. For females, the average number of repeat spawning events did not differ significantly between age groups (p = 0.112; Table 1.7) and no interaction occurred between site and age group (p = 0.991, standard least squares; Figure 1.8b). For males, the average number of repeat spawn events differed significantly between combined age groups (p = 0.043; Table 1.7), but there was also a significant interaction between age and site (p = 0.017, standard least squares). Further analysis found that the average number of repeat spawn events by ME age-3 (2.77) and MA age-2 (2.41) male smelt was significantly higher than ME age-1 (1.84), MA age-1 (1.83), and ME age-2 smelt (1.67); MA age-3 smelt did not differ significantly from either group (avg. rs = 2.24, p = 0.005, one-way ANOVA, Each Pair Student’s t; Figure 1.8c).

<table>
<thead>
<tr>
<th></th>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age-1</td>
<td>1.11</td>
<td>1.83</td>
</tr>
<tr>
<td>Age-2</td>
<td>0.82</td>
<td>2.17</td>
</tr>
<tr>
<td>Age-3</td>
<td>0.66</td>
<td>2.47</td>
</tr>
<tr>
<td>Standard Error</td>
<td>± 0.05</td>
<td>± 0.09</td>
</tr>
</tbody>
</table>

Table 1.7. Mean repeat spawn events by male and female smelt from two study sites and three sampling years.
Figure 1.8. The average number of repeat spawning events (±1SE) for (a) all tagged smelt, (b) females, and (c) males.
Of the fish that were tagged, 10 male smelt (1.0% of all tagged fish) were observed to return in the year following their initial tagging event. During this second year of detection, these fish made on average 3.1 within-season repeat spawn movements. Comparatively, male smelt that were detected in the same year as they were tagged made on average 2.2 within-season repeat spawn movements. The difference in the number of within-season repeat spawn events between these groups, however, was not significant (p = 0.111, one-way ANOVA).

Within each study year, smelt were not tagged on a single date but throughout the run as described in the Methods section. Considering fish tagged during week-1, -2, etc. of the spawning period, the average number of repeat spawn events declined significantly as the tagging periods progressed (adj $R^2 = 0.078$, p < 0.0001; Figure 1.9). Smelt that were tagged the first week of the spawning run returned on average 2.2 times, while smelt tagged in the middle of spawning period (week-4) returned 1.3 times, and those tagged at the end of the period (week-8) returned on average 0.4 times (study years and sites combined). The pattern was influenced by male behavior only. The mean

\[ y = -0.0442x + 0.4676 \]

\[ \text{adj. } R^2 = 0.0778 \]

Figure 1.9. The mean number of repeat spawn events (log normalized) compared to the study week in which smelt were tagged.

39
number of repeat spawn events did not decrease through time when only females were considered (slope = -0.0013, \( p = 0.8550 \)), but did decrease through time for males (slope = -0.0617, \( p < 0.0001 \)).

Continuous data collection made it possible to assess patterns of spawning migrations with diel and tidal cycles. All spawning movements were made during nighttime hours, where the majority of upstream movements occurred from 6pm to 11pm, and downstream movements from 3am to 6am (Figure 1.10). Daytime movements were observed 8 times and were characterized by continuous detections on or near the daytime high tide time, and were not considered spawning movements. While the majority of the spawning movements were made with the incoming tide (70.9%), upstream movements were also made during the ebb tide. At the Fore River in 2012, 35.0% of upstream movements made to the spawning grounds during nighttime hours were made against the tide or when the tide was low. At Mill Creek this pattern was also evident in both study years where 31.9% (2010) and 20.8% (2011) of assumed movements were made against the tide (Table 1.5).

<table>
<thead>
<tr>
<th>Year</th>
<th>Nighttime Movements with the Rising Tide</th>
<th>Nighttime Movements Against the Tide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fore River, MA</td>
<td>65.0% (n = 169)</td>
<td>35.0% (91)</td>
</tr>
<tr>
<td>2010</td>
<td>68.1% (49)</td>
<td>31.9% (23)</td>
</tr>
<tr>
<td>2011</td>
<td>79.2% (99)</td>
<td>20.8% (26)</td>
</tr>
<tr>
<td>Total</td>
<td>70.9% (378)</td>
<td>29.1% (155)</td>
</tr>
</tbody>
</table>

Table 1.8. The number of nighttime movements to the spawning grounds made with the tide and against the tide is shown by year where calculated. Percentage of all movements is first shown followed by the number of movements (n).
Figure 1.10. The average frequency of PIT tag detections recorded by the RFID system at Mast Landing, Freeport, ME 2010-2011 (±1SE) at each daily hour.
Discussion

This study revealed differences in within-season repeat spawning behavior of rainbow smelt by age and by gender. The study also provided information about the mortality and retention of PIT tags by rainbow smelt, and the ability of large-scale RFID systems to continuously detect smelt movements. Further, continuous monitoring enabled movements to be compared to diel and tidal cycles. We found that repeat spawning is largely a male behavior that was observed at the same rate at two sites over multiple years. Movements into and out of the spawning grounds were found to occur primarily with the night-time flood tide and the morning ebb tide, respectively.

Understanding what drives the catch composition is key to correctly interpreting data from surveys performed during the spawning run. The results from this study are consistent with findings of previous studies: there is ample evidence to suggest that the highly skewed sex ratio (male biased) during rainbow smelt spawning runs is a factor of the behavior by males that return to the spawning grounds multiple times (Langlois 1935, Hoover 1936, McKenzie 1964, Rupp 1968, Clayton 1976, Murawski et al. 1980). In a study of freshwater smelt, Rupp (1968) noted that individual males in Branch Lake, ME participated in spawning up to eight times, while females were found returning only three to four times. In the Parker River, MA, Murawski et al. (1980) found that while 2.3 – 9.5% of tagged females were recaptured on the spawning grounds, no female was recaptured more than once. In comparison, 13.0 – 24.7% of tagged males were recaptured on the spawning grounds and individual males were recaptured up to four
times. Past studies have been limited to a single river system, however, and have not considered whether similar trends occur between spawning sites. This study provided similar results at two sites: anadromous male smelt were observed to return up to ten times in the Fore River, MA, and up to eight times in Mill Creek, ME, while females returned less often (up to 5 times; Table 1.6). This study also determined that for male smelt, the average rate of repeat spawning (2.07) was consistent among multiple years and multiple sites (Figure 1.7).

The consistency in the repeat spawning rate is important when considering the difference in sex ratios recorded for smelt populations of different sizes within the Gulf of Maine. Standardized regional spawning surveys performed by state fisheries agencies in Massachusetts, New Hampshire, and Maine during 2008 – 2012 found that the sex ratio varied between 1.5:1 (M:F) at Schoppee Brook in Jonesboro, ME and 7.7:1 at the Saugus River in Saugus, MA. During the same period, the average catch-per-unit-effort (CPUE) at Schoppee Brook was 37.83 smelt/haul (the second highest of 17 surveyed sites), while the average CPUE at the Saugus River was 2.76 smelt/haul (the fifth lowest). At the sites where tagging studies were completed, a similar pattern was found where the average sex ratio was lower at Mill Creek, ME (2.7:1) compared to the Fore River, MA (4.0:1), while the CPUE value was slightly higher at Mill Creek (26.11 smelt per haul) compared to the Fore River (20.42 smelt per haul; Enterline et al. 2012a). Despite the difference in sex ratios between sites, the results of this study indicate that the rate of repeat spawning was consistent between sites (Figure 1.7). The difference between sex ratios among the regional survey sites may then indicate that fewer females are present in
less abundant runs compared to those with higher CPUE values. Populations dominated by males may be less robust than those containing a higher proportion of females because the limiting factor for population growth is often the abundance of females.

It should be noted, however, that the standardized spawning survey methods may favor the capture of males. Catch efficiency trials performed by the MA DMF at the Fore River, MA used a large net placed upstream from the standardized fyke net to block the entire river channel and catch all fish that escaped capture in the smaller survey net. Weekly trials performed during two years confirmed that female smelt were more likely to escape capture in the smaller survey net as evidenced by more even sex ratios in the efficiency net catches compared to the survey net catches (M. Ayer, MA DMF, pers. comm). Although the sex ratios resulting from the standardized fyke net surveys may be biased, they are still comparable between sites under the assumption that each site’s fyke net allows the females to escape at a similar rate. Future field studies should compare the catch efficiency among survey sites.

The conclusions regarding the average number of repeat spawn events may also be limited by tagging methods and timing. Tagging occurred throughout the spawning period at each site, and any individual smelt may have made multiple visits to the spawning grounds before it was tagged. The return rate for fish tagged at the beginning of the run was higher than for fish tagged later in the run (Figure 1.9). This trend may be reflective of tagging methods. While tagging was stratified by gender and age weekly, striving for an equal number of males and females from each age group, the actual number of smelt tagged in each category varied based on fyke net catches. In the
beginning of the smelt run, more males are present compared to females. As the spawning run progresses, the sex ratio approaches 1:1. In each year at both study sites, the male to female tagging ratio was higher earlier in the run, varying between 2.2 – 4.3:1 (M:F). As the run progressed, the male to female tagging ratio evened out, varying between 0.9 – 1.5:1. The trend of decreasing return rates over the run may be partly due to tagging methods because more males were tagged at the beginning of each season, and because males were found to have a higher return rate compared to females (Figure 1.7).

The trend of decreasing return rates over the spawning season can further be investigated through the behavior of smelt that returned in the year following their initial tagging event. During this second detection year, the complete spawning behavior was detected without interruption. Ten male smelt were observed in the year following their tagging event, and these fish made on average of 3.1 within-season repeat spawn events during this second year. Comparatively, male smelt detected and tagged within the same year made on average 2.2 within-season repeat spawn events. The difference in the number of within-season repeat spawn events between these groups was not significant (p = 0.111), however, the sample size of the second year returning smelt was very small (10) and a larger sample size may improve the comparison. It is possible, then, that study results under-estimate the number of total repeat spawn trips completed by individuals tagged later in the run.

Passive Integrated Transponder (PIT) tags were used in this study to determine the rate of repeat spawning at the study sites. Corollary laboratory studies found that the retention of 23 mm PIT tags was high (2 shed tags among 90 tagged smelt). While
mortality during the second week following tagging was significantly higher for tagged fish compared to control fish in the laboratory studies (Figure 1.6a), mortality did not differ by gender (Figure 1.6b). The field results showing a higher return rate for males and the laboratory studies indicate that these results are likely not a result of disproportionate female mortality following the tagging event. The percentage that were never detected by the RFID systems was significantly higher for females (18.1%) than for males (11.0%), however, the efficiency of the system in detecting all of the movements made by those fish that were detected did not vary between males (81.5% of all movements detected) and females (80.5%). The difference between genders in the detection proportions may be due to the unlikelihood of females to repeat spawn. After the first attempt at spawning and the tagging event, some females may have simply left the system and not returned, while males displayed repeat spawning behavior. The difference in detection proportions (while efficiency rates remained equal) therefore supports the evidence of higher repeat spawning rates for males compared to females.

This study also investigated the rate of repeat spawning in relation to age. The occurrence of repeat spawning may over- or under-estimate mortality if the rates are unequal between ages, because mortality estimates are calculated based on the percent survival of an age cohort through time (Haddon 2011). At both sites, the average number of repeat spawn events did not vary by age for females (Figure 1.8b). The frequency of repeat spawning by male smelt at different ages was not consistent between sites, but in both cases there was a trend of age-2 and age-3 fish returning more times than age-1 fish (Figure 1.8c). While the trend is not consistent, it indicates that at some sites, mortality
may be under-estimated for older smelt if estimates are calculated based on male catches during the spawning run. Mortality estimates performed using catch data for both genders combined may hold no bias, as results indicated that there was no difference in repeat spawn rate by age using combined data (Figure 1.8a).

Both laboratory and field studies found no significant difference or trend in mortality due to tagging among three length categories corresponding to age-1, age-2, and age-3+, although a trend of decreasing mortality with increasing size was apparent in the laboratory studies (Figure 1.6c). In laboratory trials, the majority of mortality occurred within the first few days, thus the proportion of smelt tagged at the study sites that were never detected by the RFID system may be a good proxy for estimating field mortality. The proportion of tagged smelt detected by the RFID systems did not differ significantly among age groups (age-1 = 85.1%, age-2 = 87.6%, age-3 = 80.8%), therefore differences in mortality among age groups are likely not a factor.

Continuous data collection made it possible to assess patterns of spawning migrations with diel and tidal cycles. Results from this study confirm the assumed pattern of nighttime spawning (Bigelow and Schroeder 1962; Murawski et al. 1980) because all movements made to the spawning grounds occurred during dark hours, or in the hours approaching darkness. Daytime observations were consistent with either movements of smelt descending from the spawning grounds after spending nighttime hours on the grounds, or slow passive drifting during daytime high tides. The latter case was observed only eight times, and was differentiated from active swimming movements by the detection patterns. Passive movements where characterized by continuous detections
recorded before, during, and directly following the daytime high tide. In contrast, active swimming movements were identified when smelt were detected by each antenna, with time lags in detections that indicated smelt were spending time above the RFID system.

Nighttime spawning events did not always coincide with nighttime high tide, in contrast to previous studies (Clayton 1976; Murawski et al. 1980). At the Fore River, MA (2012), 35.0% of upstream movements made to the spawning grounds during dark hours were made against the tide or when the tide was low. At Mill Creek, ME this pattern was also evident in 2010 (31.9%) and 2011 (20.8%; Table 1.8). This result may have implications when considering stream connectivity and access to spawning grounds under all conditions. The majority of smelt spawning streams in the U. S. Gulf of Maine are small coastal streams that are crossed by at least one road. Road crossings can impair smelt spawning migrations when culverts become perched, i.e. stream height is below culvert height. In Maine, there is an ongoing effort to ground-survey all stream barriers. At the time of this paper, 35% of the state has been surveyed. Of the 88 historical or current smelt spawning sites falling within this surveyed portion, 34 (39%) sites have potential barriers to passage. Extending the scope to the entire state, 127 historical or current spawning sites out of a total of 275 are crossed by roads at least once, and multiple times in many cases. While some of these crossings may have adequate passage under all tidal conditions, it is estimated that two-thirds of these crossings may present passage problems for smelt (A. Abbott, USFWS, pers. comm., 2012).

The use of 23 mm PIT tags in rainbow smelt provided beneficial information about repeat spawning behavior. Despite the high mortality rate during the first week
following tagging in laboratory trials, field mortality may have been lower. Of the 868 smelt that were detected by the RFID system, 602 (71.8%) were observed to return one or more times. Further, 78.3% of first returns were made the night that the fish was tagged, indicating that the act of capturing and tagging the smelt did not disrupt the normal spawning behavior. It is also important to note that mortality rate measurements varied among the three laboratory studies for both control and tagged fish. While mortality rates were comparable between the study conducted at the ME DMR laboratory and the first trial performed at the MA DMF laboratory, mortality was considerably lower for both tagged and control fish during the second study performed at the MA DMF laboratory (Table 1.3).

The higher mortality rates during the first two studies were likely a factor of the laboratory environments rather than tagging as described in the Methods section. Tank environment and water quality may have increased mortality during the ME DMR study and the first MA DMF study. It is likely, therefore, that the mortality rates recorded during the final laboratory study are most reflective of mortality due to tagging. As no replicates were performed within each study, it is not possible to assess the differences between control and tagged smelt using the data from the final study only. Considering point measurements from the final study, mortality rates were fairly even between control and tagged fish both cumulatively (control = 25.0%, tagged = 30.2%), and during the first week (control = 6.7%, tagged = 11.1%) when the majority of mortality was observed in the other two studies. Incorporating the mortality due to tagging from this final study to the field studies, if an average of 85.3% of smelt tagged in the field were never detected
by the RFID system it is possible that ~11% of the undetected 14.7% died as a result of tagging.

Studies using 23 mm PIT tags may provide some information for long-term movement patterns of rainbow smelt, as a small percentage (1-4%) of tagged smelt in this study were observed returning to the system the following year. Smaller PIT tags (ex. 12.0 x 2.12 mm, 0.1 g air weight) may cause less mortality in smelt, however, these tags were not commercially available for half-duplex RFID systems at the time this study was conducted. It should also be noted that smaller tags would not be appropriate for use in large-scale RFID systems with the antenna dimensions described in this study because the power distribution of the RFID systems would be too dispersed to detect tags smaller than 23 mm (A. Haro, USGS Conte Anadromous Fish Branch, pers. comm).

Half-duplex rather than full-duplex RFID systems were used in this study because of the former’s ability to collect data in tidal waters that are subject to changes in salinity, flow, and antenna shape. Moreover, full-duplex systems have a smaller detection range compared to half-duplex systems and thus were not appropriate for the study sites where large-scale installations were required (Figures 1.2b and 1.3b). Full-duplex systems can be advantageous because they are able to detect tags continuously, while half-duplex systems send out listening “pulses” that charge the tags, and then pause and “listen” for tag responses. Full-duplex systems can thus be more efficient, but were deemed inappropriate for the study locations. Detection rates and efficiency values for the two studies indicated that our systems had a relatively high performance rate (Fore River average detection rate = 88.6%, Mill Creek = 82.0%; Fore River average efficiency value
Detection and efficiency rates of, or approaching, 100% are not expected because the RFID system is only able to detect one tag at a time. Although the half-duplex system can detect tags at high rate (14 scans/second) only one tag can be detected during each scan. Thus, efficiency and detection rates above 80% indicate that a system is performing very well (A. Haro, USGS Conte Anadromous Fish Branch, pers. comm).

In conclusion, this study demonstrated that repeat spawning by rainbow smelt is a predominantly male behavior, that the occurrence of the behavior does not differ by age when both genders are considered, and that the use of PIT tags may be a useful technology when continuous monitoring of rainbow smelt is desired. The results of this study were consistent between two spawning sites, suggesting that differences in sex ratios in different spawning populations may be due to limited numbers of females in the spawning population and not differences in male spawning behavior. Future studies should explore whether the consistency between male spawning rates holds for other sites, and whether stressed or declining smelt runs may be limited by the number of spawning females.
CHAPTER II

MOVEMENTS AND HABITAT USE BY RAINBOW SMELT WITHIN AN EMBAYMENT DURING AND FOLLOWING THE SPAWNING PERIOD

Introduction

Identification of highly utilized habitat is central to effective species management. Degradation of important habitats or harvesting during vulnerable life history phases, including spawning (Lawson and Rose 2000) and larval development (Beck et al. 2001), can lead to, or exacerbate, population declines (Langton et al. 1996, Bigford 2013). Where these relationships are understood, management plans include the locations of important habitats and directives for their protection (e.g. ASMFC 2010), classification of critical habitat for endangered species (Sabesan et al. 2008), and recommendations for research and restoration funding (Chatwin and Charles 2012).

Diadromous species have experienced severe declines due to obstructed access to habitat, habitat alteration, and over-exploitation due to directed harvest or bycatch effects (Limburg and Waldman 2003, Jelks et al. 2008, Courmane et al. 2013). Rainbow smelt (Osmerus mordax) have supported culturally important commercial and recreational fisheries throughout New England since at least the 1800s (Kendall 1926). Historically, the range of rainbow smelt extended from Chesapeake Bay to Labrador (Buckley 1989, Kendall 1926), but over the last century the range has contracted, and smelt are now only
found east of Long Island Sound and spawning populations may be reduced to areas north of Narragansett Bay (Keller et al. 1999, Gottschall et al. 2000, Oviatt et al. 2003, Fried 2006). Researchers have suggested that factors responsible for smelt population declines may include spawning habitat degradation (Murawski and Cole 1978; Chase and Childs 2001; Wyatt et al. 2010), stream obstructions (Chase and Childs 2001), declines in water quality (Chase and Childs 2001; Fuda et al. 2007; Geffen 1990), and fishing pressure (Flagg 1983, Brown and Taylor 1995).

Initial descriptions of annual adult rainbow smelt movements were largely based on known spawning run locations and recreational and commercial catches. Migration patterns were described in a review paper by Kendall (1926), expanded upon by Bigelow and Schroeder (1953), and later summarized with few additions by Buckley (1989). From these accounts, it has been assumed that adult smelt migrate in search of optimum water temperatures, moving offshore during the summer months in search of cooler water (Buckley 1989). In the early spring, smelt spawn in the freshwater portions of coastal streams and rivers, often close to the upper extent of tidal waters (Kendall 1926). Across their geographic range, anadromous smelt spawning runs are variable in spawning period and water temperature range (Bigelow and Schroeder 1953, Hulbert 1974, Kendall 1926, Pettigrew 1997). Water temperature at the beginning of runs varies from 1.5 to 9°C, and most runs span a three to six week period (Chase 2006, Enterline et al. 2012a).

Adults are presumed to return to estuaries and coastal waters immediately after spawning based on low catches by fishermen in freshwater and larger catches in brackish and salt water in May and June, the presumed end of the spawning run (Buckley 1989).
Fishermen reported that smelt stay in near-shore coastal waters through the summer, not straying any further from shore than a distance of 2 km, or to depths greater than 6 m (Bigelow and Schroeder 1953). In contrast, recent trawl surveys found smelt as far from the coast as 50 km and in depths of 18 m (Sherman S, ME DMR, pers. comm.).

Smelt movements during the spawning season have been explored through mark and recapture studies. Murawski et al. (1980) documented limited movements by adult spawning smelt between tributaries of the Parker River, MA estuary system during a single spawning season. They hypothesized that movement in different streams may be facilitated by passive tidal transport, however, this has not been directly observed. Freshwater smelt were documented to stray between multiple spawning locations up to 6.4 km apart in a single lake (Rupp 1968). In contrast, McKenzie (1964) found little straying of marked fish between runs occurring in early spring and late spring on the Miramichi River, New Brunswick, Canada.

Several methods have recently been used to identify important diadromous species habitat. Icthyoplankton tows identified likely spawning locations (Dodson et al. 1989), and otolith microchemistry and daily growth rings have provided information about natal location, and marine vs. freshwater residence time (Limburg 1996). Movement patterns, aggregations of individuals, and temporal habitat use were described by using data collected from hydroacoustic and radio telemetry studies (Russell et al. 1998, Hightower and Sparks 2003, Dionne et al. 2013). Results of these studies have informed critical habitat designation (Shortnose Sturgeon Status Review Team 2010) and habitat restoration directives (ASMFC 2010).
The present study used hydroacoustic telemetry technology to track the movements of rainbow smelt in the Great Bay and Piscataqua River estuary complex in New Hampshire (NH). I hypothesized that adult spawning smelt would make use of multiple spawning rivers that are tributaries to Great Bay. Further, I tested the hypothesis that post-spawn smelt remain the estuary and embayment during the summer months, rather than immediately migrating into coastal waters. Hydroacoustic arrays monitored movement into and out of coastal waters via the Piscataqua River, movement between major spawning rivers, and areas of frequent use within the estuary. The primary objectives of this study were to: 1) monitor smelt movement between riverine, estuarine and marine habitats during, and following, the spawning season; 2) assess relative use of each habitat area; and 3) determine whether smelt stray between multiple spawning locations within a spawning season.

Methods

Laboratory Studies

A preliminary laboratory study was conducted March 8 – June 20, 2011 to compare retention and mortality of three hydroacoustic transmitter types: JSATS AMT designed for tagging juvenile salmon (0.3 g, 11.2x5.1x2.9 mm, 118 day life, Lotek Wireless, Inc., Newmarket, Ontario), VEMCO V6 (1.0 g, 16.5x6.3 mm, 351 day life on low power, Bedford, Nova Scotia), and VEMCO V5 (<1.0 g, ~13x5 mm, 351 day life on low power, Bedford, Nova Scotia). For each group, “dummy” tags, that did not transmit, were used.
Smelt were collected during January – February, 2011 during annual creel surveys on the Kennebec River, ME and from the Squamscott River, NH. Smelt were collected using hook and line through the ice. Commercially available small hooks (size 10-12) on monofilament fishing line were baited with small (~3 mm long) pieces of fresh bloodworm (*Glycera spp.*). Upon capture, smelt were immediately placed into a holding cooler (28 qt Coleman® Marine Cooler, Goldman, CO) containing river water oxygenated with a battery operated aerator (Bubbles® Bait Bucket Aerator, Marine Metal Products, Clearwater, FL). Within several hours, smelt were transferred to the Aquaculture Research Center laboratory at the University of NH, Durham, NH, where they were held for one to two weeks for acclimation prior to the start of the study. Fifty-two smelt were equally divided between two 460 gallon polyethylene round tanks (70” x 30”, Pentair Aquatic Eco-systems, Inc., Apopka, FL). Water was from the Piscataqua River (transported by truck) was UV sterilized and filtered (5 microns) prior to use. Salinity varied between 18-22 ppt. Water temperature was held constant between 9-16°C by a drop-in chiller (Aqualogic® Cyclone® drop-in titanium chiller, San Diego, CA). Dissolved oxygen was recorded daily and varied between 8-12mg/L.

After the acclimatization period, smelt were removed individually from the tanks and anesthetized with 200 ppm methanesulfonate (MS 222, Finquel®, Redmon, WA). Smelt were measured to the nearest millimeter (TL) and gender determined by the expression of milt or eggs with slight ventral massage. Gender was difficult to determine in some cases because many smelt were not in spawning condition, in these cases, no gender was assigned. A total of 26 smelt were placed into two study tanks as controls (13
in each), 10 smelt received a JSATS tag (5 in each tank), 10 received V6 tags (5 in each tank), and 6 received V5 tags (3 in each tank; Table 2.1). The V5 group was smaller because the distributor had limited tags of this type.

Tags were sterilized with commercially available isopropyl (99% isopropyl rubbing alcohol) and gently inserted into a small incision (<1 cm) made with a hooked scalpel in the peritoneal cavity directly posterior to the pectoral fins. Vetbond™ Tissue Adhesive (n-butyl cyanoacrylate, St. Paul, MN) was used to seal the wound. Each smelt with a tag also received a visible implant elastomer mark (Northwest Marine Technology, Inc., Shaw Island, WA) in the operculum for the purpose of visual identification, with the exception of 5 smelt receiving JSAT tags. These first 5 smelt received a 20mm floy (T-bar) tag (Hallprint, Hindmarsh Valley, Australia) directly posterior to the dorsal fin. The use of Floy tags was stopped after these first 5 fish because the tagging process caused a large wound in the dorsal musculature.

<table>
<thead>
<tr>
<th>Length</th>
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<th></th>
<th></th>
<th></th>
<th>Tank 2</th>
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<tbody>
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<td>JSATS</td>
<td>V5</td>
<td>V6</td>
<td>Control</td>
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<td>5</td>
<td>13</td>
<td>5</td>
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<td>5</td>
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</tbody>
</table>

Table 2.1. Length distribution of smelt used for hydroacoustic transmitter mortality and retention studies.
Smelt were held in two study tanks with the specifications described above. Tanks were monitored daily and any mortalities removed. Tag type, fish length (TL mm), and tank number were recorded for all mortalities. Feed was withheld for the first eight weeks of the study, after which commercially available fish feed pellets (3mm, Burris, Franklinton, LA) were offered three times a week.

**Field Studies**

The Great Bay and Piscataqua river estuary system is formed by a 17.4 km² sheltered tidal estuary (Great Bay) and approximately 7.6 km² tidal embayment that flows into marine waters through the Piscataqua River at Portsmouth, NH (Figure 2.1). Seven major rivers drain into this estuary complex: the Winnicut (watershed size = 104.6 km²), Squamscott (388.5 km²), Lamprey (554.1 km²), Oyster (79.4 km²), Bellamy (87.7 km²), Cocheco (479.8 km²), and Salmon Falls (568.7 km²). The confluence of the Cocheco and Salmon Falls rivers is located upstream of the tidal embayment – together these two rivers form the Piscataqua River. Rainbow smelt spawning has been documented within each of these river systems within the five years of this study (Enterline *et al.* 2012c). Land cover in the watershed encompassing Great Bay and the seven contributing rivers is predominantly forested (62.9%), with wetlands (9.8%), agriculture and land cultivation (9.8%), and developed land (8.3%) accounting for the majority of the remaining area.

Movement between riverine, estuary and marine habitats in the Great Bay and Piscataqua River estuary system was monitored using fourteen (2011) and fifteen (2012) VEMCO VR2W-180kHz hydroacoustic receivers (VEMCO, Bedford, Nova Scotia)
placed in six tidal rivers, two geographic chokepoints, and two locations at the confluence between estuarine and marine habitats. This arrangement allowed detection of smelt moving in and out of all spawning rivers and movements into and out of coastal waters at Portsmouth Harbor (Figure 2.1). Most receivers were placed before the initial tagging event each year (April in 2011 and March in 2012) and left in place until December in 2011 and August in 2012. The exception was the receiver placed in Little Harbor, which was left in place from April 2011 through August 2012. The receivers were removed earlier in 2012 because the battery life of the tags was shorter in that study year.

The placement of the receivers in 2011 was replicated in 2012 with the following exceptions: 1) only one receiver was placed in the Oyster River in 2012; 2) a receiver was placed in the Winnicut River; 3) only two receivers were placed at the General Sullivan Bridge area compared to three in 2011, and these two were placed in slightly different locations to minimize the distance between them; 4) a receiver was placed in the Piscataqua River at the Schiller Power Station starting in December 2011, and remained at this fixed location through the end of the study; and 5) receivers at the mouth of the Piscataqua River were placed approximately 3.5 km upstream of their 2011 locations in an effort to minimize their exposure to high flows (Figure 2.1a and 2.1b).

In 2011, four receivers were lost: two at the General Sullivan Bridge (last data downloads on June 20, 2011), and two at the mouth of the Piscataqua (last downloads April 27 and July 21, 2011). In 2012, two receivers were lost: one at the General
Figure 2.1. Deployment locations of 14 (2011, panel a) and 15 (2012, panel b) VEMCO VR2W-180kHz hydroacoustic receivers set to monitor the movement of smelt tagged with acoustic transmitters between riverine, estuary and marine habitats in the Great Bay and Piscataqua River estuary system, NH.
Sullivan Bridge (last data download May 25, 2012), and one at the mouth of the Piscataqua River (lost on deployment, no data uploaded).

Receivers were fixed to nylon line using six 11” cables ties (50 lb tensile strength, 3M™, St. Paul, MN) so that the listening end of the receiver was facing up in the water column, and positioned on the line to be approximately 5’ off the bottom substrate. Each line was anchored to the bottom substrate using two lead bricks (30 kg each) coated in rubber, attached to the line using eyebolt screws. Also anchoring the bottom of each line was a 25 lb Danforth ® Standard anchor (Atlanta, GA) attached using a shackle connected to a 5’ length of ½” galvanized chain that was in turn attached to the line. The line was buoyed on the surface using either a lobster buoy with a 2 ft toggle (all locations in 2011, spawning river locations in 2012) or 15” x 20” inflatable buoys (Adams Point, General Sullivan, and Piscataqua Mouth locations in 2012). Small (4” x 6”) buoyant toggles were fixed on the line ~1 ft above the receiver as well as ~10 ft below the surface buoy.

The range of the 180 kHz VR2W VEMCO receiver was tested at a location close to Adams Point. A receiver anchored as described above was placed and GPS marked. A V6 transmitter was fixed to a weighted line and placed in the water for 15 minutes at approximate distances of 50, 100, 150, 200, 250, 300, and 350 m away from the receiver. The depth of the transmitter could not be fixed because of strong tidal flow.

Smelt were collected as part of the regionally standardized rainbow smelt survey performed by NH Fish and Game Department (FGD) during annual spring spawning runs on the Oyster and Squamscott rivers, New Hampshire (March to May) using large fyke
nets placed directly beneath the spawning grounds (fyke nets: mouth 4’x4’ box; 11’ approximate length from mouth to cod end; three chambers; first throat tapered to 5”; second throat tapered to 3”; 2.5’ diameter hoops; wings 4’x4’ box on each side with 12’6”x4’ soft wing extensions; Memphis Net and Twine, Memphis, TN; Figure 1.1). The fyke nets were set at mid-channel in the intertidal zone below the downstream limit of smelt egg deposition. Nets were set three days each week and hauled during the morning low tide after overnight sets from the first week of March until the second week in May. The fyke net opening faced downstream to intercept the spawning movements of smelt that occur at night during the flood tide. Fyke net catches were assumed to be representative of the size and gender composition of the spawning run. All non-target species were counted and a subset (30) measured (TL mm) and released. All smelt caught as part of the fyke net survey were counted, sexed and a subset (100 males and 100 females) were measured to the nearest millimeter (TL). Gender was determined by the presence (males) or absence (females) of nuptial tubercles on their ventral surfaces (Warfel et al. 1943) or by the expression of milt with slight ventral message. Scale samples were taken from the dorsal area of the fish directly below the dorsal fin for a subset of all measured fish to create site-specific age-at-length keys. All smelt that were not tagged were released live with no additional handling. Scale samples were taken and analyzed as described above from each tagged smelt to confirm age as described in the previous chapter.

In 2011, thirty smelt were tagged with a V6 acoustic transmitter (VEMCO, 6 x 16 mm, appx. 350 day battery life). Twenty-one of these fish were captured and released
from the Oyster River and nine from the Squamscott River (Table 2.2). Of these, six females from the Oyster River were captured and held at the Aquaculture Research Center, Durham, NH for six days before being tagged and released at the Oyster River. These smelt were held before being tagged because transmitters had not yet been received from the distributor and there was concern that few females would be captured later in the run. Holding these females ensured that a fairly equal number of females and males were tagged. In 2012, thirty smelt were tagged with a V6 transmitter (VEMCO, 6 x 16 mm, appx. 232 day battery life). Nineteen of these fish were captured and released at the Oyster River and eleven from the Squamscott River. Additionally, ten smelt were tagged with a V5 transmitter (VEMCO 5 x 10 mm, appx. 150 day battery life) in 2012, the first year V5 tags were commercially available. Five of the fish with V5 transmitters were captured and released at the Oyster River and five from the Squamscott River (Table 2.2). The battery life of the transmitters was reduced in 2012 because the power of the ping signals emitted from the transmitters was increased in an effort to increase detection efficiency, and because no smelt tagged in 2011 were detected after 150 days (the projected battery life with increased ping rate). Smelt were tagged equally by gender when possible. Tagging was not stratified by age, but was random and favored the dominant age caught (Table 2.2).

Smelt were anesthetized and tagged with an acoustic transmitter as described above. Each smelt receiving a hydroacoustic transmitter was also tagged with a visible implant elastomer (Northwest Marine Technology, Inc., Shaw Island, WA) mark in the operculum for the purpose of visual identification upon recapture. All tagged smelt were
placed in a recovery tank with aerated river water for at least ten minutes after regaining consciousness before being released upstream from the capture site. Hydroacoustic receivers placed at the rivers and geographic chokepoints in the area (Figure 2.1) recorded detections continuously, and data were downloaded every two to four weeks.

*Data Analysis*

Hourly tide data covering the entire sampling period were obtained using WXTide32 (1.6.2, 2007). Tide data were queried for Seavey Island (location: 43.0767 N, 70.7246 W, receiver sites: Piscataqua mouth), Salmon Falls entrance (location: 43.1915 N, 70.8217 W, receiver site: Piscataqua River), Jaffrey Point (location: 43.0556 N, 70.7132 W, receiver site: Little Harbor), Dover Point (location: 43.1198 N, 70.8317 W, receiver sites: General Sullivan Bridge, offset for Oyster River +14 min at low tide, +18 min at high tide, offset for Adams Point +23 min for low tide, +37 min for high tide),

<table>
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<th>Year</th>
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<th>Squamscott River</th>
<th>Grand Total</th>
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<tbody>
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<td></td>
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<td>Age-1</td>
<td>Age-2</td>
<td>Age-3</td>
</tr>
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<td>V6</td>
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<td>11</td>
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<td>M</td>
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<td>5</td>
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</tr>
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<td>2</td>
</tr>
<tr>
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<td></td>
</tr>
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<td>5</td>
</tr>
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<tr>
<td></td>
<td></td>
<td>Total Tagged</td>
<td>4</td>
<td>29</td>
<td>12</td>
</tr>
</tbody>
</table>

Table 2.2. Number of smelt tagged with VEMCO transmitters in 2011 and 2012 with VEMCO V5 or V6 transmitters. Tagging by age was random and favored the dominant age caught (age-2).
Squamscott River railroad bridge (location: 43.0525 N, 70.9134 W, receiver sites: Squamscott and Lamprey rivers). No tide data were queried for the Winnicut River because no detections occurred at the receiver placed in that river.

The distances between receiver locations, and between receivers located within rivers and the spawning site at the head-of-tide of each river was measured in ArcMap™ 10.1 (ESRI© 1999-2012). Receiver locations were overlaid on the National Hydrography Dataset (NHD) hydrography network (U.S. Geological Survey, network extracted for Hydrologic Unit Code 2-01, 2010). The Utility Network Analysis Toolbar (ESRI© 1995-2012) was used to identify point locations on the NHD flowlines closest to receiver locations, and determine the path between each receiver location and every other. For each path, the sum of the length of flowlines was calculated in kilometers. The spawning site was located using the head-of-tide data available from the ME DMR (West Boothbay Harbor, Maine), and consultation with diadromous fisheries biologists at the NH FGD (J. Carloni, pers. comm.).

Statistical analyses were performed using JMP Pro 10.0.0 (Copyright © 2012 SAS Institute Inc., Cary, NC) and SYSTAT 13.1 (SYSTAT 2009, Chicago, IL). For all tests, statistical significance was considered when p < 0.05. Laboratory retention data were compared among all tag types using the Chi Square test of proportions and Likelihood Ratio to determine significance. Mortality rates for each tag type were compared to the control mortality rate using Dunnett’s test (Dunnett 1955). The null hypothesis for each comparison was that mortality did not differ significantly between tagged and control fish. The mortality and retention data for each tag type were
compared as the mean of the two replicate tanks, with one exception: mortality analyses considered smelt with JSAT tags in tank 1 and tank 2 separately because smelt tagged with JSAT tags in tank 1 were the only fish to also receive Floy tags, while those in tank 2 did not receive Floy tags.

When comparing the mortality between tagged and control fish, data in each study were divided into six time periods: period-1 = week 1; period-2 = week 1-3; period-3 = weeks 1-6; period-4 = weeks 1-9; period-5 = weeks 1-12; period-6 = weeks 1-15. Cumulative mortality at each time period was used due to the small samples sizes. The mortality for each time period ($p_i$; control or tagged fish) was calculated as the number of mortalities occurring within each time period ($m_i$) over the total number of fish within that group ($t_i$):

$$p_i = \frac{m_i}{t_i}$$

Mortality rates were non-normal and so were transformed before performing statistical analyses by taking the arcsine square root of the proportion of the number of mortalities ($m_i$) to the number fish alive ($t_i$) using correction values for the proportions to improve the normal fit of the resulting data (Anscombe 1948):

$$p' = \text{arcsin} \sqrt{\frac{X + 3/8}{n + 3/4}}$$

Hydroacoustic telemetry data were assessed to determine differences that may have occurred between study years, gender, and transmitter type. The proportion of smelt tagged was compared to the proportion of smelt detected using a Chi Square test of
proportions and the Likelihood ratio to determine significant differences. The number of detections was compared between study years, gender, and transmitter type to determine whether detection efficiencies may have varied between these groups. The null hypothesis was that the number of detected smelt did not differ within any of these variables. A least squares model was used to assess differences and interactions between all of these variables. Detection data were normalized using a log transformation:

$$x' = \log_{10}(x + 1)$$

using the correction factor $x+1$ because some values were equal to zero (Bartlett 1947).

The average number of spawning rivers visited by tagged smelt was compared between study year, tag/release river (Oyster or Squamscott River), and gender, and whether interactions existed among these variables using a least squares model. The null hypothesis was that the number of rivers visited did not vary between release locations, by gender, or by study year. Data were non-normal and first normalized using a log transformation:

$$x' = \log_{10}(x + 1)$$

using the correction factor $x+1$ because some values were equal to zero (Bartlett 1947).

The relationship between movement time (hrs) and distance between receivers (km) was explored using linear regression, using distance as the independent variable. The travel rate was calculated using the mean time for each receiver to receiver movement (hrs) over the distance between receivers. The mean travel rate was then taken as the average of all receiver to receiver travel rates.
The number of days spent at each receiver location by each tagged smelt was compared among receiver locations to estimate the relative importance of each site. For each receiver location, the number of days each tagged smelt spent at that location was first calculated. The mean number of days per fish at each receiver site was the metric used to compare differences among sites. The null hypothesis was that the mean number of days spent by each fish did not vary among the receiver sites. Data from the Oyster River mouth location were excluded because a receiver was placed at that site in only one study year (2011). There were no detections recorded on the receiver placed at Winnicut River. The daily data were non-normal, so differences among receiver locations were assessed using the non-parametric Kruskal-Wallis and Each Pair Wilcoxon tests.

Differences between gender, study year, and tag/release rivers were evaluated using Wilcoxon ranked sign, using the Chi Square p-value to determine significant differences.

Temporal patterns in the use of receiver locations by tagged smelt were evaluated using multivariate Cluster analysis (Ward’s method) and Principle Components Analysis (PCA) on correlations using four variables: the earliest (minimum) detection date at each site, the latest (maximum) detection date, median detection date, and date at which the largest number of detections were recorded. These variables were calculated for each site for each study year (2011 and 2012), and the average date value of the two study years was used in the multivariate analyses. The null hypothesis was that there was no temporal difference among detection dates at the receiver sites. The Cluster analysis assigned the sites into groups based on similarities among the four variables. The PCA provided information about the relationship of the four variables to each other, and
allowed the sites to be plotted with the variable relationships to determine which
variables were driving the cluster assignments. Finally, differences in the maximum and
median detection dates among the clusters were assessed using one-way ANOVAs and
Each Pair Student’s t test.

Temporal patterns were further identified using a heat map plotting date against
receiver location, which displayed the number of tagged smelt detected at each receiver
location on each date. A color scale was super-imposed onto the figure to illustrate
patterns of use: dates with few smelt observed were colored light gray, increasing
numbers of smelt detected on a date corresponded to darker coloration of the cell.

Results

Laboratory Studies

A preliminary laboratory study was conducted March 8 – June 20, 2011 to
compare retention and mortality between three transmitter types: Lotek JSATS AMT,
VEMCO V6, and VEMCO V5. Comparing combined results for both tanks, retention
success varied from 80% (JSAT) to 83% (V5) to 90% (V6), but not significantly different
(Likelihood Ratio p = 0.815, Chi-Square; Table 2.3). Retention also varied between
tanks: JSAT and V5 tags had higher retention than V6 tags in tank 1, however these two
tag types were retained in lower numbers than the V6 tags in Tank 2.

<table>
<thead>
<tr>
<th>Transmitter Type</th>
<th>No. Retained Tank 1</th>
<th>Percent Retention Tank 1</th>
<th>No. Retained Tank 2</th>
<th>Percent Retention Tank 2</th>
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<td>100%</td>
<td>3 (5)</td>
<td>60%</td>
<td>80%</td>
</tr>
<tr>
<td>V5</td>
<td>3 (3)</td>
<td>100%</td>
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<td>67%</td>
<td>83%</td>
</tr>
<tr>
<td>V6</td>
<td>4 (5)</td>
<td>80%</td>
<td>5 (5)</td>
<td>100%</td>
<td>90%</td>
</tr>
</tbody>
</table>

Table 2.3. Retention of three hydroacoustic transmitters by adult rainbow smelt in two replicate tanks.
Mortality did not differ significantly between replicate tanks for any tag group, and so the average mortality of the replicate tanks was used to compare mortality between each tag type and the control group (Table 2.4). Smelt in tank 1 with JSAT tags were the only group to also receive a Floy tag in the dorsal musculature. In all analyses, these fish were considered separately from smelt tagged with JSAT tags in tank 2.

Cumulative mortality over the entire study (period-6 = week 1-15) was not significantly different from the control group for any group (Dunnett’s test with control, p-values and mortality values in Table 2.5; Figure 2.2). Cumulative mortality was also compared between each tag type and the control group at five different points before the end of the 15-week study. During the first three periods, limited mortality was observed and was not significantly different between any tag type and the control (see Table 2.5 for mortality values and Dunnett’s test p-values). Mortality increased noticeably for each group during period-4 (week 9), possibly due to starvation.

Although the smelt were offered food on a regular basis starting at week 8, active feeding was not observed. Most smelt were observed to lose progressively more body mass as the study continued, and mortality also increased with time. At period-4 there was significantly higher cumulative mortality for smelt tagged with the JSAT and Floy

<table>
<thead>
<tr>
<th>Transmitter Type</th>
<th>Mortality Count</th>
<th>Percent Mortality</th>
<th>Mean Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tank 1</td>
<td>Tank 2</td>
<td>Tank 1</td>
</tr>
<tr>
<td>Control</td>
<td>3 (13)</td>
<td>7 (13)</td>
<td>23%</td>
</tr>
<tr>
<td>JSAT</td>
<td>-</td>
<td>1 (5)</td>
<td>-</td>
</tr>
<tr>
<td>JSAT+Floy</td>
<td>5 (5)</td>
<td>-</td>
<td>100%</td>
</tr>
<tr>
<td>V5</td>
<td>0 (3)</td>
<td>0 (3)</td>
<td>0%</td>
</tr>
<tr>
<td>V6</td>
<td>5 (5)</td>
<td>4 (5)</td>
<td>100%</td>
</tr>
</tbody>
</table>

Table 2.4. The number of mortalities by transmitter type is shown with the total number tagged (n), and mean mortality by tag type. Smelt in tank 1 tagged with Lotek JSAT were the only group to also receive a Floy tag.
tag compared to the control group, but no significant difference between any other tag type and the control occurred (Table 2.5, Figure 2.2). At period-5, there was again significantly higher cumulative mortality for smelt tagged with the JSAT and Floy tag compared to the control group, but no significant difference between any other tag type and the control occurred (Table 2.5, Figure 2.2). The mean length of mortalities did not differ significantly by tag type (p = 0.981; one-way ANOVA by tag type, tank type as block variable; Table 2.6).

<table>
<thead>
<tr>
<th>Dunnett's Test p-value</th>
<th>Control</th>
<th>JSAT</th>
<th>JSAT+Floy</th>
<th>V5</th>
<th>V6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period-1</td>
<td>-</td>
<td>0.999</td>
<td>0.999</td>
<td>0.634</td>
<td>0.998</td>
</tr>
<tr>
<td>Period-2</td>
<td>-</td>
<td>0.999</td>
<td>0.106</td>
<td>0.634</td>
<td>0.998</td>
</tr>
<tr>
<td>Period-3</td>
<td>-</td>
<td>0.999</td>
<td>0.39</td>
<td>0.916</td>
<td>0.667</td>
</tr>
<tr>
<td>Period-4</td>
<td>-</td>
<td>0.383</td>
<td>0.018*</td>
<td>0.55</td>
<td>0.253</td>
</tr>
<tr>
<td>Period-5</td>
<td>-</td>
<td>0.173</td>
<td>0.026*</td>
<td>0.174</td>
<td>0.088</td>
</tr>
<tr>
<td>Period-6</td>
<td>-</td>
<td>0.846</td>
<td>0.12</td>
<td>0.286</td>
<td>0.125</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mortality Rate</th>
<th>Period-1</th>
<th>Period-2</th>
<th>Period-3</th>
<th>Period-4</th>
<th>Period-5</th>
<th>Period-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.8% (+0.01)</td>
<td>-</td>
<td>20.0% (+0.04)</td>
<td>-</td>
<td>15.4% (+0.15)</td>
<td>30.8% (+0.20)</td>
</tr>
<tr>
<td>JSAT+floy</td>
<td>-</td>
<td>20.0% (+0.04)</td>
<td>-</td>
<td>10.0% (+0.04)</td>
<td>-</td>
<td>9.0% (+0.04)</td>
</tr>
<tr>
<td>JSAT</td>
<td>3.8% (+0.04)</td>
<td>-</td>
<td>20.0% (+0.04)</td>
<td>-</td>
<td>30.0% (+0.15)</td>
<td>-</td>
</tr>
<tr>
<td>V5</td>
<td>-</td>
<td>-</td>
<td>100.0% (+0.20)*</td>
<td>-</td>
<td>70.0% (+0.20)</td>
<td>-</td>
</tr>
<tr>
<td>V6</td>
<td>-</td>
<td>-</td>
<td>100.0% (+0.20)*</td>
<td>-</td>
<td>-</td>
<td>90.0% (+0.20)</td>
</tr>
</tbody>
</table>

Table 2.5. Cumulative mortality between two replicate tanks (±1SE) is shown for control fish and tagged fish at 6 periods. Comparisons between control and tagged fish were made using Dunnett's test, p-values for each comparison are shown.

<table>
<thead>
<tr>
<th>Tag</th>
<th>Tank 1</th>
<th>Tank 2</th>
<th>Both Tanks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>169.0</td>
<td>181.0</td>
<td>175.9</td>
</tr>
<tr>
<td>JSAT+floy</td>
<td>178.4</td>
<td>-</td>
<td>178.4</td>
</tr>
<tr>
<td>JSAT</td>
<td>-</td>
<td>160.0</td>
<td>160.0</td>
</tr>
<tr>
<td>V5</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>V6</td>
<td>175.0</td>
<td>161.0</td>
<td>171.4</td>
</tr>
</tbody>
</table>

Table 2.6. Mean length (TL mm) of mortalities by tag type and for the control group.
Field Studies

Assessing Study Design Variables

The range of the 180 kHz VR2W VEMCO receivers was tested at a location close to Adams Point and the results used to inform receiver placement. At a distance of 54 m, a transmitter with a ping rate of 2 minutes was detected 33 times over a period of 15 minutes (100% detection rate). At 98 m, the transmitter was detected 28 times in 15 minutes (100% detected). At 141 m, 6 detections were recorded (20% detected). No detections were recorded at ~200 m or ~250 m, likely because of uneven bathymetry. At 324 m, the transmitter was detected twice (7% detected).

The proportion of detected fish compared to the total number of tagged fish was compared between years, transmitter types, and power settings to determine if bias within any of these variables existed. The number of tagged fish that were never detected by any receiver did not vary significantly between the study years (2011 = 86.7% detected, 2012 = 92.5%, p = 0.424), gender (F = 84.4% detected, M = 94.7%, p = 0.147), nor by transmitter type (V5 = 90.0% detected, V6 = 90.0% detected, p = 1.0; Chi Square

Figure 2.2. Comparison of cumulative mortality (± 1SE) of adult smelt over fifteen weeks after six periods for four tag types.
Likelihood Ratio). Of the 63 smelt (out of 70 that were tagged) that were detected in both years, 8 were identified as possible immediate mortalities (within 1 day) because they were either detected only very few times by only the receiver at the mouth the release river, or almost continuously and drifting with the tide at only the receiver at the mouth of the release river. These eight fish were excluded from further analyses.

The mean number of detections by all receivers did not vary significantly by year (2011 avg. det. =127.5, 2012 = 328.5, p = 0.470), gender (p = 0.072), or by tag type (p = 0.942). There was no significant interaction between year and gender (p = 0.138), or tag type and gender (0.333; Standard Least Squares). Interaction between tag type and year was not tested because the number of tag types was only varied in 2012.

**Individual Movement Patterns**

Movement by tagged smelt followed three major patterns. Among the first group, smelt visited one or more spawning rivers, and were last detected by a receiver within a river 3 to 24 days after being tagged and released. These smelt generally passed through Adams Point at least once, and may have spent multiple days in the Adams Point area. Fish within this group may have also been detected within the lower part of the Piscataqua River, but subsequently returned to and was ultimately detected with a river (Figure 2.3a).

Among the second group, the majority of detections and the final detection for each smelt were recorded at the Adams Point receiver. Smelt were detected for 6 to 86 days after being tagged. While some smelt within this group visited multiple spawning rivers, or even made movements to the Piscataqua River mouth before returning to
Adams Point, others were detected at Adams Point exclusively (Figure 2.3b). When repeated detections were made at this location, the movement was considered to be active, rather than from a deceased fish, because detections were not continuous and occurred in close proximity to detections of other tagged fish (assumed schooling behavior).

Among the third group, smelt were last detected in the Piscataqua River mouth 4 to 61 days after being tagged. Some fish in this group visited other spawning rivers before moving to coastal waters through the river mouth, while others left the embayment within a few days of being tagged (Figure 2.3c).
Figure 2.3. Examples of three movements patterns represented by: (a) three individual smelt tagged with hydroacoustic transmitters whose last detections were recorded in one of the spawning rivers; (b) three smelt last detected at Adams Point; and (c) three smelt last detected at the Piscataqua River mouth.
Movement Among Rivers and Between Receiver Sites

Nearly two-thirds of the smelt that were both detected and assumed to not be immediate mortalities were observed to visit at least one other river than the one where it was captured/released. The number of rivers visited, excluding the capture/release river, varied from 0 to 3. The majority of tagged fish (23 fish, 42.6%) visited only one other river than the river in which it was tagged and released; 11 fish (20.4%) visited two other rivers; and 1 fish (1.9%) visited 3 rivers. The mean number of other rivers visited did not differ significantly by gender (F avg. rivers visited = 0.64, M = 1.03, p = 0.317), or release location (Oyster R. avg. rivers visited = 0.89, Squamscott R. = 0.84, p = 0.957), but did differ by study year (2011 avg. rivers visited = 0.48, 2012 = 1.2, p = 0.002). There was no significant interaction between gender and study year (p = 0.250), or between release location and study year (p = 0.968), indicating that the difference observed by study year was not influenced by the other variables (Least Squares). In 2011, only 9 out of 25 (36.0%) tagged smelt visited one or more rivers, while in 2012, 26 out of 30 tagged fish (89.7%) visited one or more other rivers.

The travel time between receiver locations increased as the distance between receiver locations increased (p = 0.001; linear regression; Figure 2.4). The travel time was least when movement was between receivers located at the Piscataqua and Bellamy rivers, and greatest for movements between Adams Point and the Piscataqua River mouth (Table 2.7). The mean rate of movement was 5.4 (±0.97) hrs/km, varying from 0.5 hrs/km for movements between the Piscataqua and Bellamy rivers, and 11.2 hrs/km for movements between Adams Point and the General Sullivan Bridge. The majority of
receiver to receiver movements either started or ended at Adams Point, while fewer direct
movements were made between river locations (Table 2.7). While these calculated
movement times do not consider tidal influences on movement speed, movements
between receivers were made during both incoming (98 movements) and outgoing (77
movements) tides. These movement rates are assumed to be reflective of the average
movement time of adult smelt within the estuary and embayment because fairly equal
numbers of movements were made at ebb and flow tides, and because the variability
among the rates was fairly low (Table 2.7). These rates are not considered to represent
swimming speeds of smelt because other environmental variables were not measured
(tidal flow speed, river discharge, etc.). Further, the distances between receivers was not
linear, receivers’ anchors shifted on occasion, and smelt may have made many
movements between locations outside of the receiver detection ranges (~100 m).

![Graph](image)

Figure 2.4. Relationship between the movement time between receivers (hrs, ±1SE) and
the distance between receivers (km).

\[
y = 8.6916x - 20.89 \\
\text{adj. } R^2 = 0.4090
\]
<table>
<thead>
<tr>
<th>Site 1</th>
<th>Site 2</th>
<th>Mean Time between Locations (hrs)</th>
<th>Distance between Locations (km)</th>
<th>Movement Rate (hrs/km)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adams Point</td>
<td>Gen. Sull. Bridge</td>
<td>87.4 (±29.5)</td>
<td>7.8</td>
<td>11.2</td>
</tr>
<tr>
<td></td>
<td>Piscataqua R. Mouth</td>
<td>188.7 (±175.2)</td>
<td>18.8</td>
<td>10.0</td>
</tr>
<tr>
<td></td>
<td>Squamscott R.</td>
<td>73.7 (±20.6)</td>
<td>7.1</td>
<td>10.4</td>
</tr>
<tr>
<td></td>
<td>Lamprey R.</td>
<td>58.1 (±24.4)</td>
<td>6.2</td>
<td>9.4</td>
</tr>
<tr>
<td></td>
<td>Oyster R. Mouth</td>
<td>25.6 (±5.9)</td>
<td>5.6</td>
<td>4.6</td>
</tr>
<tr>
<td></td>
<td>Oyster R.</td>
<td>20.6 (±8.3)</td>
<td>6.6</td>
<td>3.1</td>
</tr>
<tr>
<td></td>
<td>Bellamy R.</td>
<td>36.6 (±16.6)</td>
<td>8.2</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td>Piscataqua R.</td>
<td>77.7 (±59.5)</td>
<td>11.7</td>
<td>6.7</td>
</tr>
<tr>
<td>Gen. Sull. Bridge</td>
<td>Piscataqua R. Mouth</td>
<td>56.7 (±33.9)</td>
<td>11.6</td>
<td>4.9</td>
</tr>
<tr>
<td></td>
<td>Oyster R. Mouth</td>
<td>7.2 (±3.2)</td>
<td>5.6</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>Oyster R.</td>
<td>39.8 (±18.0)</td>
<td>6.5</td>
<td>6.1</td>
</tr>
<tr>
<td></td>
<td>Bellamy R.</td>
<td>1.8 (±0.2)</td>
<td>5.7</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>Piscataqua R.</td>
<td>8.0 (±2.7)</td>
<td>1.8</td>
<td>4.5</td>
</tr>
<tr>
<td>Bellamy R.</td>
<td>Oyster R. Mouth</td>
<td>5.3 (-)</td>
<td>5.8</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>Oyster R.</td>
<td>37.7 (±18.1)</td>
<td>6.9</td>
<td>5.5</td>
</tr>
<tr>
<td></td>
<td>Piscataqua R.</td>
<td>4.4 (-)</td>
<td>9.6</td>
<td>0.5</td>
</tr>
<tr>
<td>Oyster R.</td>
<td>Oyster R. Mouth</td>
<td>4.0 (±0.9)</td>
<td>1.1</td>
<td>3.6</td>
</tr>
<tr>
<td></td>
<td>Piscataqua R.</td>
<td>44.3 (±17.1)</td>
<td>9.4</td>
<td>4.7</td>
</tr>
<tr>
<td>Lamprey R.</td>
<td>Squamscott R.</td>
<td>7.6 (±0.9)</td>
<td>3.5</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>Oyster R.</td>
<td>7.0 (-)</td>
<td>11.7</td>
<td>0.6</td>
</tr>
<tr>
<td>Piscataqua R. Mouth</td>
<td>Little Harbor</td>
<td>158.2 (-)</td>
<td>8.6</td>
<td>18.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean Movement Rate (hrs/km)</td>
<td>5.4 (± 0.97)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2.7. The mean movement time between receiver locations (±1SE) is shown with the corresponding distance between locations and the calculated travel time.
Movement Patterns by Smelt Released from the Squamscott River

Smelt tagged and released at the Squamscott River were detected in all other study rivers except the Winnicut River. The greatest number of individuals were observed in the Lamprey River (9 fish, 45% of total tagged, detected, and not immediate mortality) compared to other river locations (Bellamy R. = 3 fish, 16%; Oyster R. = 1 fish, 5%; Piscataqua R. = 1 fish, 5%). The number of days spent at each river, by each fish, was then compared among the rivers as a measure of the use of each system: 21 days were spent on the Bellamy River (avg. 7 days per fish), 17 days were spent at the Lamprey River (avg. 1.9 days per fish), 6 days were spent on the Oyster River by one fish, and only one day total was spent on the Piscataqua River by one fish (Table 2.8, Figure 2.5).

Figure 2.5. The mean number of daily detections for each smelt (± 1SE) was highest at the Bellamy, Oyster, and Squamscott rivers, and Adams Point among smelt tagged/released from the Squamscott River.
The mean number of days spent at other rivers by fish released from the Squamscott River did not vary significantly among the different river sites (p = 0.310; Kruskal-Wallis), by gender (F avg. days = 8.0, M = 2.2, p = 0.618), or by study years (2011 avg. days = 1.3, 2012 = 3.7, p = 0.184, Wilcoxon rank sum, Chi Square p-value).

Among all receiver locations, the mean number days spent at each location by each fish was significantly lower at the General Sullivan Bridge compared to the Bellamy (p = 0.023), Oyster (p = 0.013), Squamscott (p = 0.025), and Lamprey rivers (p = 0.007) and to Adams Point (p = 0.001; Table 2.8, Figure 2.5). The mean number of days was lower at the Piscataqua River mouth compared to Adams Point (p = 0.009) and the Lamprey River (p = 0.028). Finally, the mean number of days was lower at the Lamprey River compared to Adams Point (p = 0.048; Kruskal-Wallis, Each Pair Wilcoxon method; Table 2.8; Figure 2.5). The mean number of days spent at all receiver locations did not vary significantly by gender (F avg. days = 5.4, M = 2.5, p = 0.220), or by study year (2011 avg. days = 2.6, 2012 = 3.0, p = 0.775; Wilcoxon ranked sign, Chi Square p-value).

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Squamscott River</td>
<td>2.8 (0.7)</td>
<td>1.9 (0.3)</td>
<td>6.0 (-)</td>
<td>7.0 (5.5)</td>
<td>1.0 (-)</td>
<td>4.1 (0.7)</td>
<td>1.0 (0.0)</td>
<td>1.0 (0.0)</td>
<td>1.0 (-)</td>
</tr>
<tr>
<td>Oyster River</td>
<td>2.9 (0.8)</td>
<td>2.8 (0.5)</td>
<td>2.6 (0.8)</td>
<td>2.5 (0.6)</td>
<td>1.8 (0.3)</td>
<td>7.0 (1.2)</td>
<td>1.7 (0.2)</td>
<td>1.0 (0.0)</td>
<td>-</td>
</tr>
<tr>
<td>Capture/Release Locations Combined</td>
<td>2.8 (0.5)</td>
<td>2.4 (0.3)</td>
<td>2.7 (0.7)</td>
<td>4.0 (1.8)</td>
<td>1.7 (0.3)</td>
<td>6.0 (0.8)</td>
<td>1.5 (0.2)</td>
<td>1.0 (0.0)</td>
<td>(-)</td>
</tr>
</tbody>
</table>

Table 2.8. The average number of days that smelt were detected at each site is shown for smelt tagged and released at two locations, the Oyster and Squamscott rivers, NH (±1SE). Where no SE is given, only one smelt was observed at that location.
Movement Patterns by Smelt Released from the Oyster River

Smelt that were tagged and released from the Oyster River were observed in all other study rivers except the Winnicut River. More individuals were observed at the Squamscott and Lamprey rivers (10 fish at each, 28% of total tagged, detected, and not immediate mortality), and fewer observed in the Bellamy and Piscataqua rivers (6 fish at each, 17%). The number of days spent at each river differed among the different locations: 15 days were spent on the Bellamy (avg. 2.5 days per fish), 28 days were spent at the Lamprey (avg. 2.8 days per fish), 29 days were spent on the Squamscott River (avg. 2.9 days per fish), and 11 days were spent on the Piscataqua River (avg. 1.8 days per fish; Table 2.8, Figure 2.6).

Figure 2.6. The mean number of days (±1SE) spent at each receiver site by smelt tagged/released at the Oyster River was highest at the Adams Point receiver and lowest at receivers located on the pathway to coastal waters.
The average number of days spent at rivers other than the Oyster River did not vary significantly (p = 0.626; Kruskal-Wallis; Table 2.8; Figure 2.6), or by study year (2011 avg. days = 3.4, 2012 = 3.5, p = 0.857), or gender (F avg. days = 3.4, M = 3.6, p = 0.523; Wilcoxon ranked sign, Chi Square p-value). Among all receiver sites, the mean number of days per fish spent at each location varied significantly (p = 0.005). Fewer days on average were spent at the General Sullivan Bridge compared to the Bellamy (p = 0.023), Oyster (p = 0.013), Lamprey (p = 0.007), and Squamscott rivers (p = 0.025), and Adams Point (p = 0.001); fewer days on average at the Piscataqua River mouth compared to the Lamprey River (p = 0.028) and Adams Point (p = 0.009); and fewer days at the Lamprey River compared to Adams Point (p = 0.048; Kruskal-Wallis, Each Pair Wilcoxon method; Table 2.8; Figure 2.6). The mean number of days per fish detected did not vary by gender (F avg. days = 3.4, M = 3.6, p = 0.220), or year (2011 avg. days = 3.4, 2012 = 3.5, p = 0.775; Wilcoxon ranked sign, Chi Square p-value).

Comparing Movement Patterns Between Release Locations

The mean number of days per fish spent at each receiver location was compared between the two tag/release sites to determine whether smelt released from one site were detected more frequently at a certain location compared to smelt released from the other site. At each location where there were enough data to perform the analysis, there was no significant difference in the number of days detected between tag/release sites (Adams Point p = 0.323; Bellamy R. p = 0.895; Gen. Sull. Bridge p = 0.061; Lamprey R. p = 0.089; Oyster R. p = 0.082; Piscataqua R. p = 0.280; Squamscott R. p = 0.411; not
enough data at Little Harbor and Piscataqua River mouth; Each Pair Wilcoxon method; Table 2.8).

Comparing Time Spent at Each Receiver Site

The relative use of the areas around each receiver site by tagged smelt was then compared using combined data from the two tag/release locations. Among all receiver sites, the average number of days spent at each location differed significantly (p < 0.0001; Kruskal-Wallis). The mean number of days per fish was significantly higher at Adams Point (avg. days = 6.0) compared to all other sites (Squamscott R. p = 0.002; Oyster R. p < 0.0001; Lamprey R. p = 0.007; Piscataqua R. p = 0.012; Gen. Sull. Bridge p < 0.0001; Piscataqua River mouth p < 0.0001) except the Bellamy River and Little

![Chart](image)

Figure 2.7. The mean number of days (±1SE) spent at each location by smelt released from both the Oyster and Squamscott rivers was highest at Adams Point and the Bellamy River and lowest at receivers placed on the pathway to coastal waters.
Harbor; higher at the Bellamy River compared to the General Sullivan Bridge ($p = 0.019$) and the Piscataqua River mouth ($p = 0.005$); higher at the Squamscott River compared to the General Sullivan Bridge ($p = 0.016$) and the Piscataqua River mouth ($p = 0.006$); higher at the Oyster River compared to the Lamprey River ($p = 0.041$) and the Piscataqua Mouth ($p = 0.040$); higher at the Lamprey River compared to the General Sullivan Bridge ($p = 0.001$) and the Piscataqua River mouth ($p = 0.0004$); and higher at the Piscataqua River compared to the Piscataqua River mouth ($p = 0.014$; Each Pair Wilcoxon method; Table 2.8; Figure 2.7). At Little Harbor, only one movement was detected by a fish captured/released from the Squamscott River. No movements or detections were recorded in the Winnicut River.

**Temporal Patterns in Habitat Use**

Temporal patterns were assessed between the sites using the earliest, median and latest detection dates at each site, and the date on which the highest number of detections were recorded at each site. The mean date value of the two study years for each of these variables was used for analysis. Cluster analysis (Ward’s method) identified three groups

<table>
<thead>
<tr>
<th>Site</th>
<th>Min. Detect. Date</th>
<th>Max. Detect. Date</th>
<th>Median Detect. Date</th>
<th>Date with Most Detect. Date</th>
<th>Cluster Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lamprey R.</td>
<td>4/25</td>
<td>5/1</td>
<td>4/26</td>
<td>4/24</td>
<td>3</td>
</tr>
<tr>
<td>Oyster R.</td>
<td>4/5</td>
<td>4/7</td>
<td>4/3</td>
<td>4/4</td>
<td>3</td>
</tr>
<tr>
<td>Bellamy R.</td>
<td>4/25</td>
<td>5/1</td>
<td>4/26</td>
<td>4/24</td>
<td>3</td>
</tr>
<tr>
<td>Adams Point</td>
<td>4/1</td>
<td>5/4</td>
<td>4/21</td>
<td>4/21</td>
<td>1</td>
</tr>
<tr>
<td>Piscataqua Mouth</td>
<td>4/19</td>
<td>4/23</td>
<td>4/17</td>
<td>4/23</td>
<td>2</td>
</tr>
<tr>
<td>Little Harbor</td>
<td>4/12</td>
<td>4/17</td>
<td>4/7</td>
<td>4/9</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 2.9. Temporal variation among the sites is compared using four variables. Cluster assignments (Ward’s method) based on these variables found that spawning rivers, Adams Point, and locations leading to coastal waters grouped separately.

84
based on these variables: Cluster 1 – Adams Point; Cluster 2 – General Sullivan Bridge, Little Harbor, and the Piscataqua River mouth; and Cluster 3 – Bellamy, Lamprey, Oyster, Piscataqua, and Squamscott rivers (Table 2.9). A Principle Components Analysis (PCA) on correlations informed which variables were driving the cluster separations (Figure 2.8). The first component was responsible for 76.8% of the variation with all variables, loading in the positive direction (right quadrants). The second component was responsible for 22.1% of the variation, with the maximum date loading in the positive direction (upper right quadrant), the minimum date loading in the negative direction (bottom right quadrant). The median detection date and the date with the highest number

![Figure 2.8. Biplot showing cluster assignments (Ward's method) with PCA results. The first cluster's placement was associated with later detection dates (symbol = •; Adams Point). The second cluster (symbol = +; General Sullivan Bridge, Little Harbor, and the Piscataqua Mouth) had later median dates and later dates with the highest number of detections. The third cluster (symbol = 0; Bellamy, Lamprey, Oyster, Piscataqua, and Squamscott rivers) had earlier first, median, and last detection dates, and the date with the highest number of detections was also earlier.](image-url)
of detections loaded together close to the zero-axis. A biplot allowed the results of the
cluster analysis and the PCA to be interpreted together. The first cluster (Adams Point)
positioned in the upper right quadrant, driven by later detection dates. The third cluster
(the Bellamy, Lamprey, Oyster, Piscataqua, and Squamscott rivers) was located in the
bottom left quadrant. This cluster had earlier first, median, and last detection dates, and
the date with the highest number of detections occurred earlier. The second cluster
(General Sullivan Bridge, Little Harbor, and the Piscataqua River mouth) fell close to the
zero-axis in the bottom right quadrant, with later median dates, and dates with the highest
number of detections (Figure 2.8).

Among clusters, the mean latest and median detection dates differed significantly
(p < 0.0001 and 0.003, respectively; one-way ANOVA). Each cluster’s mean latest
detection date was significantly unique (Cluster 1 avg. max. det. date = May 26; Cluster 2
= May 2; Cluster 3 = April 11). The median detection dates were significantly different
between Cluster 2 (avg. median date = April 28) and Cluster 3 (avg. median date = April
8), but neither was different from Cluster 1 (median date = April 17; Each Pair Student’s
t; Figure 2.9).

Movement and temporal patterns were further examined by considering the
number of individual fish detected at each receiver site daily. In both study years, daily
movement during the first period following release was concentrated in the spawning
rivers (Figure 2.10). Tagged smelt were observed most frequently in the tag/release
rivers (Oyster and Squamscott rivers), with up to 7 fish detected on March 15, 2012 in the
Oyster River. The number of smelt detected daily at the Piscataqua and Bellamy rivers
was comparatively smaller: 1-2 smelt were detected on a fairly daily basis at the Bellamy River while the Piscataqua River was visited sporadically. In contrast, more tagged smelt were observed at the Lamprey River compared to the other non-release sites: up to 5 on March 16 and 22, 2012. In both 2011 and 2012, few smelt were observed in the spawning rivers after April 26. In both years, a comparatively high number of individual smelt were observed daily at Adams Point, up to 13 in 2011 and 6 in 2012. In 2011 the number of fish detected daily at Adams Point was higher in late April, while in 2012 higher numbers of smelt were observed in late March and early April (Figure 2.10).

![Figure 2.9](image)

Figure 2.9. The mean (a) latest detection dates ±1SE and (b) median detection dates ±1SE differed among three clusters of receiver sites: Cluster 1 = Adams Point; Cluster 2 = Gen. Sull. Bridge, Little Harbor, and the Piscataqua mouth; Cluster 3 = Bellamy, Lamprey, Oyster, Piscataqua, and Squamscott rivers (one-way ANOVA).
At the General Sullivan Bridge, Piscataqua River mouth, and Little Harbor, fewer tagged smelt were observed daily. Up to 3 fish were detected on a single day at the General Sullivan Bridge on multiple dates, while no more than 1 fish per day were observed at the Piscataqua River mouth and Little Harbor (Figure 2.10). Considering the 2011-2012 mean number of fish detected daily, it appears that movement was concentrated in the spawning rivers from March to mid-April, at Adams Point from mid-March to early May, and at the General Sullivan Bridge, Piscataqua River mouth, and Little Harbor from late April to late May (Figure 2.11).

Despite this pattern, tagged smelt were not always observed to exit the Great Bay and Piscataqua River complex and return to coastal waters. In 2011, 14 out of 25 tagged fish (56.0%) were last detected at a receiver located on the pathway to coastal waters (General Sullivan Bridge, Piscataqua River mouth, or Little Harbor). In 2012, 11 out of 30 (36.7%) were last detected at one of these receivers. Considering both years combined, 45.5% of all tagged smelt were detected moving into marine waters. This portion was composed of a relatively equal number of males (11 fish) and females (14 fish). A proportion of tagged smelt (16.0% in 2011, 43.3% in 2012) were last detected at one river locations (Squamscott, Lamprey, Oyster, Bellamy, or Piscataqua River) before the end of April. Comparatively more males were last detected in rivers (16 fish) compared to females (9 fish). The remaining portion (28.0% in 2011, 20.0% in 2012) were last observed at Adams Point, the last detection dates occurring anywhere between April 13 – May 15 in 2011 and March 23 – June 6 in 2012. Though the sample sizes were low, this behavior was more associated with males (9 fish) than females (4 fish).
Table 2.2. The number of individual tagged smelt detected at each receiver location daily varied between the sites and study years. Cells are colored by density of fish detected daily: light gray = few smelt; black = higher numbers.
Figure 2.11. The 2011-2012 mean number of tagged smelt detected daily. A higher number were observed before mid-April at the spawning rivers, in mid to late April at Adams Point, in late April and through May and at pathways to coastal waters. Cells are colored by density of fish detected daily: light gray = few smelt; black = higher numbers.
Discussion

Effective species management relies upon understanding annual movement patterns and identifying important habitats (Beck et al. 2001). Given the decline in rainbow smelt populations along the U. S. east coast, and specifically in coastal New Hampshire (Enterline et al. 2012a), active management of important habitat may be necessary to safe-guard the species from further decline. While anadromous rainbow smelt spawning habitat in riverine systems has been thoroughly described and identified (Kendall 1926, Bigelow and Schroeder 1953, Hulbert 1974, Lawton et al. 1990, Pettigrew 1997, Chase and Childs 2001, Enterline et al. 2012a), an understanding of smelt habitat-use outside of the spawning season is less clear.

The current study used hydroacoustic telemetry to monitor anadromous adult rainbow smelt movements during and following the spawning season among six rivers, a tidal estuary, and coastal waters in the Great Bay and Piscataqua River complex, NH. Our results show that smelt of both genders visited multiple rivers within an embayment during the spawning season and made use of a tidal estuary after spawning activity ceased. The study also provided information about the ability of rainbow smelt to be monitored using hydroacoustic telemetry, as well as estimates of transmitter retention and mortality due to tagging.

Movement Among Spawning Rivers

Movement between spawning sites has been previously documented for other rainbow smelt populations. Murawski et al. (1980) recaptured <1 – 20% of marked smelt at spawning sites other than the release location, and found that recaptures were more
likely to be males. The current study documented comparatively more ‘wandering’: between 36.0% (2011) and 89.7% (2012) of tagged fish visited rivers other than the release river. In 2011, fewer rivers may have been visited because the study was started later in the spawning run (early April), while in 2012 the study began at the beginning of the spawning run in early March. If wandering between rivers was associated with repeat spawning, fewer rivers may have been visited in 2011 because half of the spawning run had already progressed. Considering both years’ data, the majority of tagged fish (23 fish, 42.6%) visited only one other river besides the river in which it was tagged and released; 11 fish (20.4%) visited two other rivers; and 1 fish (1.9%) visited 3 rivers.

This study found no significant difference in behavior between genders (F avg. rivers visited = 0.64, M = 1.03, p = 0.317) unlike previous studies that found movement between spawning rivers to be a predominantly male behavior (Rupp 1968, Murawski et al. 1980). The first chapter of the thesis documented repeat spawning behavior for both male and female smelt within a single river system, however, the behavior was more pronounced for males (Figure 1.9). The contrast with previous studies may be explained by differences in study design. The current study’s detection sites were located at the mouths of rivers, on average 6.6 km downstream of spawning locations (Table 2.10), whereas the recapture sites in the previous studies and the sites used in the PIT tag study were located at the head-of-tide of rivers at smelt spawning grounds.

<table>
<thead>
<tr>
<th>Squamscott R.</th>
<th>Lamprey R.</th>
<th>Oyster R.</th>
<th>Bellamy R.</th>
<th>Piscataqua R.</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.2 km</td>
<td>4.4 km</td>
<td>5.3 km</td>
<td>7.5 km</td>
<td>12.5 km</td>
</tr>
</tbody>
</table>

Table 2.10. Distance (km) between receiver location and smelt spawning grounds at the head-of-tide of each river.
Active wandering by spawning smelt has been documented for both freshwater and anadromous populations. Rupp (1968) observed this behavior prior to and during the spawning season in a fresh water system in Maine, documenting movements between spawning sites up to 6.4 km apart. Murawski et al. (1980) described movement by anadromous smelt between spawning sites up to 16 km apart within a 48 period (3 hrs/km). The current study found movement between receivers located within the lower portions of spawning rivers up to 13.3 km apart (Squamscott and Oyster rivers). It was not possible to determine whether these smelt were making migrations to the spawning grounds located at the head-of-tide of these rivers, however, if smelt did travel between the spawning sites, the total distance traveled would be 21.8 km. The average rate of travel between all receiver to receiver locations (5.4 hrs/km) was faster than described by Murawski et al. (1980). This divergence may be due to differences in tidal flow between the study areas.

Movement between spawning rivers may be directed by tidal currents (Murawski et al. 1980). As schooling fish (Buckley 1989), smelt may be moving between various spawning rivers as mixed groups of pre- and post-spawn fish of both genders. While spawning smelt actively migrate to the spawning grounds, post-spawn smelt may drop out of the school somewhere between the mouth of the river and the head-of-tide.

The variation between the study years in both the average time spent at each location (Table 2.8, Figures 2.5 and 2.6), and the average number of rivers visited (2011 avg. rivers visited = 0.48, 2012 = 1.2, p = 0.002) may indicate that movement between rivers is partially driven by tidal processes, and may vary annually based on circulation...
patterns. Future work to determine whether movement is coincident with tidal circulation is necessary to confirm this inference.

Post-Spawning Habitat Use

Bigelow and Schroeder (1953) inferred that smelt return to coastal water immediately following spawning. In contrast, the current study documented that only a portion left the system at the mouth of the Piscataqua River (45.5% of all fish tagged, 56.0% in 2011, 36.7% in 2012). The latest smelt catches in fyke net surveys performed at two spawning rivers in the study area (Oyster and Squamscott rivers) occurred on April 17, 30, 28, and 21 in 2008-2011, consecutively (J. Carloni, NH FGD, pers. comm.), indicating that the fish remaining in the estuary after late April were likely not spawning but making use of the habitat for other purposes.

The remaining tagged smelt were last detected within one of the spawning rivers (30.9%), or at the geographic choke point, Adams Point (23.6%). It is possible that these fish passed through the mouth of the Piscataqua River but were not detected due to lower detection effectiveness at certain receiver sites, because receivers at the General Sullivan Bridge and the mouth of the Piscataqua River were lost in both study years, or because these smelt became prey or died from other causes while they were within the estuary. No detections were recorded after May 25, 2011 and June 7, 2012 even though the receivers remained in place and transmitters were active until December, 2011 and August, 2012. If smelt were not detected as they left the estuary, the results of this study over-estimate the proportion of post-spawn smelt remaining in the estuary during the summer months.
Smelt last detected within rivers (16.0% in 2011, 43.3% in 2012) likely experienced mortality because the final detections for each fish occurred before the end of April, while the spawning runs were still ongoing. While smelt are iteroparous and do not generally experience mortality following the act of spawning, some individuals do not survive the event due to exhaustion, similar to other anadromous species (*Oncorhynchus* spp.; Quinn and Myers 2004, Osmeridae; Christiansen *et al.* 2008).

A portion of the tagged smelt were last detected at Adams Point (28.0% in 2011, 20.0% in 2012), a result that may be supported by other surveys. Annual beach-seine surveys performed in the Great Bay and Piscataqua River complex from June to October document some catches of adult smelt. The largest abundance occurs in June, when up to 13.0% of the smelt catch has been composed of adults (in proportion to juvenile smelt). During the 17 survey years, adult smelt have been caught in June in 9 years, July in 2 years, August in 3 years, September in 5 years, and October in 5 years. Over the entire study period, the average adult catch proportion (in relation to juvenile smelt catch) was 5.1% (J. Carloni, NH FGD, pers. comm.). In a similar survey performed in the Kennebec Estuary, Maine, adults contribute little (<1%) to summer smelt catches although larger catches of adult smelt (~33%) have occurred in the fall months (G. Wippelhauser, ME DMR, pers. comm.). Performed at shoreline locations, these surveys are designed to target juvenile fish and as a result may under-represent the abundance of adult smelt within the estuary.

Near-shore trawl surveys performed close to the mouth of the Piscataqua River annually 2000-2011 in late May and October catch very few adult smelt. At two sites
trawled in late May off coastal New Hampshire (Foss Ledges and Hampton Shoals), only 1 smelt has been caught in each of 6 survey years and 15 were caught during one survey year. During October, smelt have been caught in only two survey years in very low quantities (1-2 fish, S. Sherman, ME DMR, pers. comm.). Bringing together the information from the trawl survey, the beach-seine surveys, and the current telemetry study, there is evidence that the majority of post-spawn adult smelt migrate to coastal waters within 5 weeks of the end of the spawning run, while a portion remains within the estuary. A smaller portion likely experiences mortality within the rivers following spawning.

Residence within the estuary or movement into coastal waters may vary annually depending on water temperatures, as smelt are known to prefer colder waters (Buckley 1989). Annual beach seine and trawl surveys may provide some information about trends in habitat use correlated with water temperature. It is unfortunate that receivers in place through December 2011 did not detect any smelt returning into the Piscataqua River and Great Bay system, however as a marine prey species, the likelihood of the tagged smelt returning was fairly low due to the small number of fish tagged. Further study using otolith microchemistry could provide information about residence time within coastal waters and the embayment.

Relative Use of Habitats within the Study Area

Within the Great Bay and Piscataqua River complex, the use of habitat areas around the receiver locations can be assessed through the amount of time tagged fish spent at each. Among all receiver locations, significantly more days on average were
spent at Adams Point compared to all other sites, with the exception of Little Harbor where not enough data were available to make comparisons (Table 2.8, Figures 2.5 – 2.6). The frequent use of Adams Point is logical based on its geographic location (Figure 2.1). The Great Bay estuary and Squamscott, Winnicut, and Lamprey rivers are located on the south side of this location. On the north side, the Oyster, Bellamy, and Piscataqua rivers are located, as well as the pathway to coastal waters. Each of the aforementioned rivers support smelt spawning (Enterline et al. 2012c). Movement data collected during this study found that nearly two-thirds of the tagged smelt that were both detected and assumed to not be immediate mortalities were observed to visit at least one other river besides the river where it was captured/released (35 out of 55 fish, 64.8%). The frequent movement at Adams Point during April can, therefore, be partially explained by the observed movement between spawning rivers.

After detections ceased in the spawning rivers in late April, frequent use of the Adams Point area continued (Figures 2.11 and 2.12). Cluster analysis and PCA showed that temporal use of spawning rivers, the area at Adams Point, and the pathway to coastal waters was unique (Table 2.9, Figure 2.8). Use of spawning rivers occurred significantly earlier compared to the pathway to coastal waters, while use of the area around Adams Point spanned the study period (Figure 2.11). Observations at Adams Point from late April until June may represent movements towards coastal waters from sites within the Great Bay estuary (Figures 2.10 and 2.11).

Some smelt, however, were not observed to return to coastal waters but were last detected at Adams Point. Further, this site had the latest maximum detection dates
compared to spawning rivers and the pathway to coastal waters (Figure 2.9). Continued use of the Adams Point area may be attributed to movement between the Great Bay estuary on the south, and the sheltered embayment to the north. Behavior (feeding, resting, passive or active swimming) during this period remains unknown and was not documented as part of this study. Future work to assess behavior could include hydroacoustics and mid-water trawling to locate aggregations of adult smelt and fine-tune habitat use descriptions, performing stomach contents analysis, and placement of multiple receivers or directional hydrophones to determine swimming behavior (for example of multi-dimensional telemetry see Redden and Broome 2012).

While more days were spent at Adams Point compared to other locations, relatively high use was also made of the spawning rivers. The average number of days spent at each river varied between 1.7 days/fish (Piscataqua R.) and 4.0 days/fish (Bellamy R.), with some variation in the number of days spent at each location based on the tag/release location (Table 2.8). Despite this variance, the average amount of time spent at each spawning river location did not vary significantly (Figures 2.5 – 2.6).

The least amount of time was spent at receiver locations located on the lower Piscataqua River (General Sullivan Bridge, Piscataqua River mouth, and Little Harbor; Table 2.8, Figures 2.5 – 2.6). It is possible that smelt spent less time in this area as they were migrating into coastal waters and remaining in this area was difficult due to strong tidal currents. Detection effectiveness may have also been lower at these sites where conditions including high flow, heavy boat traffic, and larger detection areas, all of which can result in lower detection efficiency (J. Mullock, VEMCO, pers. comm).
Relevance of Mortality Due to Tagging and Tag Retention

Based on laboratory trials and field data, some mortality due to tagging likely occurred, however, field studies still provided useful data. Low mortality and high retention were found in laboratory trials (Tables 2.3 and 2.5, Figure 2.2), and a high proportion of tagged fish were detected (63 out of 70). Some detected smelt (8) were excluded from analysis because of possible tagging mortality, yet the remaining proportion of assumed live tagged fish (55 out of 70, 79%) provided useful movement data. Conclusions in regard to behavior by gender and study year remain valid because the proportion detected did not vary by study year (p = 0.424), or gender (p = 0.0147).

In laboratory trials, mortality increased over time for smelt tagged with the V6 transmitter. Where no mortality was observed before week-3, 10% mortality was recorded at week-6, and 30% at week-9 (Figure 2.2). Increasing mortality over time was likely due to starvation rather than tagging. Wild smelt are known to refuse commercial fish feed (H. Colburn, UNH, pers. comm.), and therefore withholding feed for the first 8 weeks of the study was thought to entice appetite. Unfortunately, at week 8 many of the study smelt had already lost most of their body mass and continued to refuse food. In wild populations, feeding may be a secondary behavior during the spawning season, but feeding is likely to remain active throughout the period similar to other anadromous species (*Alosa aestivalis*; McBride *et al.* 2010). Based on these laboratory trials and the high proportion of tagged smelt (55 out of 70) that were detected and determined not to be immediate mortalities in the field study, acoustic telemetry transmitters were determined to provide valuable data to describe adult rainbow smelt movement.
In summary, acoustic telemetry provided useful data about the movements and relative habitat use by rainbow smelt during and following the spawning period in the Great Bay and Piscataqua River estuary complex, NH. Movement between multiple spawning rivers did not vary by gender or between smelt released from different spawning locations. Final detection locations provide some evidence that some post-spawn smelt may remain within the embayment through the summer months, but future studies, including otolith microchemistry and mid-water trawling, would provide better support for this conclusion. Temporal use of the study area differed among locations: use of spawning rivers occurred in April, use of the lower Piscataqua River and movements into coastal waters occurred in mid-May to June, and use of Adams Point, the geographic choke point between Great Bay and the Piscataqua River embayment, was used most frequently and over the longest period of time April – June.
CONCLUSIONS

This study quantified within-season repeat spawning behavior by rainbow smelt at two spawning sites in the Gulf of Maine and described movements and habitat use during and following the spawning season in the Great Bay and Piscataqua River estuary complex, NH. Repeat spawning behavior was found to be a predominantly male behavior, consistent with past studies (Langlois 1935, Hoover 1936, McKenzie 1964, Rupp 1968, Clayton 1976, Murawski et al. 1980). The rate of repeat spawning by males was consistent among years and between two study sites. Regarding larger scale movements, smelt of both genders visited multiple rivers within an embayment during the spawning season, and further, made use of a tidal estuary system after spawning activity ceased.

The results from the first study provide insight into the underlying population structure of different spawning populations. If the rate of repeat spawning by males is consistent among populations, comparing sex ratio among sites can be one tool to identify stressed populations. At sites with comparatively higher sex ratios, the number of spawning females will be limited, which can lead to population decline over time. The results from the second study provide information about movement between river systems, habitat use, and post-spawning movements between estuarine and coastal waters. Considered together, these studies offer more detailed information regarding
smelt life history and behavior. The results may be utilized by fisheries managers to inform analyses of survey data and to identify areas of important habitat.

Extending the conclusions of these results beyond the study areas may be appropriate in some situations, but should take into account several considerations. Several genetic populations of rainbow smelt have been described for the U. S. Gulf of Maine region (Kovach et al. 2012), and further, divergent populations have been documented within a single river system (Bradbury et al. 2006, Coulson and Bentzen 2009). In the latter case, patterns in genetic divergence have been correlated to differences in behavior including spawning site selection and spawning timing (Coulson et al. 2006). It cannot be assumed, therefore, that the behaviors described in these studies apply to all populations.

These studies provided information about spawning and post-spawning adult rainbow smelt, but not information about marine habitat use or areas important for larval development. Effective management of this species of concern must consider these other life stages. Marine predation, prey availability, bycatch within small mesh fisheries (e.g. shrimp, Atlantic herring, and white hake), and range truncation due to climate change may affect smelt populations within the marine environment. While current near-shore trawl surveys provide some information about smelt presence in marine waters, the survey does not target pelagic species and may occur too far offshore to accurately show smelt presence. Considering larval and juvenile habitat, seine surveys are performed in only two locations within the extant range of rainbow smelt, the Great Bay and Piscataqua estuary complex, NH and the Kennebec Estuary, ME. These locations are
somewhat unique in the region as large tidal estuaries. The majority of smelt spawning, and in turn larval development areas, are within smaller coastal streams and rivers.

The behavior and movement data reported here as well as on-going fisheries independent surveys show that the estuarine environment and coastal embayments are important habitats to spawning and post-spawn smelt. Historic and recent population declines may have been influenced by coastal development and degradation of these habitat areas in mid-Atlantic and southern New England states. Further study is necessary to determine marine habitat types and temporal use, and the threats that may impact smelt while in marine waters.
LIST OF REFERENCES


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APPENDIX A – IACUC APPROVAL
The Institutional Animal Care and Use Committee (IACUC) reviewed and approved the protocol submitted for this study under Category D on Page 5 of the Application for Review of Vertebrate Animal Use in Research or Instruction - Animal use activities that involve accompanying pain or distress to the animals for which appropriate anesthetic, analgesic, tranquilizing drugs or other methods for relieving pain or distress are used.

Approval is granted for a period of three years from the approval date above. Continued approval throughout the three year period is contingent upon completion of annual reports on the use of animals. At the end of the three year approval period you may submit a new application and request for extension to continue this project. Requests for extension must be filed prior to the expiration of the original approval.

Please Note:
1. All cage, pen, or other animal identification records must include your IACUC # listed above.
2. Use of animals in research and instruction is approved contingent upon participation in the UNH Occupational Health Program for persons handling animals. Participation is mandatory for all principal investigators and their affiliated personnel, employees of the University and students alike. A Medical History Questionnaire accompanies this approval; please copy and distribute to all listed project staff who have not completed this form already. Completed questionnaires should be sent to Dr. Gladis Porche, UNH Health Services.

If you have any questions, please contact either Dean Elder at 862-4629 or Julie Simpson at 862-2003.

For the IACUC,

Jessica Bolker, Ph.D.
Chair

cc: File