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Assessment of a Hatchery Based Rainbow Smelt Supplementation Effort

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ASSESSMENT OF A HATCHERY BASED RAINBOW SMELT SUPPLEMENTATION EFFORT

By

Andrew James O'Malley

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THESIS ACCEPTANCE STATEMENT

On behalf of the Graduate Committee for Andrew J. O'Malley, I affirm that this manuscript is the final and accepted thesis. Signatures of all committee members are on file with the Graduate School at the University of Maine, 42 Stodder Hall, Orono, Maine.

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ASSESSMENT OF A HATCHERY BASED RAINBOW SMELT

SUPPLEMENTATION EFFORT

By Andrew O'Malley

Thesis Advisor: Dr. Joseph Zydlewski

An Abstract of the Thesis Presented
in Partial Fulfillment of the Requirements for the
Degree of Master of Science
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Rainbow Smelt (*Osmerus mordax*) are an important fish distributed throughout northeastern North America with both anadromous and landlocked populations. Abundance, size at age, and maximum size vary widely among populations and life histories. In order to compare anadromous and landlocked populations, we collected spawning adults in 2014 from four anadromous and three landlocked populations. Scales and otoliths from the anadromous fish were examined and compared for estimates of bias and precision in ageing. Analysis of both scales and otoliths provided age estimates that were acceptable, but estimates from scales were more precise and had less bias. Otoliths were used to estimate mean size at age and von Bertalanffy growth parameters for each population. Compared to landlocked populations, anadromous fish exhibited a greater and more variable size at age, and asymptotic size. While anadromous fish generally grew faster than landlocked fish, von Bertalanffy growth parameters were variable across life histories. Age analysis showed that populations of both anadromous and landlocked

Rainbow Smelt were comprised of fish age 1 to 4, and were typically dominated by a single age class. These data suggest considerable plasticity associated with tradeoffs between growth and reproduction among different populations and life histories.

Commercially reared Rainbow Smelt larvae have recently become available for supplementation for this species that is known to be highly variable in abundance. We stocked smelt larvae into two small ponds in central Maine at a density of approximately 30,000 fish per hectare to assess survival of hatchery reared fish. Fish were double marked with thermal and oxytetracycline marks. We subsequently sampled for stocked larval Rainbow Smelt with ichthyoplankton tows, both day and night for the first four weeks after stocking, capturing more than 1,800 Rainbow smelt in one pond, and two in the other. Capture rate was higher at night than the day, and decreased over the duration of the study. Otoliths were examined from a subset of 339 larval Rainbow Smelt. The median hatch date of all fish was two days after the observed hatch date of our stocked fish. The mean daily growth rate was calculated to vary from a low of 0.3 mm per day at 7 days after hatching, to a high of 0.5 mm per day at 14 days after hatching. There were no distinct marks consistent with oxtretracycline marking found on any of the larval Rainbow Smelt otoliths examined. Potential thermal marks and stocking checks were found on otoliths from 80% and 45% of fish examined respectively. Larval Rainbow Smelt density and distribution was estimated with a linear model and was significantly related to depth, time of day, and sample event. This model was to estimate the population and mortality of Rainbow Smelt. The large difference between the observed and predicted catches of Rainbow Smelt in one of our study waters lends evidence to the poor success of stocking on this water.

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This project would not have been possible without the support and expertise of John Whalen and the Maine Smelt Hatchery division of Harmon Brook Farm. John's fish rearing capabilities and expertise gathered through a lifetime of careful observation allowed us to stock literally millions of smelt larvae in accordance with our objectives. He spent some long nights spawning and caring for fish to make this project possible.

Many nights of sleep were forgone and long hours were spent staring through microscopes by research technicians for this project. Special thanks go out to Graham Griffin, Cris Introne, Josh and Joe Kocik, Rebecca Fontes, Cody Kennedy, and Heather Brinson, and the other graduate and undergraduate volunteers associated with the Maine Cooperative Fish and Wildlife Research Unit.

Anadromous Rainbow Smelt were provided by staff from the Maine Department of Marine Resources. Support for this research was from the Maine Cooperative Fish and Wildlife Research Unit, Orono Maine, the Maine Department Inland Fisheries and Wildlife, Augusta Maine and the Maine Outdoor Heritage Fund. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government. This study was performed under the auspices of University of Maine IACUC protocol A2013-02-04.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

SIZE AND AGE STRUCTURE OF ANADROMOUS AND LANDLOCKED POPULATIONS OF RAINBOW SMELT (OSMERUS MORDAX)

Chapter Abstract

Rainbow Smelt *Osmerus mordax*, are an important fish distributed throughout northeastern North America with both anadromous and landlocked populations. Abundance, size at age, and maximum size vary widely among populations and life histories. To compare anadromous and landlocked populations, we collected spawning adults in 2014 from four anadromous and three landlocked populations. Scales and otoliths from the anadromous fish were examined and compared for estimates of bias and precision in ageing. Otoliths were used to assess age and growth for both anadromous and landlocked populations. Whereas analysis of both scales and otoliths provided age estimates that had acceptable levels of precision and bias, estimates from scales were more precise and had lower bias. Mean size at age and von Bertalanffy growth parameters were estimated for each population. Anadromous fish exhibited a greater and more variable size at age and asymptotic size than landlocked populations. Age analysis determined that populations of both anadromous and landlocked Rainbow Smelt were comprised of fish ages 1 to 4, and were typically dominated by a single age class. These data suggest considerable plasticity in characteristics associated with tradeoffs between growth and reproduction among different populations and life histories.

Introduction

Rainbow Smelt *Osmerus mordax* are widely distributed throughout northeastern North America and are targeted by commercial and recreational fisheries. Ecologically these fish are a major food source for other piscivores (Havey 1973; Sayers et al. 1989). Rainbow Smelt have a short life cycle and high fecundity often resulting in highly variable abundance (Gorman 2007; Stritzel Thomson et al. 2010). Populations of Rainbow Smelt exhibit flexibility in life history strategies, either as anadromous fish along the coast, or landlocked fish in cold lakes (Nellbring 1989). Anadromous populations of Rainbow Smelt were once found along the coast from Labrador, Canada to New Jersey, U.S.A. but have experienced a northward range contraction of 500 kilometers in the last 200 years (Scott 1973). This is likely attributable to a suite of anthropogenic perturbations including pollution, loss of spawning habitat, and fishing pressure, as this region is one of the most heavily developed areas of the continent (McKenzie 1947; Brown and Taylor 1995; Fuda et al. 2007). Contemporarily, resilient anadromous populations are only found from Maine northward, while the populations to the south are in decline.

Within Maine, landlocked Rainbow Smelt occur naturally in a few lakes along coastal areas that were accessible at the end of the last ice age (approximately 13,000 years before present). In contrast to the anadromous populations, landlocked populations have proliferated in recent times (Nellbring 1989). They have spread throughout the Great Lakes, as well as many smaller waters in the Hudson Bay and Mississippi River watersheds as the result of intentional and unintentional introductions (Kendall 1918; Evans and Loftus 1987; Mercado-Silva et al. 2006). These introductions are often the progeny of anadromous adults, no distinction has been made to the success of stocking from anadromous or landlocked sources (Bridges 1971). This range expansion to new lakes has been associated with significant ecological and economic impacts (Havey 1973; Hrabik et al. 1998). Rainbow Smelt can outcompete some native species such as yellow perch *Perca flavescens* and cisco *Coregonus artedii* while simultaneously providing forage for other native and introduced species such as Atlantic and Pacific salmon *Salmo salar, Oncorhynchus* spp. (Hoover 1936a; Havey 1973; Hrabik et al. 1998).

Both anadromous and landlocked Rainbow Smelt exhibit sexual dimorphism, with female Rainbow Smelt being longer lived and a larger size at age then males (McKenzie 1958; Bailey 1964). In many populations, the males mature a year earlier than the females (McKenzie 1958; McKenzie 1964; Nellbring 1989). Numerous studies have reported that sex distributions are heavily skewed towards females in older age classes (McKenzie 1958; Bailey 1964; Murawski and Cole 1978). The higher mortality of males is likely attributable to the younger age at maturity and the longer duration of time spent on the spawning grounds during which they incur a higher risk of predation and experience a large amount of stress related to spawning activities (Hoover 1936b; Murawski et al. 1980; Schaefer et al. 1981).

Within and among populations, age at maturity can differ widely. Some landlocked populations of Rainbow Smelt may be dominated by age 1 spawners, while other runs are comprised of individuals ages 2 – 4 (McKenzie 1958; Murawski and Cole 1978). Whereas Rainbow Smelt ages 4 and older make up only a small proportion of the population, a few individuals have reached age 8 (Bailey 1964; Kirn and Labar 1996). Also, older and larger fish spawn earlier in the run, with both overall size and size at age of returning fish decreasing throughout the duration of the spawning run (McKenzie 1958; Bailey 1964).

Rainbow Smelt exhibit a wide range of maximum body size both within and among populations (Beckman 1942; McKenzie 1958; Rupp and Redmond 1966; Kirn and Labar 1996). The non-linear relation between size and fecundity means that maximum size has important implications for both eggs produced and recruitment (McKenzie 1964; Feiner et al. 2015). Additionally, body size plays an important role in prey selection and predation risk (McCullough and Stanley 1981; Lantry and Stewart 1993; Kirn and Labar 1996). There exists a large difference in growth potential between the highly productive marine environment and the oligotrophic lakes in which landlocked populations reside. Anadromous Rainbow Smelt are able to exploit the greater gross productivity of coastal waters and attain a greater body size than landlocked Rainbow Smelt (Rupp 1959; Murawski and Cole 1978). These differences in growth opportunity and growth rates are important to understand when characterizing size and age of Rainbow Smelt populations in different locations.

The differences in size, age, and longevity of Rainbow Smelt from different populations can be important background information for management considerations. Age and growth can be obtained from retrospective aging and measuring of previous growth via hard parts such as scales, otoliths, and fin rays (Campana and Thorrold 2001). Rainbow Smelt, as in many species of fish can show distinct patterns within these hard structures (Brooks et al. 1994; Volk et al. 1994; Campana 1999). These patterns of growth can correspond to daily or seasonal patterns of growth (Sepulveda 1994; Walsh et al. 2008). The accuracy and precision of these measurements can vary between structures and between populations (Walsh et al. 2008). It is important that these measurements of age and growth be as precise, accurate, and easily measured as possible (Secor et al. 1991a; McBride 2015). Scales are the most commonly used structure for ageing Rainbow Smelt. This procedure is well described by McKenzie (1958). Walsh et al. (2008) compared fin rays with whole cleared and uncleared otoliths. Their conclusion was that fin rays were superior to whole otoliths, however, the small size of fin rays and damage to the fish makes them a less desirable method to use. Sectioned sagittal otoliths have not been compared with scales or fin rays for Rainbow Smelt, but are commonly used for many other species of fish (Power 1978).

We used age estimates from scales and sectioned otoliths to compare accuracy and precision of ageing Rainbow Smelt. In addition, we sought to compare the growth, maximum size, and age structure of Rainbow Smelt between and among both freshwater and landlocked populations in Maine.

Methods

Anadromous fish collection

Anadromous Rainbow Smelt were captured from four coastal streams with naturally occurring spawning populations of Rainbow Smelt: Mast Landing (A), Deer Meadow Brook (B), Tannery Brook (C), and Schoppee Brook (D; Figure 1). The study streams are separated from each other by 70 – 100 km and span the coast of Maine. For the purposes of this paper, the fish in each stream are considered separate spawning populations, although the only barrier to movement between locations is distance.

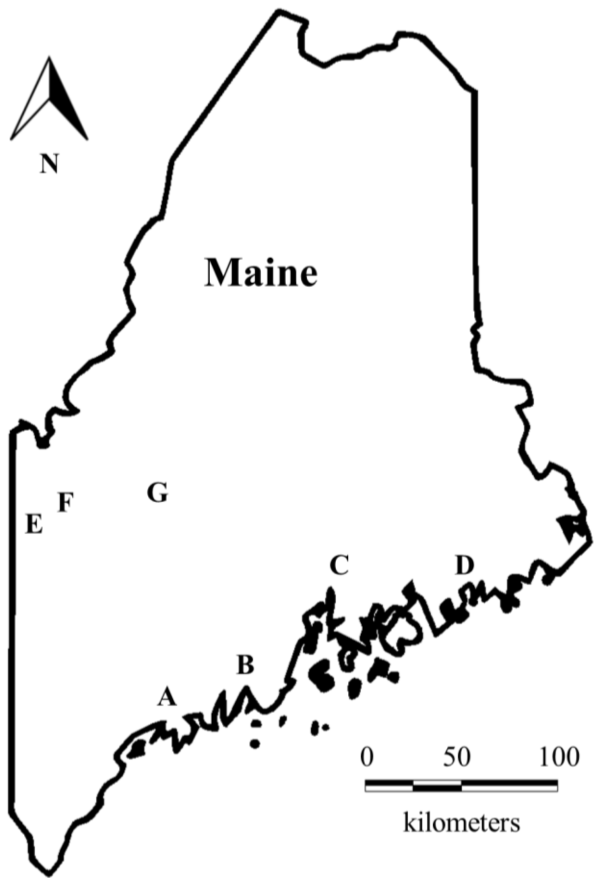

Figure 1. Sample collection locations for four anadromous populations: Mast Landing (A), Deer Meadow (B), Tannery Brook (C), Schoppee Brook (D), and three landlocked populations: Wyman Lake (E), Rangeley Lake (F), and Richardson Lake (G) of Rainbow Smelt collected within the state of Maine, U.S.A during the spring of 2014.

Anadromous Rainbow Smelt were collected with fyke nets set in the intertidal zone near the mouth of each brook. The nets were set mid-channel with the opening facing downstream. Nets were checked during morning low tide to record the catch during the previous high tide. The fyke nets were tended three consecutive days a week for the duration of the 2015 spawning season, and were closed to fish entry for the remainder of the week (Table 1). These nets are operated annually as an established long-term monitoring project for Rainbow Smelt (C. Enterline, unpublished data). Total length was recorded from the first 100 males and 100 females each day, and a count was made for all remaining individuals. A subsample of these fish was sacrificed for use in this study. Up to 15 fish of each sex were collected from each of three size bins (<15 cm, 15 – 20 cm, >20 cm total length) based on presumed ages from previous work (Enterline, unpublished data), allowing for up to 90 Rainbow Smelt collected per population (Table 1). We attempted to stratify fish collection throughout the run by not collecting more than five fish per size and sex category per week. Each fish was individually labeled, stored, and frozen until processing.

Landlocked fish collection

We collected landlocked Rainbow Smelt from three lakes: Rangeley (E), Richardson (F) and Wyman lakes (G; Figure 1). All three lakes are large, oligotrophic lakes located in Western Maine (Table 2). Rangeley and Richardson lakes are both part of the Androscoggin River watershed, but separated by enough distance (23 km) that we assume that the two populations are isolated from one another. Rainbow Smelt became established in these lakes around 1900 from an undocumented source (Cooper 1940). The third lake, Wyman Lake, is a reservoir in the Kennebec River watershed and is isolated from the other two lakes. The impoundment was created in the 1930's with Rainbow Smelt likely becoming naturally established around the 1950's from other landlocked populations located upstream (R. VanRiper, Maine Department of Inland Fisheries and Wildlife, unpublished data).

Table 1. Count of anadromous Rainbow Smelt sampled from four coastal streams: Mast Landing (A), Deer Meadow (B), Tannery Brook (C), Schoppee Brook (D) during spring 2014. Included are the start and end dates of the survey, the number of fish sacrificed for age and growth analysis and total number of fish measured for modeling year class contribution to the run. The fish collected for the age and growth analysis are broken down by sex (male (M), female (F) or immature/unknown (U)), and three length bins (<150, 150 – 200, and >200 mm) to stratify sampling across observed sizes.

Source	UTM	Sample Dates	Sex	Number Aged by Size			Total Number Measured
				< 150 mm	150 - 200 mm	> 200 mm	
Mast Landing (A)	4856550 m N, 412770 m E	4/15-5/20	M	20	3	5	65
			F	15	1	2	31
			U	2	0	0	4
Deer Meadow (B)	4876300 m N, 453000 m E	4/14-5/29	M	12	18	11	393
			F	7	17	7	50
			U	5	0	0	5
Tannery Brook (C)	4935300 m N, 516780 m E	4/15-6/19	M	18	16	2	500
			F	18	17	6	223
			U	2	0	0	2
Schoppee Brook (D)	4946500 m N, 614730 m E	4/29-6/26	M	15	21	11	1009
			F	2	16	20	532
			U	0	0	0	172

Table 2. Count and location of landlocked Rainbow Smelt collected from tributaries to Wyman (E), Rangeley (F), and Richardson (G) lakes during spring 2014. Included are the dates of collection, the number of fish sacrificed for age and growth analysis, and total number of fish measured for modeling of year class contribution to the run. Sex was recorded as male (M), female (F) or unknown/immature (U). Also included are the coordinates, area, and max depth of the lakes.

Source	UTM	Area (ha)	Max depth (m)	Sample dates	Sex	Number aged	Total number measured
Wyman (E)	5000000 m N, 425000 m E	736	42.7	4/27-4/29	M	95	95
					F	78	78
					U	0	0
Rangeley (F)	4978000 m N, 370000 m E	2550	45.4	5/6	M	89	103
					F	75	75
					U	0	197
Richardson (G)	4973000 m N, 350000 m E	3137	33	5/5	M	106	134
					F	81	82
					U	0	272

In these lakes, collection sites were located in a single spawning tributary just upstream from the confluence with the lake. We captured fish via dip net during 1-3 consecutive nights near the peak of the spawning run in the spring of 2015 (Table 2). All captured Rainbow Smelt were transported live to a commercial fish hatchery for gamete extraction. After spawning, all Rainbow Smelt were non-selectively frozen in batches of 10-20 individuals. A target subsample of 200 fish per site was taken by sampling all individuals in selected bags (Table 2).

Length correction

After defrosting, fish were sexed and measured for total and standard length. Total length of fish decreased by approximately 5% after freezing (n = 58, r^2 = 0.996, *P*-value < 0.01). The total length before freezing was used for the computations throughout the remainder of this paper. If the total length before freezing was missing, it was estimated from the standard length after freezing using a simple linear regression (n = 792, $r^2 = 0.994$, P-value < 0.01).

Otolith removal, preparation, and reading

Sagittal otoliths were removed via the "up through the gills method" by cutting through the gill isthmus then breaking through the prootic bone to remove the sagittae (Secor et al. 1991a). Otoliths were cleaned with a 10% sodium hypochlorite solution to remove soft tissue, placed in deionized water to remove the bleach, and then allowed to dry (Secor et al. 1991a). Otoliths were mounted in a two-part epoxy for sectioning (Epo-Fix™, Electron Microscopy Sciences). A transverse section encompassing or close to the primordium was taken from each otolith using a slow speed saw (IsoMet™, Buehler). The sections were mounted on microscope slides using thermoplastic glue (Crystalbond™, Structure Probe), and imaged with a digital camera (Spot Insight 2, Spot Imaging Solutions) attached to a stereo microscope (EMZ-13TR, Meiji Techno) and viewed under transmitted light at 30x magnification. Immersion oil was used to improve the contrast of the otolith and obviate sanding.

Otolith reading and measurement

When possible, the left otolith was used for all fish (n = 726), but if this otolith was damaged or unreadable then the right one was used (n = 118). If neither otolith was readable, the fish was excluded from the growth analysis (n = 3). The otoliths in this study display two distinct regions of growth when viewed under transmitted light: a wide, opaque continuous zone that corresponds to summer growth, and a narrowed, translucent, discontinuous zone of winter growth. Each pair of continuous and discontinuous zones represent one complete year of growth. Each annual growth increment was measured to the outer edge of the discontinuous zone, which is assumed to correspond with the onset of spring growth. Because fish were captured in early spring, they did not show signs of a partial growing season. These fish were captured during spawning so all annuli are assumed to represent a complete year's growth.

Two readers aged one otolith from each fish. After aging was complete, otolith images were measured using ImageJ imaging software (version 1.48, Research Services Branch, National Institute of Health). The annual growth increments were measured along a straight line from the centrum to dorsal margin of the otoliths and calibrated using a stage micrometer.

Scales reading for anadromous fish

In addition to reading otoliths from anadromous fish, scales were also read. Scales were cleaned in a sonicator (Model 32V118, Lab Safety Supply) while immersed in a 5% pancreatin solution (NOW FOODS) as described by Whaley (1991). Scales were mounted on glass slides with a coverslip and read under a microscope with transmitted light. Ageing of scales followed the methods of McKenzie (1958), using "shiny lines" and incomplete circuli as the primary indicators of annuli. The same two readers aged both the scales and the otoliths.

Assignment and analysis of ages

Both readers examined all scales and otoliths to make initial age estimates for each structure independently. After the initial ageing, if both readers agreed upon the age of a particular structure, it was given a structure consensus age. Secondly, the following decision tree was used to assign a consensus age to the fish, which was used in lieu of a true age. If all four assigned ages matched (both readers for both structures), or if three of four matched and the remaining age differed by no more than one year, the fish was given a consensus age matching the majority of the assigned ages. If only one structure was present, and both of those readings agreed, a consensus age was assigned. For fish that did not show agreement between the readers, the consensus age was estimated using all information available, which included which population the fish was from, body length, and sex. This consensus age for the fish was used in comparisons of ages from each reader to the fish consensus age. Comparison of ages between scales and otoliths from the same fish utilized only fish where the readers agreed on the age of each structure.

Precision and bias between the two readers and between scales and otoliths were determined by the FSA package (version 0.8.4, D. Ogle, personal communication; available at www.fishr.wordpress.com/fsa/) using Program R statistical software (version 3.2.0, R Core Team; available at www.r-project.org). Precision and bias both between readers for a given structure, and between scales and otoliths were examined using the average coefficient of variation (*ACV*) for precision and a Bowker's test of symmetry for bias (Bowker 1948; Chang 1982). The critical value for a statistical difference was set at P-value ≤ 0.05 for bias, and $ACV < 5.0\%$ (McBride 2015).

Size at age and von Bertalanffy growth

We estimated individual size at age for both anadromous and landlocked fish using the Fraser-Lee back-calculation method and rounded the results to the nearest millimeter (Lee 1920). Growth trajectories for size at age (L_t) were estimated for each population using a von Bertalanffy growth function (von Bertalanffy 1938), expressed as:

$$L_t = L_\infty[1 - e^{-K(t-t_0)}]$$

Three parameters: maximum size (L_∞), intercept age (t_0), and growth rate (K) were estimated for males and females for each population and compared using a log likelihood ratio test for differences. These parameter estimates were made using the Fishmethods package for R software (version 1.7-0, G. Nelson, personal communication; available at www. cran.r-project.org/web/packages/fishmethods).

Bayesian mixture models of age class contribution to spawning

The aged fish are just a subsample of those captured and aged from each of the anadromous and landlocked populations. The distribution of sizes for all fish measured was different the distribution of sizes for the aged subsample of fish. We used a Bayesian mixture model to estimate the proportion by age of all captured fish. The distribution of total lengths of the population was modeled as a weighted mixture of the observed age classes as:

$$f(y) = \sum_{1}^{i} \pi_i f_i(y)$$

where π_i is the age class proportion and $f_i(y)$ are the total length probability density functions for each age class observed. The mixture model was implemented in a Bayesian framework using the Mixdist package for R software (version 0.5-4, P. Macdonald and J Du, personal communication; available at www. cran.r-project.org/web/packages/mixdist). Model parameters were the mean (μ_i) and standard deviation (σ_i) for each age class (i) and the proportion of the measured fish belonging to each age class (π_i). Two populations had a single age 1 individual, so the mean standard deviation for age from the other populations was used as a prior. Uncertainty for each parameter was characterized by an estimated 95% credible interval. Parameters were estimated using an expectation maximization algorithm.

Results

Precision and Bias between readers and structures

A global test for bias combining all populations of Rainbow Smelt found a statistical bias (*P*-value < 0.03) between the two readers for both scales and otoliths, as well as between scale and otolith consensus ages (Table 3). Readers tended to underestimate the age from scales when compared with estimates from otoliths for fish age 3 and older (Figure 2). The two readers showed biases in opposite directions but similar magnitudes for both scales and otoliths (Figure 3). This bias was stronger for otoliths than for scales. Readings between scales and otoliths, and between readers for each structure, still had a high precision with an *ACV* ≤ 5% (Table 3).

Table 3. Comparison of estimated ages from scales vs. otoliths from four anadromous populations, and between two readers for four anadromous and three landlocked populations of Rainbow Smelt using a Bowker's test of symmetry and average coefficient of variation (*ACV*). All Rainbow Smelt were collected in Maine, Spring 2014. Asterisks denote significance levels; *P*-value < 0.05*, *P*-value < 0.01**.

Comparison	Bowker's Test p-value	ACV (%)	Sample size
Scales vs. Otoliths	0.03*	2.9	168
Reader 1 vs. Reader 2			
Scales	0.04*	2.3	263
Otoliths	<0.01**	5.0	834

After determining there was an overall bias between readers, the bias in ages between readers for each population was assessed. Individual populations had a smaller sample size, but a bias between readers was detected (*P*-value < 0.05) in one in four comparisons for scales, three of four comparisons for anadromous otoliths, and one of three comparisons for landlocked otoliths, (Table 4). Precision was generally high (0 – 7.5% *ACV*) with only four of 22 comparisons having an *ACV* > 5% between a reader and the consensus age (Table 4); (McBride 2015). All comparisons with low precision were from anadromous fish, one from scales and the other three from otoliths. Precision was highest for otoliths from landlocked fish, followed by scales from anadromous fish, and finally otoliths from anadromous fish. High precision is indicated by the mean of the *ACV* for each for each series of comparisons (Table 4).

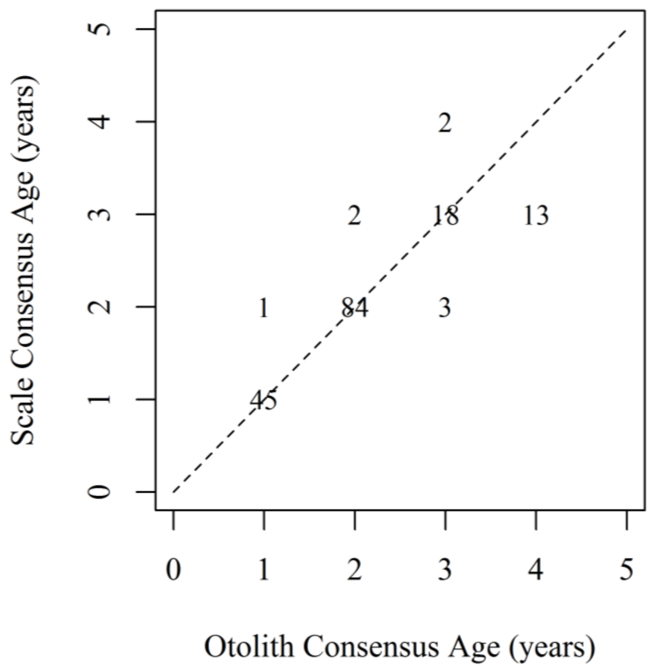

Figure 2. An age bias plot for consensus scale and sectioned otolith ages (in years) from four anadromous populations of Rainbow Smelt collected during spawning in Maine, spring 2014. Two readers aged each structure. If readers did not agree on the apparent age of the structure, the fish was excluded from the analysis. The dotted line represents the 1:1 line for agreement between the ages.

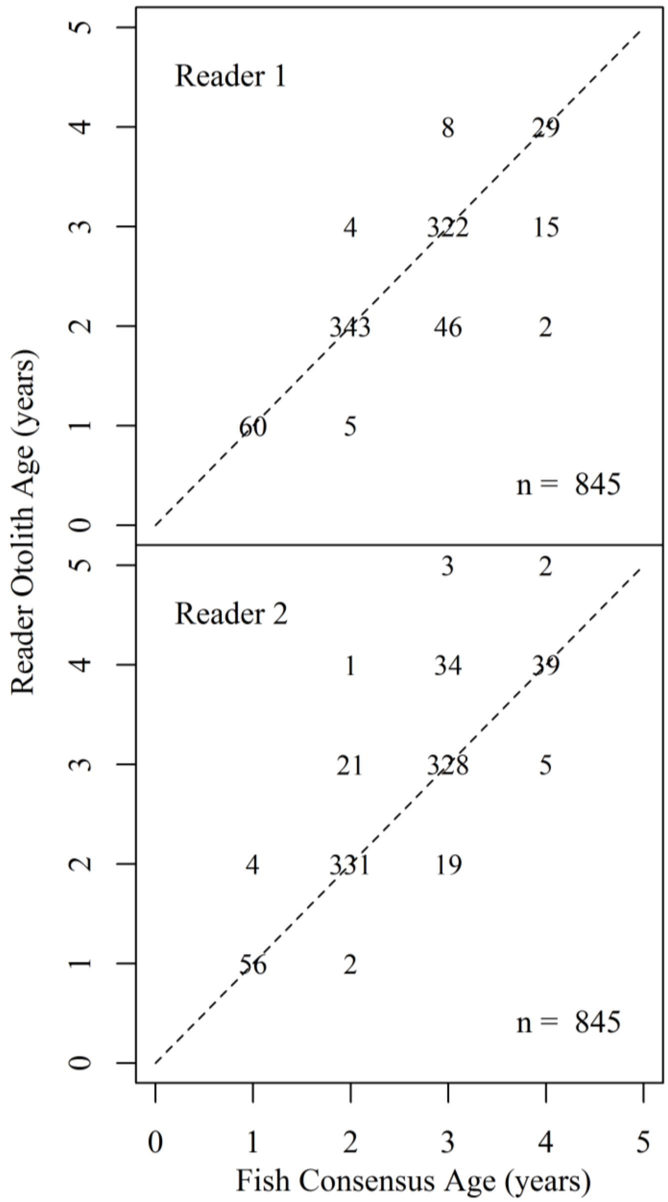

Figure 3. Age bias plots for ages (years) estimated from otoliths compared with fish consensus age from seven populations of Rainbow Smelt collected during spawning in Maine, spring 2014. Consensus ages were reached via agreement of scales, otoliths, and an age-length key. The dotted line represents the 1:1 line for agreement between the ages. Reader 1 shows a bias toward underestimating age, Reader 2 shows a bias toward over estimating age. These biases were consistent across populations.

Table 4. Tests of symmetry and bias in ageing for two readers of scales and otoliths collected from four populations of anadromous (Anad.) and three populations of landlocked (LL.) Rainbow Smelt captured during spawning in Maine, spring 2014. Test results include a Bowker's test of symmetry to test for bias between readers, and the average coefficient of variation (*ACV*) between each reader and the consensus age for the fish, and the sample size. Asterisks denote significance levels; *P*-value < 0.05*, *P*-value < 0.01**.

Life strategy	Structure	Source	Bowker's Test p-value	ACV (%)		Sample size
				Reader 1	Reader 2	
Anad.	Scales	A	0.317	1.9	3.7	51
		B	0.416	5.0	3.4	69
		C	0.135	0.3	1.4	67
		D	0.030*	5.9	4.4	76
			Average:	3.3	3.2	66
	Otolith	A	0.513	1.9	0.0	48
		B	0.025*	5.7	7.5	76
		C	0.006**	3.0	4.7	79
		D	<0.001**	4.6	6.1	85
			Average:	3.8	4.6	72
LL.	Otolith	E	<0.001**	3.7	2.3	164
		F	0.083	1.5	2.6	198
		G	0.102	0.8	0.1	184
			Average:	2.0	1.7	182

Back calculated growth

Size at age was back-calculated using the intercept corrected proportional method. The slope and intercept were estimated as 0.0073 mm and 0.241 mm, respectively, from the ratio of otolith radius to total body size from 846 Rainbow Smelt (r^2 = 0.843, *P*-value < 0.001). Rainbow Smelt show variation in the size at age both among populations and between ecotypes. Anadromous fish are characterized by a greater body size and have more variation among individuals than those from landlocked populations (Table 5).The mean back-calculated size at age agrees closely with observed sizes for ages 2 – 4 (Table 5). Calculated size at age 1 had a larger departure between observed and calculated, with a departure of up to 26 mm and a mean of 15.6 mm across populations (Table 5). This shows an occurrence of a positive Lee’s phenomenon (Lee 1920).

Our mean back-calculated size at age for each of the anadromous populations was relatively similar. One anadromous run, population C had a smaller size at age 1 than the others, but this difference decreased in older age classes (Table 5). The most southerly run, population A was much larger at ages 1 and 2 than the other anadromous runs but this difference decreased in older age classes as well (Table 5). In contrast, the three landlocked populations showed variation in size at age 1 and these relative magnitudes of these differences were maintained throughout all age classes observed (Table 5).

Von Bertalanffy growth modeling

As we had individual growth data from several different populations and two different life history strategies, we sought to determine growth rate and asymptotic size to characterize variability among different populations. These populations demonstrate a large difference in size at age (Table 6).

Back-calculated size at age produced growth trajectories for each individual fish. These data were used to fit a von Bertalanffy growth model to each population. Model parameters were estimated for male and female Rainbow Smelt from each population separately. A stepwise model selection was used to test for statistically significant

Table 5. The mean ± SD observed size at age and difference between observed and back-calculated size at age for ages 1 – 4 of Rainbow Smelt from four anadromous runs (Anad.) and three landlocked runs (LL.) captured during spawning in Maine, spring 2014. The number of fish observed and back-calculated sizes from is below the observed sizes and differences, respectively. A positive value indicates observed size at age was larger than the back-calculated size. Bolded values are back-calculated size at age for ages where no fish were available for observed size at age. Asterisks denote significance levels; *P*-value < 0.05*, *P*-value < 0.01**.

Life strategy	Source	Age 1		Age 2		Age 3		Age 4	
Anad.	A	135 ± 13 (45)	1 (48)	198 ± 5 (5)	13 (9)	216 ± 13 (2)	16 (4)	216 ± 16 (2)	0 (2)
	B	111 ± 17 (17)	17** (77)	158 ± 20 (22)	5 (60)	190 ± 16 (30)	3 (38)	208 ± 12 (9)	0 (9)
	C	100 ± - (1)	25 (79)	145 ± 17 (54)	-1 (78)	185 ± 20 (22)	0 (24)	208 ± 2 (2)	0 (2)
	D	105 ± - (1)	9 (85)	168 ± 19 (42)	7 (84)	200 ± 15 (26)	8 (42)	215 ± 13 (16)	0 (16)
LL.	E	111 ± 5 (3)	26* (173)	137 ± 7 (18)	8** (170)	153 ± 8 (148)	0 (152)	180 ± 27 (4)	0 (4)
	F	**66 ± 10** **(198)**	- -	121 ± 8 (154)	3** (198)	127 ± 6 (41)	1 (44)	130 ± 2 (3)	0 (3)
	G	**52 ± 10** **(186)**	- -	100 ± 5 (60)	8** (186)	106 ± 5 (116)	0 (126)	114 ± 6 (10)	0 (10)

Table 6. Estimated von Bertalanffy growth parameters for four anadromous (Anad.) and three landlocked (LL.) populations of Rainbow Smelt collected in Maine, spring 2014. Estimates of asymptotic length ($L\infty$), growth rate (K), and intercept value (t_0) and ± 1 SE are presented. Models were run for all fish combined (A), and for females (F) and males (M) separately. Sample size for each model is in parentheses.

Life Strategy	Source	Sex	L_∞	K	t_0	Sample size
Anad.	A	A	216 ± 24	0.9 ± 0.6	0.0 ± 0.5	(54)
		♀	301 ± 183	0.3 ± 0.4	-1.3 ± 1.6	(22)
		♂	199 ± 15	1.7 ± 1.2	0.3 ± 0.4	(30)
	B	A	237 ± 25	0.5 ± 0.1	0.1 ± 0.1	(78)
		♀	238 ± 32	0.5 ± 0.2	0.0 ± 0.2	(31)
		♂	237 ± 37	0.5 ± 0.2	0.1 ± 0.2	(42)
	C	A	231 ± 24	0.6 ± 0.1	0.4 ± 0.1	(79)
		♀	250 ± 36	0.5 ± 0.2	0.3 ± 0.1	(41)
		♂	199 ± 23	0.8 ± 0.3	0.4 ± 0.1	(36)
	D	A	232 ± 14	0.6 ± 0.1	0.2 ± 0.1	(85)
		♀	240 ± 20	0.6 ± 0.1	0.1 ± 0.1	(38)
		♂	215 ± 17	0.8 ± 0.2	0.3 ± 0.1	(47)
LL.	E	A	186 ± 8	0.6 ± 0.1	-0.1 ± 0.1	(173)
		♀	200 ± 17	0.5 ± 0.1	-0.2 ± 0.1	(78)
		♂	177 ± 8	0.6 ± 0.1	0.0 ± 0.1	(95)
	F	A	128 ± 3	1.8 ± 0.3	0.6 ± 0.1	(198)
		♀	130 ± 6	1.6 ± 0.4	0.6 ± 0.1	(74)
		♂	126 ± 4	2.0 ± 0.5	0.6 ± 0.1	(89)
	G	A	116 ± 3	1.0 ± 0.1	0.4 ± 0.0	(186)
		♀	117 ± 5	0.9 ± 0.1	0.4 ± 0.1	(81)
		♂	115 ± 4	1.0 ± 0.1	0.4 ± 0.1	(105)

differences (*P*-value < 0.05) between sexes and populations. A statistical difference was found between males and females in two of four anadromous populations, and none of the three landlocked populations. Although a statistical difference was only found in two comparisons, the mean estimate for asymptotic size was larger for females in all seven populations.

There is a marked difference in the estimated growth parameters between populations (Table 6). The parameter estimates for the four anadromous populations are very similar to each other. Two of the landlocked runs, populations F and G are similar to each other. The model parameter estimates for the third landlocked run, population E, lay between those of the anadromous and the landlocked populations.

Run proportion by age

The Rainbow Smelt that were aged were a small snapshot of the total number of fish sampled from each population. Using the body size and assigned ages from the subsample of fish, we used a mixture model to estimate the proportional contribution of each age class to their respective populations. This was done for all populations except population E that had no additional fish measured so the proportional contribution of each age class was assumed to reflect the population. The model was constrained by forcing the mean size at age to fit a von Bertalanffy growth curve. Most populations are predominantly ages 2 or 3, constituting 78 – 98% of the observed fish in each run for six of the seven populations (Figure 4). The outlier, anadromous population A, was dominated by age 1 fish (89%), which comprised 0 to 10% of the other six populations. Fish age 4 and older comprised a small part of the run (Figure 4).

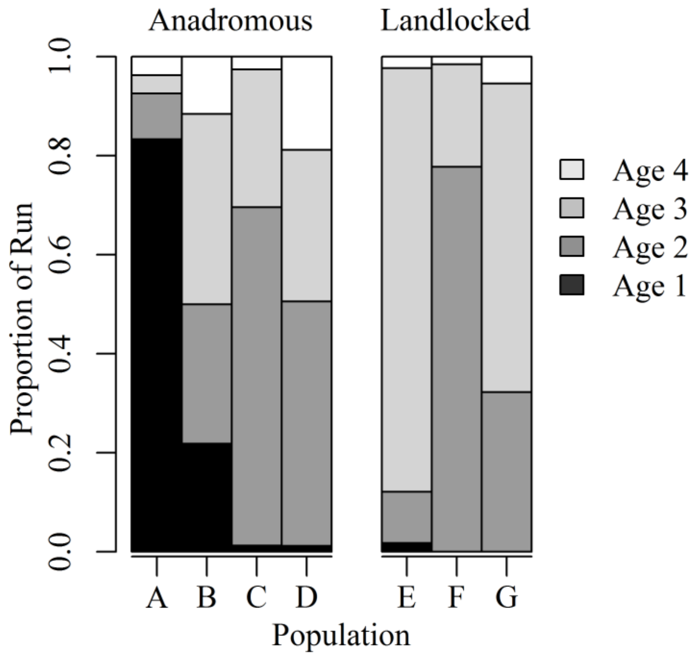

Figure 4. Proportional contribution by age class of Rainbow smelt from four anadromous and three landlocked populations collected during spawning in Maine, spring 2014. Proportions were estimated by applying a Bayesian mixture model of fish of known size and age to a larger number of fish of unknown age. Population E did not have any fish of unknown age so proportions are of observed ages.

Discussion

There was a noted difference in the clarity and readability of otoliths from different populations despite the same handling and processing procedures. Two of the landlocked populations had a sharp transition between winter growth and summer growth, whereas the anadromous populations and the remaining landlocked population displayed a gradual transition. This sharp transition facilitated ageing with the readers showing a high degree of precision and a lack of bias on these two populations (Table 4). The otoliths of the third landlocked population closely resembled those of the anadromous populations with growth regions that are less distinct and a higher incidence of presumed false annuli, which is reflected in the very comparable estimates of bias and precision between three of the anadromous populations (B, C, and D) and the landlocked population E. The only anadromous population without any evidence of a bias between readers and a very high *ACV* was dominated by age 1 fish (population A) which minimizes the potential for errors. The difference in readability between the anadromous populations and the two landlocked populations is likely a result of a stronger seasonal pattern of growth in the landlocked populations. This strong seasonal pattern is likely due to the long, ice covered winter experienced by the fish in the lakes and a more abrupt change in temperature in lakes than the thermally stable ocean.

The scale ages from the anadromous fish had a higher precision and less bias compared to the otoliths from the same fish. The ability to mount and read a larger number of scales (approx. 10 scales per fish were used) from each fish helped in the detection of check marks and false annuli. It is unfortunate that we did not collect scales from our landlocked fish to compare with the precision of the anadromous scales. The high precision between readers of otoliths from landlocked fish suggests that estimates from the scales of these fish would have been very precise and the scale-otolith comparison that was made from anadromous fish may be more reflective of a “worst-case scenario.” The greater precision between readers of landlocked fish than the anadromous fish is evidence of the variation in readability of otoliths among populations. As a result of the lower precision of otoliths, scales may be advantageous for ageing of Rainbow Smelt, but otoliths may still be superior for comparisons of growth due to the potential resorption of scales in older individuals (Hernandez et al. 2014).

The results of our comparison of sectioned otoliths to scales are a parallel to the findings of Walsh et. al. (2008) who found whole otoliths less precise than fin rays for ageing Rainbow Smelt. The findings of Walsh et al. (2008) and the present study both find otoliths (whole or sectioned) to have lower precision than other available structures such as scales or fin rays for ageing Rainbow Smelt. Collection of scales is a minimally invasive procedure, which is less damaging to the fish than the collection of fin rays or otoliths. The sampling of scales allows the sampler to collect data without sacrificing the fish as must be done for sampling otoliths. Although fin rays and scales have not been directly compared, we report higher precision for ageing scales than reported for fin rays suggesting that scales may be the optimal method of the two (Table 4); (Walsh et al. 2008).

On average, the anadromous populations had a larger back-calculated size at each age and a larger asymptotic size than the landlocked populations. This is consistent with the anadromous fish living in the more productive coastal environment having greater growth potential. The back-calculated size at age shows that the mean size at age 1 for our fastest growing population (anadromous) was 160% larger than the means size at age 1 from the slowest growing population (landlocked; Table 5). Furthermore, the fish from the fastest growing population were larger at age 1 than the asymptotic size for our two slowest growing populations (Table 6). In addition to being larger at a given age, the anadromous populations had a greater variation size at age than the landlocked populations.

The back-calculated sizes at age for our four anadromous populations were similar to those reported by other studies. In a population to the north, McKenzie (1958) reported a smaller average size at age for Rainbow Smelt in the Miramichi estuary, New Brunswick, Canada. In a population to the south, in the Parker River estuary, Massachusetts, U.S.A size at age was similar to but slightly larger than our fastest growing population (Murawski and Cole 1978). These findings are consistent with a growth following a temperature or latitudinal gradient along the coast from Massachusetts to New Brunswick.

Our back-calculated sizes at ages for landlocked populations are generally smaller than those reported in the literature are. Bailey (1964), Frie and Spangler (1985), Kirn and Lebar (1996), and Rupp and Redmond (1966) all report size at ages for Rainbow Smelt at or above the upper limit of the growth rates seen by landlocked populations in this study. The two slower growing populations sampled are reflective of the size of Rainbow Smelt seen in many of the waters in the state of Maine (S. Davis, Maine Department of Inland Fisheries and Wildlife, personal communication). This discrepancy in growth rates may be due to the greater productivity of the large, deep waterbodies when compared to our study lakes (Bailey 1964; Frie and Spangler 1985; Kirn and Labar 1996). Rainbow Smelt inhabiting smaller waters show decreased growth than those from the Great Lakes and other very large waterbodies, which is an important consideration for those estimating population dynamics on these waters (Rupp and Redmond 1966).

Interestingly, populations of the fastest growing (population A) and slowest growing (population F and G) individuals had a very high growth coefficient (K) of 0.9 – 1.8. These individuals do much of their growing in their first year of life then show little sustained growth. The three anadromous and one remaining landlocked populations displayed a lower growth coefficient of 0.5 – 0.6, indicating that they grew less early in life but sustained growth through older age classes (Table 6).This suggests that populations are responding to the tradeoffs between somatic growth, reproduction, and survival in different ways. The populations with high growth coefficients were dominated by fish ages 1 and 2 whereas the populations with low growth coefficients were predominantly age 3 fish at the remaining sites (Figure 4). The larger overall body size of anadromous populations than the landlocked populations for both high and low coefficients of growth is strong evidence that the anadromous populations experience conditions that are more bioenergetically profitable to support continued growth.

Recruitment to spawning is linked to individual growth opportunity (Morgan and Colbourne 1999). Three of four anadromous populations and the remaining landlocked populations show a sizeable difference between the mean observed and mean back-calculated sizes at age 1. The difference between observed and calculated sizes for these populations ranges from 9 – 33% of the mean calculated sizes, which is known as Lee's phenomenon (Lee 1920). This difference between observed and calculated size is indicative that the age 1 fish are not fully recruited to the spawning run. We see strong evidence of this for the landlocked populations, where ages 1 fish were not observed at all in two of the three populations (Figure 4). The anadromous populations generally show greater proportion of age 1 fish than the landlocked populations. Age 1 fish dominated one run which resulted in a small difference between observed and back-calculated size (<1%). The other anadromous runs showed similar evidence of Lee's phenomenon to the landlocked run (Table 5). The similar nature and size of Lee's phenomenon between anadromous and landlocked runs show evidence that in most populations only the largest age 1 fish were recruited to spawning (Lee 1920).

Mixture modeling showed that Rainbow Smelt of ages 2 and 3, which comprise 82 – 99 % of individual runs, dominate both anadromous and landlocked spawning populations. This pattern of dominance of age 2 and 3 fish is similar to that described by Bailey (1964), Gorman (2007), and Murawski and Cole (1978) among others. Fish age 4 and older comprise < 18% of any population with no clear trend between anadromous and landlocked populations (Figure 4).

As mentioned earlier, one population was unusual in that it was dominated by age 1 fish (89% of population A). This run is near the southern extent of the range of anadromous Rainbow Smelt and may reflect a transition in life history strategy to cope with warmer waters, a longer growing season, and other factors that have caused the collapse of other populations farther south. The exceptional growth of these fish may be driving the earlier maturation than that of other populations. The low survival to older age classes is likely linked to earlier maturation, but it would be difficult to say which effect is driving the other (Trippel 1995; Morgan and Colbourne 1999).

CHAPTER 2

ASSESSMENT OF A HATCHERY BASED RAINBOW SMELT (*OSMERUS MORDAX*) SUPPLEMENTATION EFFORT

Chapter Abstract

Rainbow Smelt *Osmerus Mordax* are a common freshwater fish in coldwater lakes and ponds in the northeastern North America. A new method of supplementation, commercially reared larvae, has recently become available. We experimentally stocked Rainbow Smelt larvae into two small ponds in central Maine in late May to assess survival and relative contribution of hatchery-reared fish at a density of approximately 30,000 fish/ha. Fish were double marked (thermal and oxytetracycline) to distinguish between wild and hatchery origin. Sampling consisted of day and night ichthyoplankton sampling for the first four weeks after stocking, which resulted in the capture of 2 Rainbow Smelt in one pond and 1,800 in the other. Capture rate was higher at night, and decreased ovcer the duration of the study. Otoliths were examined from a subset of fish to estimate age, growth, and to examine for marks. The median hatch date of all fish was within two days of stocking. Daily growth rate ranged between 0.3 mm/d to 0.5 mm/d. No otoliths had distinct oxtretracycline marks, but 80% had putative thermal marks and 45% had stocking checks. Larval Rainbow Smelt distribution was estimated with a linear model based on trawl depth, day vs. night, and week after stocking. This model generated a population index, to estimate daily mortality rate (Z) for the pond with a high catch rate, and compare the large difference between predicted and observed catches from the pond with a low catch rate. These data indicate that hatchery larval Rainbow Smelt

supplementation was successful in one pond, yet largely ineffective in the other, and differences between ponds are potential explanatory factors.

Introduction

Rainbow Smelt *Osmerus mordax* are a common anadromous fish in northeastern North America that are readily adaptable to living in cold ponds (Nellbring 1989). Such landlocked populations are the result of natural occurrences as well as intentional and unintentional introductions (Mercado-Silva et al. 2006). Life history plasticity and early age at maturation of Rainbow Smelt make them well suited to colonizing new bodies of water quickly (Evans and Loftus 1987; Hrabik et al. 1998; Gaeta et al. 2012). As a result, Rainbow Smelt have expanded their range over the past 200 years throughout the Great Lakes and into the Hudson Bay and Mississippi River watersheds (Evans and Loftus 1987; Mercado-Silva et al. 2006). Intentional introductions have typically been carried out by the transport of eggs, often with burlap as a substrate. Alternatively, the transfer of live adult fish between waters has been used (Rupp and Redmond 1966). The transfer of wild fish and material between waterbodies, however, carries the risk of transferring pathogens and other aquatic organisms between waters.

Rainbow Smelt are actively managed as forage for sportfish species such as lake trout (*Salvelinus namaycush*) and landlocked salmon (*Salmo salar*; Gaeta et al. 2012; Hrabik et al. 1998). The strong correlation between the growth of salmonines and Rainbow Smelt abundance makes the management of this fish a priority in the State of Maine (Havey 1973; Havey 1974; Kirn and Labar 1996; Boucher 2004). Rainbow Smelt can be of commercial importance in some areas (e.g. the Great Lakes), making robust populations desirable. This is complicated by been known to undergo large changes in abundance and can exhibit regular cyclic trends in some waters associated with cannibalism (Gorman 2007; Parker Stetter et al. 2007; O'Brien 2010; Stritzel Thomson et al. 2010). Management of this fish is often accomplished by reduction in sportfish stocking or increases in angling catch limits in response to trophic limitations (Boucher 2004). Both of these actions are generally unwelcomed by anglers who have promoted hatchery-supplementation in Maine as an alternative to maintain Rainbow Smelt populations. An effective means of commercially hatching Rainbow Smelt has been developed recently and these hatchery-produced larvae have been stocked in Moosehead and East Grand Lakes, Maine, U.S.A. in an effort to improve existing but declining Rainbow Smelt populations (Hobbs 2010). The success of these efforts has been equivocal largely because of the inability to capture and discriminate between wild and hatchery-stocked fish.

Early survival of Rainbow Smelt is critical for year class strength within a population and is linked to growth opportunity. Larval Rainbow Smelt are residents of the epilimnion throughout their first summer, staying above the thermocline. Their depth range is limited by high temperatures near the surface and increased risk of predation at the thermocline (He and LaBar 1994; Lantry and Stewart 2000; Parker Stetter et al. 2007). Rainbow Smelt are more dispersed and higher in the water column at night, then move deeper during the day (Ferguson 1965; Parker Stetter et al. 2007; Simonin et al. 2012). Researchers have effectively used fish distribution and waterbody volume to estimate populations, and allow estimates of survival (He and LaBar 1994; Lantry and Stewart 2000; Gorman 2007).

We sought to assess the method of larval stocking by supplementing two small ponds in Maine with hatchery-produced Rainbow Smelt. We used both day and night ichthyoplankton trawling to sample for stocked Rainbow Smelt. We used otolith marking in conjunction with age estimates to characterize the probability of hatchery origin. We modeled fish density based on trawl sampling to generate a population index and estimate survival during the first month after stocking. Modeled Rainbow Smelt density was compared to observed catches to compare the efficacy of stocking in the two ponds. Together these data serve as an informative tool for persons considering larval Rainbow Smelt stocking as a management option.

Study Areas

We stocked two small ponds (Tilton and Egypt) located in central Maine, U.S.A (Table 7). Tilton Pond has a surface area of 42 ha and a maximum depth of 14 m. Egypt Pond has a surface area of 28 ha and a maximum depth of 17 m. Both ponds have a thermocline at approximately 6 m depth in the summer and both ponds were previously stocked with Rainbow Smelt approximately 40 years prior to this study. In Tilton Pond, Rainbow Smelt were abundant and self-sustaining in the 1980's but had not been observed recently and were considered to be extirpated (J. Seiders, MEDIFW, personal communication). The prior stockings in Egypt Pond resulted in a robust and persistent Rainbow Smelt population.

Table 7. The location, size, depth, and species inhabiting two small ponds in central Maine, U.S.A. stocked with Rainbow Smelt in 2014. Species list is from state of Maine pond survey data (MEDIFW, unpublished data). UTM are in Zone 19.

Pond	Tilton	Egypt
Northing (m)	4923000	4930000
Easting (m)	415000	416600
Area (ha)	41.2	27.6
Elev. (m)	148	123
Depth: max [average] (m)	12.5 [4.3]	17.1 [5.4]
Estimated volume (m^3)	1,757,000	1,486,000
List of known species present:		
Rainbow Smelt (*Osmerus mordax*)	x	x
White Sucker (*Catostomus commersonii*)	x	x
Chain Pickerel (*Esox niger*)	x	
Largemouth Bass (*Micropterus salmoides*)	x	
Smallmouth Bass (*Micropterus dolomieu*)	x	
Brook Trout (*Salvelinus fontinalis*)		x
Yellow Perch (*Perca flavescens*)	x	
Pumpkinseed Sunfish (*Lepomis gibbosus*)	x	x
American Eel (*Anguilla rostrata*)	x	x
Brown Bullhead (*Ameiurus nebulosus*)	x	
Golden Shiner (*Notemigonus crysoleucas*)	x	x
Redbelly Dace (*Chrosomus eos*)		x
Fathead Minnow (*Pimephales promelas*)		x
Banded Killifish (*Fundulus diaphanus*)	x	x

Methods

Rainbow Smelt incubation and marking

Approximately 2.3 million Rainbow Smelt larvae were reared for stocking in the two study ponds in May 2014. These larvae were sourced from the Maine Smelt Hatchery at Harmon Brook Farm, Canaan, Maine, and administered a thermal and fluorescent otoliths mark prior to release. All fertilized eggs were the progeny of wild landlocked broodstock from three lakes in Northwestern Maine where the timing of spawning matched Egypt Pond. Broodstock from Wyman Lake were manually spawned, while fish from Rangeley and Richardson Lakes were allowed to spawn in captivity. Embryos were incubated in 6-L hatching jars with aeration in a water bath maintained at 10° C. Eight days after fertilization the embryos were subjected to a thermal marking protocol. Fish were subjected to two cycles of transfer from 10° C to 6° C at 24-h intervals and then returned to 10° C until hatching. This was accomplished by transferring jars between water baths in conjunction with an immediate exchange of 80% of hatching jar water volume.

After hatching, estimates of the number of larvae stocked were made by taking at volumetric samples from each aquarium with a pipette and counting the number of larvae captured. A chemical mark was applied by bathing the fish in a solution of oxytetracycline (Agrimycin-343, AGRI Laboratories LTD. St. Joseph, MO, U.S.A) at a concentration of 500 mg/L for 6 hours immediately prior to stocking. A reference set of fish was set aside for hatchery rearing in an outdoor pond, however, all control fish (both marked and unmarked) died prior to sampling.

Larval Rainbow Smelt stocking

Larvae were transported to and stocked in the study waters 1-3 days after hatching. The bags were floated in the pond for 30 minutes to equilibrate prior to release at dusk, away from shore in water chest-deep. Stocking took place on Egypt Pond on May 15, approximately 2.5 weeks after ice-out (Table 8). Tilton Pond was stocked on May 22 and 23, approximately 3.5 weeks after ice-out (Table 8).

Table 8. Estimated total number and density (fish/ha) of Rainbow Smelt larvae stocked,and the associated 95% confidence intervals for two small ponds stocked in central Maine in the spring of 2014.

Waterbody	Estimated stocked			Density		
Tilton	1,452,000	±	422,000	35,200	±	10,200
Egypt	790,000	±	414,000	28,600	±	15,000
Total	2,242,000	±	836,000	32,600	±	12,200

Larval Rainbow Smelt collection

We attempted to recapture stocked fish to estimate survival using ichthyoplankton sampling with a net having an oval mouth opening of 0.7 m^2 and 500-micron mesh towed at 1.2 m/s with a depth logger and flow meter mounted at the mouth. Weekly sampling was conducted during the day and night at three depth strata: 0.5, 1.2, and 2.3 m with the addition of 0-20 kg of weight to the net. No sampling was conducted within one hour of sunrise or sunset to allow the completion of diel vertical movements (Ferguson 1965; Kirn and Labar 1991).

Tows were conducted in a stratified random sample design to cover all sections of the ponds. We attempted to collect a minimum of 10 tows per depth strata for each sample event (30 tows/event) unless we could not complete sampling due to inclement weather (Table 9). Individual tows ranged from 57 to 314 m in length with a mean of 160 m. If the net made contact with the bottom, that sample was discarded and the tow was repeated. All organisms collected in each tow were stored in 70% ethanol for later examination. Tow samples had large quantities of large zooplankton and invertebrates, which necessitated careful examination for larval fish.

Table 9. Sampling effort, in number of tows and volume of water filtered (m^3) for larval fish for day and night sampling on four successive weeks in two small ponds in central Maine, 2014. Sampling began the week of May 24 on Egypt Pond, and June 1 on Tilton Pond. Tows were collected with a 0.7-m^2 net with 500-micron mesh towed at 0 – 3 m depth at a velocity of 1.2 m/s. Column totals represent the total number of samples collected for each pond, and average volume per sampling event.

Timing	Sample Event	Pond			
		Tilton		Egypt	
		Tows	Volume (m^3)	Tows	Volume (m^3)
Day	1	6	804	30	3393
	2	30	3738	30	3263
	3	30	2835	30	3898
	4	30	3518	30	2892
Night	1	30	3751	26	2866
	2	31	2803	30	2910
	3	30	2797	30	3223
	4	35	3857	30	3173
Total:		222	3013	236	3202

Otolith extraction and determination of applied marks

Captured Rainbow Smelt were counted and standard length was measured. A subsample of three fish was arbitrarily selected from each successful sample for otolith examination. The sagittal otoliths were removed from the head with very fine dissecting needles, and embedded in thermoplastic glue (Crystalbond™, Structure Probe Inc., West Chester, U.S.A.). Otoliths were coated with immersion oil, and examined along the dextro-sinster axis under 400 – 1,000x magnification using a compound microscope (Zeiss Axioplan, Carl Zeiss Microscopy, LLC. Thornwood, NY, U.S.A). Images were captured with an attached digital camera (Spot Insight 2, Spot Imaging Solutions, Sterling

Heights, U.S.A.) and measured with ImageJ imaging software (version 1.48, Research Services Branch, National Institute of Health, Bethdesa, U.S.A.).

Otoliths were assessed for thermal, stocking, and hatching marks and daily growth rings with transmitted white light. The hatching mark distinctly separated two zones of the otolith. Outward of the hatching mark was characterized by a distinct pattern of daily growth bands extending out to the margin. Putative stocking marks were observed at 1 – 4 days after hatching, and were observed opportunistically, as no mark was intentionally applied at that time. Inside of the hatching mark there was no distinct banding pattern, but some otoliths displayed a broad, dark region that was interpreted as a putative thermal mark. The radius of all marks and daily growth increments were measured. The otoliths of older fish were more asymmetrical. To correct for the difference in radius along different axes of the otolith, all measurements were scaled to the average of the longest and shortest radius along the sagittal plane.

Otoliths were also examined for the presence of an oxytetracycline mark using fluorescent microscopy. The specimens were illuminated with a 100-watt mercury vapor lamp, which was passed through a 440-nm excitation filter, a 510-nm barrier filter, and a 520-nm dichroic mirror. This microscope setup was tested and confirmed using oxytetracycline-marked walleye (*Sander vitreus*) otoliths from another source that were independently identified as having visible marks (courtesy of Vermont Department of Fish and Wildlife).

Estimating growth

Size at age and daily growth was back calculated for subsampled fish. Counts of daily growth increments were used to estimate the age and expected hatch data of each fish. Size at age and daily growth was calculated using the intercept corrected proportional back calculation method (Lee 1920). Otolith radius and body size have a distinct non-linear relation so otolith radii were transformed using a Box-Cox transformation (Box and Cox 1964). Daily growth rate was calculated by the difference in back calculated daily size at successive increments.

Pond mapping and water temperature

Physical characteristics of the ponds were recorded to estimate the available habitat for Rainbow Smelt. Bathymetry was mapped by recording pond depth using a chart plotter (Humminbird 385ci or 899ci, Eufaula, AL, U.S.A). Sonar tracks were uploaded to GIS software to create a contour map and intersected with a 2 x 2 m spatial grid to estimate area and volume at each 0.2-m depth interval (version 2.14.0, QGIS Development Team). Water temperature data were collected at 1-m intervals with a handheld temperate probe (YSI 550A Dissolved Oxygen Instrument, YSI, Inc, Yellow Springs, OH, U.S.A) from the surface to 10 m during sampling.

Predicted Rainbow Smelt catch rate

Trawling efforts on Egypt Pond were successful in capturing larval Rainbow Smelt and were used to create a linear model of the expected catch rate. This model was used to compare predicted and observed catches for Tilton and Egypt ponds for all sampling events. The model includes trawl depth (D), day vs. night (N), weekly sample event (E), and a second order interaction between day vs. night and the other variables (N * D and N * E). All terms were significantly correlated (p-value <0.05) with predicted density (r^2 = 0.286, p-value < 0.001). The model followed the general formula:

$$\text{Density} = \text{N} + \text{D} + \text{E} + \text{N} * \text{D} + \text{N} * \text{E}$$

Index of larval Rainbow Smelt abundance and mortality

The model used predicted catch rate to estimate an index of the total abundance of larval Rainbow Smelt in the upper epilimnion of Egypt Pond. This index was calculated by summing the predicted density at the midpoint of each 0.2-m depth bin for the top 3 m of the bathymetric volume model. The instantaneous daily mortality rate (Z) was estimated from the slope of the log population index and days after stocking (Guy and Brown 2007).

Results

Larval Rainbow Smelt collection

Rainbow Smelt comprised 47% of the total catch of fish across both ponds (Table 10). Only two Rainbow Smelt were captured in Tilton Pond (one each during the third and fourth night sample events), while 1,800 fish were captured in Egypt Pond, where fish were caught both day and night across all sample weeks (Table 10). The catch rate was higher at night than the day, and decreased in successive sampling weeks. In addition to Rainbow Smelt, trawling resulted in the capture of Yellow Perch *Perca flavescens*, Golden Shiner *Notemigonus crysoleucas*, and Pumpkinseed Sunfish *Lepomis gibbosus* which comprised 47%, 6%, and <1% of the total catch of fish respectively (Table 10). Catch rates for Yellow Perch and Pumpkinseed Sunfish declined over the sample period, while the catch of Golden Shiners increased (Table 10). Notably, Yellow Perch were relatively abundant in Tilton Pond while this species was absent in Egypt Pond.

Table 10. Catch of larval and juvenile fish from ichthyoplankton trawling on two small ponds in central Maine, summer 2014. Catch is by day and night for four weekly sampling events, starting the week of May 24 on Egypt Pond, and June 1on Tilton Pond. Species encountered were: GS- Golden Shiner (*Notemigonus crysoleucas*), YP- Yellow Perch (*Perca flavescens*), PS- Pumpkinseed Sunfish (*Lepomis gibbosus*), and SL- Rainbow Smelt (*Osmerus mordax*).

Pond	Timing	Sample Event	Species				Event Total
			GS	YP	PS	SL	
Tilton	Day	1	-	24	-	-	24
		2	-	6	-	-	6
		3	7	1	-	-	8
		4	56	-	-	-	56
		Subtotal:	63	31	0	0	94
	Night	1	-	1621	-	-	1621
		2	-	50	-	-	50
		3	1	74	-	1	76
		4	8	37	-	1	46
		Subtotal:	9	1782	0	2	1793
Egypt	Day	1	-	-	-	490	490
		2	-	-	-	117	117
		3	46	-	-	2	48
		4	35	-	-	1	36
		Subtotal:	81	0	0	610	691
	Night	1	-	-	11	266	277
		2	-	-	1	600	601
		3	58	-	-	227	285
		4	8	-	-	120	128
		Subtotal:	66	0	12	1213	1291
Species Total:			219	1813	12	1825	3869

Presence of applied marks on larval Rainbow Smelt

Sagittal otoliths were removed from 339 larval Rainbow Smelt from Egypt Pond for determining the date of hatching and the presence of applied marks. Ten fish were excluded because neither otolith could be read. Another 59 were removed from the analysis because only one otolith was readable. The hatching mark was the most distinct mark on the otolith at a mean radius of 9.8 µm and marked the initiation of daily increment formation. Putative stocking marks were observed on both otoliths from 21% of fish at a mean radius of 11.6 µm, and putative thermal marks occurred on both otoliths from 67% of fish at a mean radius of 7.7 µm. Both types of marks were observed on both otoliths from 11% of fish and at least one mark was observed on both otoliths from 77% of fish. There were no distinct oxytetracycline marks observed in any sample. Three out of the 329 fish observed had a "possible mark" on only one otolith. These marks were faint and located at the hatching mark, which may have caused a small but detectable amount of autofluorescence. The two fish from Tilton Pond were not aged because their otoliths were not readable.

Larval growth and age

The size at capture was measured for all fish. Standard body lengths of fish captured in Egypt Pond were unimodal consistent with a single cohort. The mean observed size ranged from 9 mm during the week of May 24, to 25 mm during the week of June 16 (Table 11). The two fish captured during the third and fourth sample events (17.2 mm and 25.9 mm, respectively) in Tilton Pond were similar in size to those captured during the third and fourth sample events in Egypt Pond. Size at age and daily growth rate was calculated from a linear model had an intercept of 0.022 $mm^{0.42}$ and a slope of 0.018 $mm^{0.42}$ (r^2 = 0.969, p-value < 0.001; Figure 5). We observed a standard body length growth rate of 0.3 – 0.6 mm/d (Figure 6). The daily growth rate followed an “S” shaped curve with respect to time. Growth was slowest around day 4 (0.3 mm/d) and greatest between days 12 – 20 (0.6mm/d; Figure 6).

Table 11. Standard length (mean ± standard deviation (mm)) of Rainbow Smelt larvae captured in Egypt Pond in 2014. The mean age (days) was calculated from otoliths from 329 fish. The sample size for fish length and fish age of each sample event is in parentheses. Results of day and night sampling are presented separately.

Timing	Sample Event	Date	Standard Length		n	Mean Age	n
			Mean	Sd.			
Day	1	May 24	8.8	1.2	490	8	71
	2	Jun 1	12.7	1.7	117	16	31
	3	Jun 10	17.3	3.3	2	25	2
	4	Jun 17	23.5	-	1	32	1
Night	1	May 24	9.5	1.4	266	8	50
	2	Jun 2	14.6	1.9	600	17	78
	3	Jun 12	20.5	2.7	227	27	69
	4	Jun 19	24.8	2.5	120	34	29

Hatch date was estimated by subtracting the number of growth increments from the date of capture for the subsample of fish examined for the presence of marks. The estimation error in hatch date ranged from 0 – 4 d (SD= 1.6) when both otoliths were read. The observed hatch dates ranged from May 11 to 27 with the mean and modal hatch date on May 16. This is one day after the supplemental stocking occurred and two to three days after the expected hatch date of the stocked fish (Figure 7). A Wilcoxon signed rank test found no statistical difference (p-value=0.09) between the median observed hatch date and the expected hatch date of the stocked fish.

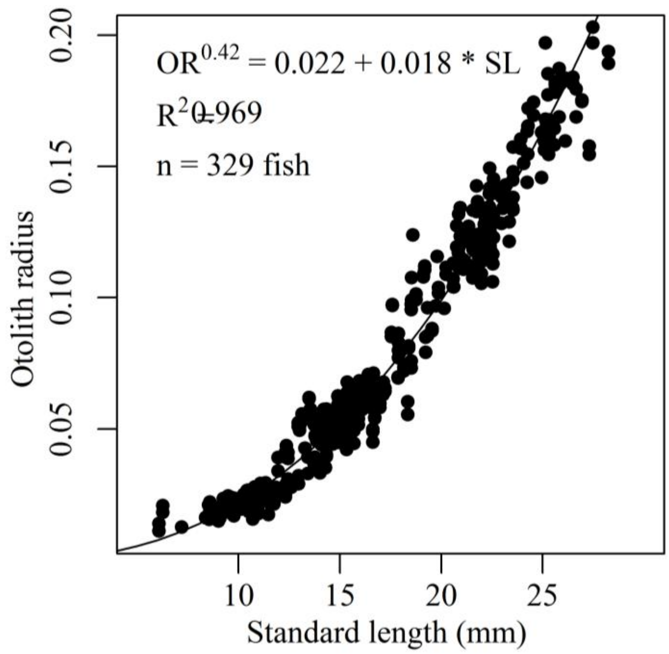

Figure 5. Relation between standard body length (SL) and sagittal otolith radius (OR) for larval Rainbow Smelt collected in Egypt Pond, spring 2014.

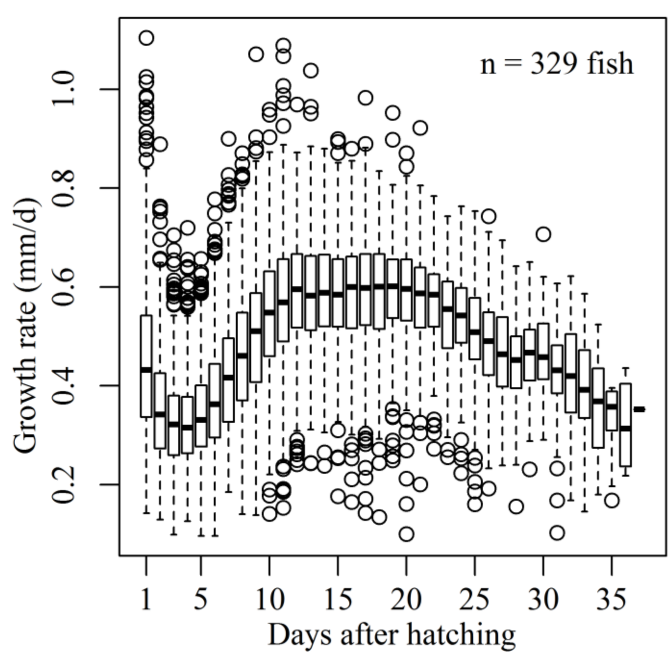

Figure 6. Daily growth rates estimated from sagittal otoliths of larval Rainbow Smelt (mm) calculated from sagittal otolith of 329 larval Rainbow Smelt captured during trawling in Egypt pond, 2014. Box edges and whiskers mark the quartiles and 95th percentiles respectively.

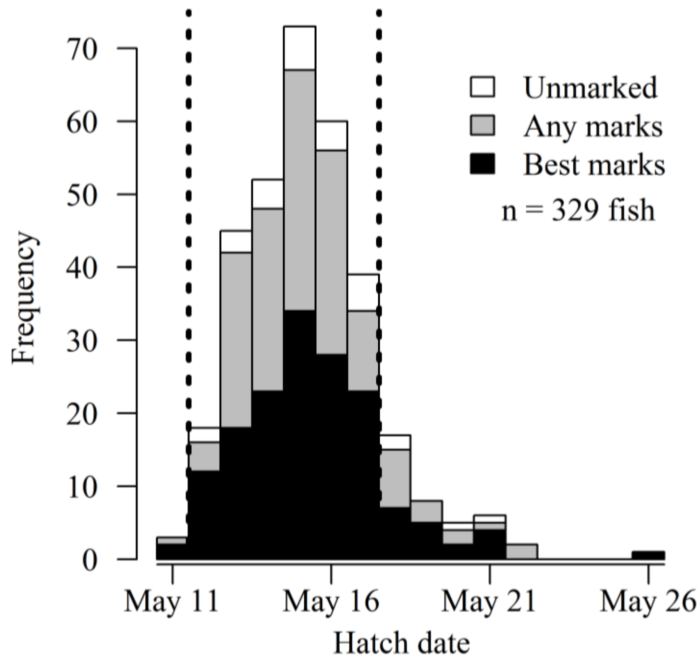

Figure 7. Histogram of hatch date estimated from the otoliths of larval Rainbow Smelt collected from Egypt Pond, spring 2014. The hatch dates of stocked fish are expected to fall between the dotted lines. Otoliths were also examined for the presence of putative marks associated with thermal marking or stocking.

Modeled Rainbow Smelt catch

Because the Egypt Pond data were used to generate the predicted catch values of Rainbow Smelt, the observed and predicted catches agree very well ($r^2 = 0.997$, p-value < 0.001). This model was extended to the Tilton Pond capture efforts to predict the expected catch based upon the sampling effort and environmental conditions. These predicted catches assume the fish in Tilton Pond would follow the same relation with depth, day vs. night and sample event (as a proxy for age and/or date), and occur at the same density as in Egypt Pond. Our total predicted catch at Tilton Pond is very similar to that of Egypt Pond (1,800 vs. 2,100), but our observed total catch is much lower (2 vs. 1,800; Table 12). This large difference between predicted and observed catch provides evidence that Rainbow Smelt occurred at a much lower density in Tilton Pond than Egypt Pond, rather than simply not being captured.

Table 12. Predicted and observed catch of larval Rainbow Smelt from day and night sampling on two study ponds located in Maine, 2014. Sampling began the week of May 24 on Egypt Pond, and June 1on Tilton Pond. Predicted densities are from applying the modeled Rainbow Smelt observed in Egypt Pond to the trawls from both ponds.

Timing	Sample Event	Date	Tilton		Egypt	
			Predicted	Observed	Predicted	Observed
Day	1	May 24	109	0	558	490
	2	Jun 1	141	0	126	117
	3	Jun 10	32	0	29	2
	4	Jun 17	23	0	27	1
Night	1	May 24	371	0	311	266
	2	Jun 2	687	0	676	600
	3	Jun 12	232	1	240	227
	4	Jun 19	196	1	143	120
Total			1791	2	2110	1823

Population index and mortality

Our modeled population index estimated as many as 160,000 larval Rainbow Smelt in the upper epilimnion of Egypt Pond during sampling (Figure 8). The index showed greater abundance of fish in the surface waters at night than during the day. The daily mortality rate (Z) was estimated from the daytime and nighttime indices, both separately and combined. Nighttime estimates result in a lower daily mortality rate ($Z = -0.037$, $r^2 = 0.279$) compared to the daytime derived estimates ($Z = -0.121$, $r^2 = 0.937$; Figure 8). By extrapolating these estimates back to the date of stocking (also mean hatch date), there were 253,000 larval Rainbow Smelt in the top 3 m in the daytime and 176,000 at night.

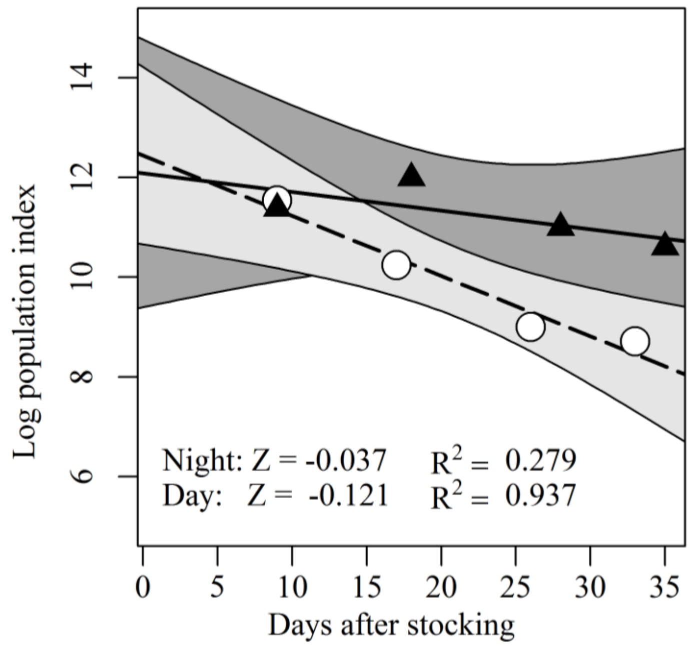

Figure 8. Plot of logged population index for Egypt Pond during night (black triangles) and day (open circles) sampling events in spring 2014. The population index is the estimated population of larval Rainbow Smelt in the top 3 m of the water column. The solid line and dark grey region represents the estimated daily mortality rate and 95% confidence region from the nighttime estimates; the dashed line and light grey region is from the daytime estimates.

Discussion

Based on our estimates in conjunction with the observations outlined below, we conclude that hatchery-origin fish accounted for the majority of the high Rainbow Smelt captures in Egypt Pond. In addition, though there was a high recapture rate of probable hatchery fish in Egypt Pond, stocking in Tilton Pond had negligible captures of larval Rainbow Smelt in spite of comparable stocking and sampling efforts. Though we observed a substantial difference in capture rated between ponds, we demonstrated the ability to effectively sample for Rainbow Smelt larvae in Egypt Pond. These data were used to generate an index of abundance in the study in the absence of full depth distributional information.

We believe this index is both representative and conservative for several reasons. First, we extensively sampled the top half of the epilimnion (thermocline depth ~ 5 – 6 m). We generally found increasing larval abundance with increasing depth consistent with distributions limited by the temperature (Lantry and Stewart 1993; Simonin et al. 2012). Several sources describe the depth distribution of larval Rainbow Smelt as skewed toward the upper portion within the epilimnion, thus we were likely sampling at or near the depth of highest density (Ferguson 1965; Pientka and Parrish 2002; Parker Stetter et al. 2007; Simonin et al. 2012). Second, the calculated index for Egypt Pond ranged as high as 0.27 fish/m^3 during the sample period, which is comparable to reported densities in years of high abundance (Brown 1994; Sirois and Julian 2000; Parker Stetter et al. 2007; O'Brien et al. 2012).

Our efforts did not provide conclusive results on the efficacy of marking Rainbow Smelt with either OTC or thermal techniques. To our knowledge, no published studies have attempted to mark embryonic and larval Rainbow Smelt though otolith marking is widely used for many species of fish, including the marking of embryos (Brooks et al. 1994; Volk et al. 1994; Brown 1995; Beckman and Schulz 1996). There was no clear evidence that fluorescent marking with oxytetracycline was successful, although this method has been used successfully in many other species (Secor et al. 1991b; Brooks et al. 1994; Isermann et al. 2002). A longer exposure time or greater concentration has shown better mark detectability in other fish and may work in Rainbow Smelt (Brooks et al. 1994). The lack of distinct banding patterns in the otoliths prior to hatching suggests that thermal marking of Rainbow Smelt embryos may also be more difficult than anticipated. However, we observed a large number of pre-hatch marks that we attribute to our thermal manipulation. The observed putative stocking marks suggest that thermal marking may be very effective several days after hatching and may be a productive direction in testing marking methods. Regrettably, however, the loss of control fish in this study greatly limits our conclusions on these methods. We therefore base our conclusions on a “weight of evidence” approach.

Our estimation of hatch dates reveals that most of the fish captured in Egypt Pond hatched at a date at or near the known hatch dates for stocked hatchery fish. The median observed hatch date of captured fish does not differ for the expected hatch date for stocked fish. Such a result was unlikely because we were successful in matching the timing of hatchery spawning with the wild population in Egypt Pond. As a result, the distribution of hatch dates matches with the expected distribution of the wild fish and observed dates of our stocked fish (Figure 7; A. O’Malley, unpublished) and is not particularly helpful in diagnosing the contribution of hatchery fish given the resulting unimodal distribution. In combination with putative thermal marks, the data suggest a high proportion of the observed fish were the result of hatchery-supplementation (Figure 7). The distribution of putative marks broadly matches the overall distribution of estimated hatch dates.

There was a large difference in catch between the two ponds despite similar stocking rates and sampling effort. Fish were captured during every sampling event on Egypt Pond, demonstrating the effectiveness of the sampling technique but the catch was meager on Tilton Pond despite stocking nearly 1.5 million larvae (Table 10). There are several potential reasons for the difference in apparent survival of larval Rainbow Smelt between the two ponds. It is possible, though improbable, that the larval Rainbow Smelt were present but were outside of our sampling range. Given the high rate of success on Egypt Pond, and the temporal overlap of sampling, it seems unlikely that all larval Rainbow Smelt would remain below our sampling depth, especially at night when they are more dispersed (Ferguson 1965; Lantry and Stewart 1993; Parker Stetter et al. 2007). It is also unlikely that the fish stayed too close to shore and aquatic vegetation to be captured because Rainbow Smelt are a pelagic species that are most abundant in open water, often several kilometers from the shoreline (Ferguson 1965; Kirn and Labar 1991; Simonin et al. 2012).

It is more likely that the existing fish community in these two ponds was a determining factor. Tilton Pond has a robust population of yellow perch, as indicated by their larval abundance in our sampling. Other large, piscivorous fish, including Chain Pickerel *Esox niger*, Smallmouth Bass *Micropterus dolomieu*, and Largemouth Bass *Micropterus salmoides*, are present in Tilton Pond but not in Egypt Pond. Together, the presence of this suite of species may have been an insurmountable gauntlet for even the high stocking rates used here.

The growth rates of larval fish from Egypt Pond were comparable to those from previous studies on Rainbow Smelt. The observed pattern of fast initial growth, a period of slow growth, and another period of rapid growth matched the pattern seen in the St. Lawrence estuary (Sirois et al. 1998). The measured absolute growth rates in our study matched those reported for wild (0.05 – 0.4 mm/d) and captive-reared (0.1 – 1.7 mm/d) fish from the St. Lawrence estuary, and were faster than those reported from Lake

Superior (Brown 1994; Sirois et al. 1998). Our power transformation (0.42) for back calculating size at age falls within the range used by Sirois et al. (1998) to back calculate growth of four captive reared populations of Rainbow Smelt (range: 0.36 – 0.48). Therefore, it is unlikely that food availability was strongly limiting for these fish.

The two Rainbow Smelt captured in Tilton Pond are likely of hatchery origin. These fish were of comparable size to the fish captured in Egypt Pond the previous week, which indicates a similar age and rate of growth; although no estimated hatch dates from otolith increments are available to confirm this conjecture. It is possible that these fish are wild but the expected hatch date for wild fish would be one week earlier, matching Egypt Pond, and the lack of any confirmed observations of Rainbow Smelt in this water in the last 15 years makes this unlikely.

We feel confident that the population index and associated estimates of mortality are reflective of the study systems despite the limited depth range, though our night-derived estimates are likely to be more robust. Our nighttime population index was substantially higher than our daytime index for three of the four sample periods, which is consistent with fish moving vertically into and out of our sample range during the night to feed (Figure 8); (Ferguson 1965; Brown 1994; Simonin et al. 2012). Although we only have an index of the population, our estimated daily mortality rates (Z: 0.037 – 0.121) are comparable to those from other studies. Studies by O'Brien et al. (2012) and Sirois and Julian (2000) both report mortality rates at the lower end of the scale (Z: 0.045 – 0.050 and 0.032 – 0.036, respectively) while Brown (1994) reports mortality rates near the estimates of our daytime samples (Z: 0.098 – 0.169).

The near absence of larval Rainbow Smelt captured in Tilton Pond in 2014 suggests that stocking was not effective on this water, despite extensive effort. In contrast, Egypt Pond produced excellent catch rates. Growth and survival estimates likewise indicate success when compared to literature values. Estimates of survival from our Egypt Pond population index are very similar to those from other waters despite the technological challenge of trawl sampling in such small ponds. The study was designed to use otolith marks to indicate known hatchery fish. While circumstances necessitated the use of a weight of evidence approach, the high frequency of individuals with putative marks in conjunction with the hatch dates is suggestive that hatchery-supplementation contributed substantially in Egypt Pond. The difference in stocking efficacy between these two waters indicates poor survival in some waters may be insurmountable even at high stocking rates. These results also underscore the importance of surveying the fish community prior to stocking and subsequently conducting an assessment of hatchery-supplementation for management.

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BIOGRAPHY OF THE AUTHOR

The author was born in Springfield Massachusetts and raised on a small hobby farm in the nearby town of Westfield where he was able to play in small streams to his heart's content and foster a passion for fish and other aquatic life. He graduated from Westfield High in 2006 and came to the University of Maine where he earned his B.S. in Ecology and environmental Science in 2010. Having fallen in love with Orono, he decided to stay for as long as possible and continue his academic career by accepting a role as a graduate student. During his time as a graduate student, Andrew has held several teaching assistantships, served as proud member of the American Fisheries Society subunit, and waterfowler-extraordinaire. He learned many things about Rainbow Smelt during the course of this study, chiefly among them: "Smelt are tasty." He is a candidate for the Masters of Science degree in Wildlife Ecology from The University of Maine in May 2016.