

# **Fourth North American Workshop on Rainbow Smelt**

Holiday Inn by the Bay in Portland, Maine, January 24-25, 2011

## **Extended Abstract Proceedings**

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## Workshop Summary

The 4<sup>th</sup> North American Workshop on Rainbow Smelt was held in Portland, Maine on January 24-25, 2011. This symposium was hosted by the Maine Department of Marine Resources, Massachusetts Division of Marine Fisheries, and the New Hampshire Department of Fish and Game, with major funding provided by a grant through the National Marine Fisheries Service Office of Protected Resources.

Building off of the success of past rainbow smelt workshops, this meeting provided a forum for attendees from universities, government agencies, and conservation organizations to exchange information about current research and management efforts directed towards both inland and anadromous rainbow smelt. This information was conveyed through more than 28 presentations that focused on the population status, biological distinctions, ecological interactions, and restoration approaches for rainbow smelt.

Abstracts from each of these presentations are compiled in this document, and we provide a brief summary of the work presented by theme below. In addition to these proceedings, the extended abstracts will be published through the Massachusetts Division of Marine Fisheries Technical Report Series. This publication will be freely available online in the fall of 2012 at: <http://www.mass.gov/dfwele/dmf/publications/technical.htm>. Once published, extended abstracts may be cited through the Technical Report Series.

### **Anadromous Spawning Strategies and Population Assessments**

Understanding spawning strategies and population structure is necessary to effectively manage a species. Lecomte (p. 26) explored spawning strategies of the Osmeridae family, focusing on anadromous rainbow smelt, using a

synopsis of scientific literature and a case study of smelt life history in the St. Lawrence estuary in Quebec. Two sympatric smelt populations co-exist within the St. Lawrence Middle Estuary; one population uses “traditional” streambed spawning grounds and another uses shoal habitats. Legault and Lecomte (p. 36) quantified the use of the shallow shoals for spawning, a strategy previously not described. Their work aimed to determine the scale of shoal spawning and to genetically identify the origin of the eggs. They found that alternative spawning strategies may be more important than previously thought, and that they may be used in concert with traditional spawning strategies. Further examining the population composition in the St. Lawrence River, Fortin *et al.* (p.76) used otolith microchemistry to distinguish the origin of larval smelt in the mainstem river, examining the contribution of each known tributary river spawning location to the overall population.

As anadromous rainbow smelt populations appear to have been extirpated in the southern portion of their range and to have declined in many extant spawning rivers, documenting their population status and life history information is critical for developing effective management and conservation efforts. Multi-party collaborative research efforts require standardized methods to ensure the comparability of data collected by different parties. Enterline *et al.* (p. 79) described standardized ageing methods that have been developed and applied by Maine, New Hampshire, and Massachusetts to characterize spawning runs of anadromous rainbow smelt. These region-wide efforts have been supported by the use of digital imaging technology that makes it possible to share reference collections, train staff, and compare results across different labs. Chase *et al.* (p. 12) discussed the population monitoring program being

implemented by this same three-state collaborative. Spanning 15 rainbow smelt spawning runs in the Gulf of Maine region, this survey indicates latitudinal influences on run timing and duration. A comparison to historical studies indicates that the age structure and survival rate of smelt spawning populations have changed over time, shifting towards a younger age distribution and lower survival rates. In an effort to better project smelt population data, catch data, and mortality estimates, Enterline *et al.* (p. 23) characterized spawning season movement using Passive Integrated Transponder (PIT) technology. Preliminary results indicate that males return to the spawning grounds at a significantly higher rate than females. This finding will enable managers to tune annual monitoring data and adjust frequency-at-age tables and mortality estimates.

Efforts are also underway to collect information about rainbow smelt populations on the Penobscot River, Maine's largest river, before the removal of dams that block access to original spawning grounds. The Penobscot River Restoration Project will be restoring nearly 1,000 miles of sea-run fish habitat by removing two hydroelectric dams and improving fish passage at a third dam. Arter and Dietert (p. 84) described collaborative research projects being conducted by members of the Diadromous Species Restoration Research Network that study how the opening of this sea-run fish habitat may affect sturgeon, bird assemblages, sea lamprey, water quality, alewives, salmon, and rainbow smelt populations and movement. As part of this effort, Stevens *et al.* (p. 70) initiated a multi-gear survey of the fish community in the Penobscot estuarine system. This survey will describe the spatial and temporal distribution of fish in the estuary and may be particularly useful for quantifying runs of diadromous species.

### **Inland Smelt Populations, Trophic Interactions and Management**

Landlocked smelt populations are found in the Northeastern US, Canada, and Great Lakes. With concerns about populations in Lake Huron at historic low levels, O'Brien *et al.* (p. 47) studied densities of larval stages of smelt and modeled stock recruitment based on bottom trawl survey data. These results indicate that variable growth rates and survival of early hatching cohorts strongly influence year class formation. In Lake Saint-Jean in Québec, Canada, sub-adult rainbow smelt are both large consumers of zooplankton and a plentiful prey source for larger species. Plourde *et al.* (p. 43) examined ingestion rates by young-of-the-year and yearling smelt in Lake Saint-Jean using a mercury mass balance model to better understand zooplankton uptake and growth during this important life phase. In an effort to increase smelt production in Lake Saint-Jean, Sirois *et al.* (p. 46) quantified smelt carrying capacity by describing smelt diet and comparing it to the rate of production of the four main prey groups in the lake. From their findings, it was determined that the zooplankton production in Lake Saint-Jean could support higher biomass of smelt. In inland Maine, smelt have been stocked in many lakes as a prey species for popular sportfish such as landlocked Atlantic salmon. Halliwell and Boucher (p. 48) described efforts by the state to selectively stock certain lakes, and to control or eradicate smelt from lakes where they were illegally stocked and upset the natural species composition.

Worldwide, smelt species are important forage fishes. Common smelt (*Retropinna retropinna*) are an important prey species for rainbow trout (*Oncorhynchus mykiss*) in Lake Rotoiti in New Zealand. There is interest in increasing stocking of rainbow trout, but the possible impact on the smelt population is unknown. Blair *et al.* (p. 83) assessed smelt

abundance and concluded that smelt reproduce locally and appear to provide a sustainable food source for brook trout.

### **Fisheries: Historical and Current**

Once supporting strong fisheries rivalling the economic value of Atlantic cod and American lobster, rainbow smelt have declined along the eastern seaboard of the United States. Wood *et al.* (p. 66) summarized information about the once prominent commercial fisheries in states from New Jersey to Maine, described their subsequent decline, and offered explanations for this drastic contraction in range. The Pleasant River in eastern coastal Maine remains one of the last successful commercial smelt fisheries in the United States; Shaw (p. 54) discussed how fishermen and multiple agencies have collaborated to collect data that quantify and protect the fishery. Despite the decline in commercial fishing for smelt in the United States, recreational fishing remains a popular and long standing tradition. State agencies in New Hampshire and Maine survey these fisheries to estimate annual population trends and gather population data. Enterline and Uraneck (p. 75) compared survey data collected on the Kennebec River, ME from 2009-2011 to survey data from 1979-1982 and found that catch rates have declined slightly.

### **Laboratory Culture**

Laboratory culture of rainbow smelt provides a controlled environment for testing the effects of certain conditions on various life stages. The results of such studies are valuable for aquaculture ventures, can lead to inferences about environmental impacts on wild populations, or may generate new methods that can be adapted for species management. Berlinksy *et al.* (p. 26) conducted a series of experiments to evaluate growth and survival of juvenile rainbow smelt reared in recirculating

systems under different environmental conditions. Water temperature, salinity and dietary protein levels were varied and physiological responses were recorded. Smelt fed the highest percent protein diet and maintained at the lowest temperature had a significantly higher growth rate compared to the other treatment groups. Ayer *et al.* (p. 62) compared methods to mark the otoliths of reared smelt using oxytetracycline hydrochloride (OTC) and found marking to be most effective when exposing the chemical to larval fish, as opposed to embryos. As part of an effort to restore smelt populations in Massachusetts, larvae were marked and released into a coastal stream. In subsequent years, adult smelt were captured from the research stream and marks were found on otoliths, thereby confirming the success of using OTC as a practical method to document smelt stocking success.

### **Genetic Research and Management Implications**

Understanding genetic structure, variation, and gene flow can lead to conclusions about the evolutionary history of species, draw attention to unique or isolated populations, and inform management strategies. In the keynote address of the workshop, Dr. Julian Dodson from Laval University, Québec described work to determine how genetic composition and contemporary environmental factors may affect population-specific morphology of rainbow smelt on the St. Lawrence River (p. 8). In order to quantify nuclear gene flow among smelt populations of the St. Lawrence estuary complex, Colbeck *et al.* (p. 41) utilized AFLP technology to compare smelt populations from sites throughout the estuary. Results show that while two distinct genetic populations are evident, genetic intermediates were also found, suggesting gene flow between the clusters.

Along the American coast of the Gulf of Maine, Kovach *et al.* (p. 14) distinguished four to six genetic groups of rainbow smelt between Buzzards Bay, Massachusetts and Downeast Maine. These results can be used to inform the designation of genetically distinct management units, which may guide restoration and restocking efforts. Within Maine, Van Gorden and Zegers (p. 79) sought to determine whether population structure existed among rainbow smelt in four rivers of the Pleasant Bay watershed. They found that microsatellites indicated polymorphism among their samples, but analyses using a major histocompatibility complex (MHC) marker suggested that the samples were monomorphic. Also in Maine, following five years of transferring anadromous smelt eggs from Casco Bay to Sebago Lake, Campbell *et al.* (p. 78) estimated gene flow between these locations. Gene diversity indices showed limited gene flow, and analyses indicated that the direction of flow was from Casco Bay to Sebago Lake.

### **Threat Identification**

Anadromous rainbow smelt face a variety of potential threats that arise from conditions and activities both within and beyond the streams they use for spawning. Because anadromous rainbow smelt may spawn immediately above the head of tide of coastal rivers and streams and are not strong swimmers, they are often blocked from spawning grounds by road crossings, dams, and fishways that are designed for other species. Clément *et al.* (p. 56) quantified smelt passage success through an alternative nature-like fishway utilizing Passive Integrated Transponder technology. They found that smelt were indeed

able to move up through the fishway, but movement was limited during high flows.

Chase *et al.* (p. 49) investigated how water and habitat quality varied across 18 smelt spawning sites in Massachusetts, New Hampshire, and Maine and classified sites as “suitable” or “impaired” based on the relationship of observed conditions to water quality criteria. They documented that in-stream pH, turbidity, total nitrogen, total phosphorus, and periphyton growth exceeded proposed thresholds and could degrade conditions for rainbow smelt spawning. Mills and Enterline (p. 15) looked at watershed conditions adjacent to smelt spawning sites in Maine. They did not detect a strong direct relationship between land cover and smelt spawning abundance, but these influences may be translated indirectly through changes in water quality and streambed features.

### **Restoration Initiatives**

In response to notable population declines, attention has increasingly focused on restoring rainbow smelt and other diadromous species in recent years. Verreault *et al.* (p. 64) described a successful restoration effort on the St. Lawrence River. Following water quality improvements and fishing mortality reductions, rainbow smelt have returned to previously abandoned spawning tributaries, and adult abundance and mean spawning age have increased. Restoration efforts are also underway in rivers along the US portion of the Gulf of Maine coast. Arter and Dietert (p. 83) explained the role of the Diadromous Species Restoration Research Network (DSRRN) in advancing the science needed to guide these restoration efforts and enhance their potential for success.

# Past and present influences shaping intraspecific morphological divergence in rainbow smelt (*Osmerus mordax*)

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## Introduction

Intra-specific phenotypic variation may be the result of divergence involving the partitioning of restricted resources among specialists adapted to different ecological niches. In freshwater fishes, there is no shortage of examples of limnetic ecotypes filtering plankton in the water column and benthic ecotypes feeding on macroinvertebrates. Such ecological divergence has often resulted in reproductive isolation, opening the door to eventual speciation. However, there are also many examples of species that are composed of morphologically distinct phylogenetic lineages that have been isolated at some time in the past and have developed more or less independently over long periods of time, particularly during the last or previous Pleistocene glaciations. Such vicariant differentiation may have arisen through genetic drift and-or adaptation to distinct environments in historical refugia. Thus, when studying species today, we are faced with the problem of disentangling to what extent variation in morphological traits in contemporary populations is attributable to adaptation to the current environment or the result of occupying some past, unknown environment.

Native to northeastern North America, rainbow smelt is composed of two mitochondrial clades (Bernatchez & Martin, 1996; Bernatchez, 1997). Given the enormous error associated with molecular clocks, the clades may have diverged

anytime between a million to 35 thousand years ago. However, it is most probable that they diverged as recently as the last glaciation. A geographical dichotomy exists in the distribution of the two clades, with the so-called B-clade occurring to the northwest of the Appalachian mountains, and the A-clade to the east in primarily Atlantic drainages. In addition, contemporary smelt populations show considerable phenotypic variation and at least 3 morphotypes are recognized: estuarine anadromous, microphageous freshwater, and macrophageous freshwater. Other morphological variants have been found in estuaries, including the superficially pelagic vs. benthic morphotypes of the St. Lawrence (Lecomte & Dodson, 2004, 2005) and the microphageous estuarine dwarf smelt found in Newfoundland.

Principal question. To what extent is variation in morphological traits among contemporary populations attributable to adaptation to the current environment or to previous history? In short, how important is the past in structuring the present? To answer this question, we evaluate the extent to which intraspecific history (as indicated by clade identity) influences morphological divergence within historical lineage when exposed to alternative selective pressures in lakes and estuaries (as indicated by morphotype identity).

## Results

We start with a morphological analysis of 13 lake populations, including allopatric and

sympatric micro- and macrophageous smelt derived from both historical lineages, located throughout the range of smelt in NE North America (Barrette *et al.* 2009). A discriminant function analysis, based on 37 morphometric and 9 meristic traits, was used to isolate the relative importance of ancestral (A vs. B clade) and contemporary (macro- vs. microphageous forms) influences. First and foremost, smelt ecotypes in lakes are more similar within lineages than between lineages. In fact, within the B lineage, there is no difference between macro- and microphageous smelt other than the overall gill raker counts and body length that we used to initially classify the ecotypes. The B-phenotype is characterized by big jaws, big eyes and a generally deeper head and longer gill arches. In contrast, all of these traits are smaller in the A-phenotype, which is also characterized by a greater number of dorsal gill rakers and bigger fins. These tendencies are significantly more pronounced for the A-microphageous smelt than the A-macrophageous smelt. Historical morphological features thus appear to dominate contemporary trophic adaptations (macro- vs. microphagy).

To a lesser degree, the larger jaw structures and longer gill arches (and hence lower raker density) we associate with the B lineage characterizes macrophageous smelt of both A and B lineages, whereas greater gill raker counts and fin sizes characterize the microphageous smelt.

To summarize.

- Smelt ecotypes (micro- and macrophageous) are morphologically more similar within their historical lineages than between lineages (more so in the case of the B lineage)
- Both lineages have experienced parallel morphological changes in producing alternative ecotypes (big jawed macrophageous ecotypes, higher

gill raker density microphageous ecotypes).

Now we ask if similar tendencies occur within the estuarine environment. However, in estuaries there appears to be very little evidence for feeding specialists, although different morphotypes have been observed. Rather, we exploit the observation that many estuarine populations are in fact mixes of A and B haplotypes, indicating some degree of introgression between ancestral lineages. We used 14 estuarine populations: 3 pure A populations, 2 pure B populations, 6 populations predominantly A with some B haplotypes, 3 populations predominantly B with some A haplotypes. We excluded a sample from the north shore of the St. Lawrence, as its morphology is so distinct that we did not want to risk biasing the comparisons (Lecomte and Dodson 2004). We aimed at seeing how the introgressed estuarine populations compared morphologically with the pure (A or B) estuarine populations.

Two quite surprising results emerged from this analysis. The major source of phenotypic variation was generated by the Ba introgressed populations that significantly diverged from all other groups. In these introgressed populations, we see characteristics of the typical B morphology greatly amplified: big jaws, big eyes, big heads, long gill arches. This is the morphology Lecomte & Dodson (2004) defined as the St. Lawrence North shore morphotype. It thus appears that this morphology is typical of populations dominated by the B lineage and introgressed with the A lineage. In opposition on this discriminant function, these same traits are reduced in size and accompanied by those traits typically associated with the A morphology: bigger fins and fin bases, deeper and somewhat larger body proportions forming a less streamlined shape. These latter characteristics define the “chunky” body morphology of the St.

Lawrence south shore morphotype (Lecomte and Dodson 2004), but it appears common to the introgressed populations dominated by the A lineage.

The second source of phenotypic variation was generated by Ab introgressed populations that significantly diverged from all other groups. We see a typical A morphology characterised by deeper and bigger body proportions (the “chunky” morphology). In opposition, these traits are smaller and define a more streamlined morphology.

To summarize.

- Populations dominated by or composed purely of either clade A or B retain an ancestral morphology whether they live in lakes or estuaries.
- Lacustrine smelt ecotypes are morphologically more similar within their historical lineages than between lineages (more so in the case of the B lineage)
- The analysis of estuarine populations showing evidence of introgression between the two historical lineages relative to pure lineages reveals an even greater divergence in clade-specific morphological traits.

This leads to the speculation that if introgression increases genetic variance, and in particular additive genetic variance, then selection may be acting on the increased phenotypic variance within contemporary ecological settings to generate estuarine morphotypes. Is there really any evidence for strong selection in these settings? To answer this question, we used AFLP nuclear markers to document the level of genetic variation among sites in the Saint Lawrence estuary where the two, mtDNA lineages occur sympatrically. In particular, we looked for outlier loci: those that differ more than expected under neutral models of divergence, and are thus potentially

associated with divergent selection. We have analyzed to date 100 polymorphic loci of which 4 loci are far more divergent than expected under neutral models of divergence. They thus serve as markers of divergent selection. Using these 4 markers, we defined 4 genetically distinct populations in the St Lawrence estuary associated with divergent selection: south shore downstream, south shore upstream, Saguenay, north shore. Thus, local selection along the south shore, in the Saguenay and on the north shore, could be contributing to limited gene flow among populations. These results provide preliminary evidence that contemporary selection is acting on historical morphologies along the north and south shores.

## **Conclusion**

- Variation in morphological traits in smelt is largely attributable to historical lineages
- In both lakes and estuaries, smelt descended from the B lineage are characterized by big jaws and big eyes whereas smelt descended from the A lineage are characterized by smaller heads and eyes, bigger fins and less streamlined bodies.
- In lakes, both lineages have experienced parallel morphological changes in producing alternative ecotypes (big jawed macrophageous ecotypes, higher gill raker density microphageous ecotypes).
- Morphological differences characterizing the St. Lawrence morphotypes probably evolved long before the post-glacial colonization of the estuary by the two founding lineages.
- Their continued divergence appears related to introgression of the two historical lineages and divergent selection acting on contemporary populations.

- Divergent selection contributes to limiting gene flow among populations and maintaining the differential distribution of the mtDNA clades along the two coasts.

Future Directions. The next step in this investigation is to fully document the genetic divergence of the estuarine populations to confirm the preliminary evidence supporting the hypothesis that divergent selection contributes to limiting gene flow among contemporary populations. Once the true population genetic structure is defined, an important objective will be to breed and raise smelt of different morphologies from different genetic populations to see if morphological differences persist under common environmental conditions. If this proves to be the case, we may then expose different genetic populations to a gradient of environmental conditions to document if reaction norms (the phenotypic expression of a genetic population under different environmental conditions) differ among populations, a first step in demonstrating local adaptation. Considerable development in the culture of rainbow smelt to reproductive age is an absolute prerequisite to achieving these goals.

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## **Rainbow smelt (*Osmerus mordax*) spawning population monitoring on the Gulf of Maine coast of New England**

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Anadromous rainbow smelt have traditionally supported popular recreational fisheries and small-scale commercial fisheries along the Northeast coast of the United States. However, concerns have grown over the health of smelt populations throughout much of their range. Rainbow smelt appear to have been extirpated from the southern extent of their range (Chesapeake Bay to Southern New England), and fisheries in the northern part of their US range along the Gulf of Maine coast have declined. The states of Maine, New Hampshire and Massachusetts received a grant from the National Marine Fisheries Service's Office of Protected Resources to investigate the status of smelt and to develop a conservation plan for New England. Information on the present status of smelt in New England is limited. One component of the investigation was to monitor spring spawning runs to record biological data that can be developed into fishery independent indices of population abundance. Analyses will be conducted on size and age composition, catch per unit effort, and mortality; and comparisons of smelt population demographics will be made among rivers and to previous studies. Preliminary results of the smelt population monitoring were presented at the workshop and are briefly summarized here.

Rivers and fyke net stations were selected in each state for monitoring during 2008-2010.

Eight fyke net stations were monitored in Massachusetts using a box-frame net (4'x4' entrance, 4'x4' wings, and ¼ inch delta mesh) with a hoop-framed cod end. The fyke was set on rebar poles at mid-channel in the intertidal zone below the downstream limit of smelt egg deposition. The fykes were deployed on Monday each week, hauled for the next three days and removed until the next week. The sampling period targeted 11 weeks from the first week of March to the third week of May to coincide with the smelt spawning period. Two fyke stations were monitored in New Hampshire and five in Maine: both states deployed a similar net design with extended wings at sites with wider channels. The sampling duration in New Hampshire and Maine also varied due to a later ice-out and spawning season that occurs later with increasing latitude. With each haul, smelt were counted, sexed, measured (total length) and released. Weekly scale subsamples were recorded at some stations for aging. Smelt were captured at all fyke stations except the Westport River in Massachusetts during 2008-2010. Smelt catches among stations displayed distinct characteristics of run peak, run duration, and size composition.

Seasonality. Spatial and annual variability were noted in the duration and peak of spawning. The onset of smelt spawning consistently occurred in the first three weeks of

March during 2008-2010 in Massachusetts and New Hampshire. Spawning was delayed with increasing latitude in Maine, starting in late April and early May at the easternmost stations. Stations in southern Massachusetts had unique spawning seasonality with spawning peaks near mid-April and longer run duration. In contrast, smelt runs in northern Massachusetts and New Hampshire had late-March to early April peaks with sharply declining catches after the peaks and shorter run duration. Smelt run duration declined progressively from northern Massachusetts to Maine, a pattern that may continue with increasing latitude as evident by very brief spawning runs at the northern end of their range (McKenzie 1964).

Catch Per Unit Effort (CPUE). Fyke net catch rates varied widely among rivers and years. Analyses to date have focused on Massachusetts stations with consistent catch data during 2005-2010. The geometric mean of peak season catch-per-haul was estimated with 95% confidence intervals. Nominal and transformed CPUE data had high variance that could obscure the detection of population changes. The catch data did detect and track stronger than average cohorts in several rivers. Additional years of CPUE data are needed in order to demonstrate the value of fyke net catch data as abundance indices.

Length and Age Composition. Smelt are fast growing fish that mature at small size and are fully recruited to the spawning stock at age-2 in the study area. Smelt length data at most fyke stations displayed two age modes: one comprised of age-1 smelt and an age-2+ mode that was mainly age-2 smelt, with limited presence of older smelt. In Massachusetts, the age-1 mode was prominent in most years at most stations and comprised a majority of the length frequency at some rivers. This observation differs from the smelt population studies during the 1970s in Massachusetts, in which age-1

participation in smelt runs was intermittent and occurred at a lower frequency (Murawski and Cole 1978; Lawton et al. 1990). Overall, catches in southern Gulf of Maine showed evidence of reduced presence of older smelt and higher occurrence of age-1 smelt.

Mortality. Limited work has been done on population metrics for anadromous rainbow smelt throughout their range. A few studies have calculated population mortality and survival rates based on age structure (Murawski and Cole 1978; and Pouliot 2002). Annual survival rates (S) and instantaneous total mortality (Z) were calculated using the Chapman and Robson equation (Chapman and Robson 1960) for this study. This analysis has biases that may limit the accuracy of mortality estimates. Few age cohorts are available for the assessment: the age-1 cohort is excluded from mortality estimates because these fish are partially recruited to the spawning run, and age-4 smelt are presently uncommon. Secondly, the sampling method cannot distinguish the occurrence of repeated spawning movements of individual smelt. This behavior, as demonstrated by Murawski et al. (1980), could bias measurements of population mortality and survival. Under the assumption that these biases were consistent among studies, we calculated mortality and survival estimates and compared them to previous studies. These preliminary analyses found higher survival in Jones River and Parker River smelt populations in Massachusetts in the 1970s (Murawski and Cole 1978, Lawton et al. 1990) than in 2008-2010. In addition to having lower survival than the previous estimates in Massachusetts, the average survival of the smelt in the present study is lower than found in earlier studies in the Miramichi River (McKenzie 1964) and the Fouquette River in Quebec (Pouliot 2002).

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## Population genetic structure of anadromous rainbow smelt (*Osmerus mordax*) in the Northeast U.S.

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Understanding the genetic structure of marine and anadromous fish populations is important for their successful management and conservation. This objective is germane to the rainbow smelt (*Osmerus mordax*), a Species of Concern in the Northeast U.S. Due to their close association with estuarine habitats and the potential for estuarine retention of larvae, anadromous smelt are expected to exhibit population structuring at the scale of estuaries or retention areas. The goal of this study was to determine the genetic variation among rainbow smelt from multiple river systems in New England. Smelt were sampled during the spring spawning season in 2006–2010 from 18 river

systems: the Weweantic, Jones, Fore, Saugus, and Parker rivers in Massachusetts, the Salmon Falls, Bellamy, Oyster, Lamprey, and Squamscott rivers in New Hampshire, and Long Creek, the Harraseeket, Kennebec, Sheepscot, Penobscot, Pleasant, and Chandler rivers, and Cobscook Bay in Maine. Genetic analysis was conducted using 11 microsatellite markers. We found a temporally stable genetic structure, with weak but significant genetic differentiation among smelt from most river systems ( $F_{ST} = 0.017$ ), with the exception of the ones in closest geographic proximity (e.g. the five rivers of the Great Bay estuary in New Hampshire). Genetic structure followed an isolation-by-distance

model, as smelt from the most distant locations displayed the highest levels of differentiation, and genetic connectivity was highest within regional river groupings. Bayesian clustering approaches identified 4-6 population groupings, with genetic discontinuities coinciding with topographic features, such as capes and enclosed

bays that might promote larval retention. Genetic diversity was slightly reduced in the most northern (Cobscook) and southern (Weweantic) rivers. These results can be used to inform the designation of genetically distinct management units, which may guide restoration and restocking efforts.

## **Relationships between watershed conditions and rainbow smelt spawning populations in Maine, USA**

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### **Introduction**

Changes in land cover in a watershed can affect receiving waters in ways that alter habitat conditions, water quality, and biological communities (Burcher et al. 2007, Allan 2004). Urbanization and agricultural activities contribute to erratic flow levels, warmer water temperatures, channel alterations, sedimentation, chemical and bacterial pollution, and nutrient loading (Wang et al. 2001, Allan 2004). These physical and chemical changes can secondarily induce biological responses that affect fish and invertebrate populations and communities (Wang et al. 2001, Burcher et al. 2007).

Anadromous fish are susceptible to impacts from landscape alterations in the watersheds of rivers they use for spawning and rearing. Limburg and Schmidt (1990) found that the density of alewife eggs and larvae was inversely related to the proportion of urban land use in a watershed. Chinook salmon recruitment in the Columbia River also declined as the percentage of urban land in the watershed increased (Regetz 2003). Further, median densities of spawning

coho salmon in forest-dominated areas were 1.5-3.5 times greater than in urban or agricultural areas (Pess et al. 2002). These examples indicate that anadromous species can be influenced by land cover, but these linkages have not yet been investigated for rainbow smelt.

Rainbow smelt spawn at the head of tide of streams and rivers, an area likely to be affected by the characteristics of the contributing watershed. Changes in flow, temperature, nutrients, and chemical loadings to estuaries and rivers could affect smelt spawning location and success. Some of these effects could accrue directly; for example, increased flows may inhibit upstream spawning migrations. Others are likely to act through indirect pathways; as an example, increased nutrient loading may enhance periphyton growth and reduce smelt egg survival (Chase 2006; Wyatt et al. 2010).

In this study, we investigate relationships between watershed land cover and rainbow smelt spawning habitat use along the coast of Maine. We seek to understand land cover characteristics that inhibit as well as support

rainbow smelt spawning. Our ultimate objective is to evaluate whether land cover characteristics can be used to prioritize conservation areas for rainbow smelt.

## Methods

Field Methods. The Maine coast has historically supported over 250 unique rainbow smelt spawning sites. In 2005 and again in 2007-2009, the Maine Department of Marine Resources worked with the Maine Marine Patrol to survey each historically documented rainbow smelt spawning site to confirm the current status of spawning.

Patrol officers visited streams during the nighttime high tide to confirm the presence or absence of spawning adults and the daytime low tide to confirm the presence or absence of eggs. Smelt eggs can be easily identified because they are adhesive, demersal, and no other species' eggs are present in the same habitat in early spring. Officers visited the streams one to three times a week for the duration of the spawning run – March 1 to mid-May west of the Penobscot River and late April to mid-June to the east. At each visit, officers recorded the date, time, approximate number of adult smelt (0, 10s, 100s, 1,000s, 10,000s, 100,000s), and the approximate egg abundance in one square foot (0, 10s, 100s, 1,000s, 10,000s, millions).

The data for each site were compiled for the four survey years. Each site was assigned a rank (0-5) based on the absence or relative strength of spawning activity:

- 0 – no eggs or adults observed
- 1 – 10s of eggs and/or adults
- 2 – 100s of eggs and/or adults
- 3 – 1,000s of eggs and/or adults
- 4 – 10,000s of eggs and/or adults
- 5 – millions of eggs and/or 100,000s of adults

Within each year, the observation with the largest amount of eggs and/or adults was used to assign the rank. The final rank for each site was the average integer rank over the four survey years. While many sites were surveyed in more than one year, the rank for some sites was assigned using only one year of data. Sites that were visited infrequently or not at all were not assigned a rank and were excluded from further analysis.

Watershed Characteristics. Using the geographic information system (GIS) program ArcGIS 9.3, watershed characteristics for each ranked spawning site were determined. The National Hydrographic Dataset was used in conjunction with the Network Analyst extension to delineate the upstream flow network for each site. This network was then overlaid on the MaineGIS small watershed layer, and the entire drainage area for each site was delineated.

We used widely available land cover layers to describe watershed characteristics that may impact rainbow smelt spawning habitat. Land cover data (2005, 30-meter resolution) from NOAA's Coastal Change Analysis Program were used to determine percent forest, developed area, open space development, agriculture, and wetland for each watershed (NOAA 2008). Developed low, medium, and high were reclassified as a single "developed" class; deciduous, mixed, and evergreen forests as "forest"; palustrine forested wetland, palustrine shrub/scrub wetland, palustrine emergent wetland, estuarine forested wetland, estuarine shrub/scrub wetland, and estuarine emergent wetland as "wetland"; and pasture/hay and cultivated crops as "agriculture". Developed open space was left as an independent category that may indicate the amount of heavily fertilized land (e.g., golf courses) within a watershed.

Statistical Analysis. The full data set was first reduced to include only watersheds up to

the 75<sup>th</sup> quartile in size. This eliminated the influence of a few very large watersheds so that more localized watershed relationships to smelt spawning activity could be captured. From this reduced data set, outliers were evaluated and two sites that were affected by high numbers of upstream crossings were removed.

Land cover characteristics in the watersheds were first described using a principal components analysis (PCA). Our intent in this analysis was not to reduce the variables used in subsequent analyses but to understand the relationships among the variables and their relative contributions towards explaining variance in land cover among watersheds. This analysis was conducted in JMP (version 9.0.0) using the correlation matrix and a restricted maximum likelihood estimation procedure to compute the eigenvectors.

A cluster analysis was used to group the sampled spawning sites based on their land use characteristics. This analysis was also conducted in JMP (version 9.0.0). We applied hierarchical clustering using a complete linkage method to the Euclidean distances derived from the standardized land cover data. We interpreted five clusters from this analysis and used those clusters to graphically depict relationships among three land cover types and the ranked smelt spawning index.

Finally, we explored relationships between the land use variables and the ranked prevalence of smelt spawning using an ordered logistic regression. The developed open space land cover category was not used in the analysis due to a high collinearity with the developed category. Land cover data for the developed, agricultural, and wetland categories were log-transformed to stabilize the variance; forest was not transformed prior to inclusion in the analysis. The model was run using the `lrm` function from the `Design` package in R (version 2.12.1).

## Results

Of 218 historical spawning sites that were surveyed during the 2000s, 67% (146 sites) showed signs of current use by rainbow smelt. However, spawning activity was fairly low at most of these recently confirmed active sites; 62% received a ranking of 1 or 2 using the scheme outlined above, 37% were ranked as 3 or 4, and only two sites scored a ranking of 5.

The small watersheds used for detailed analyses represented a broad range of forest and wetland conditions, with each category ranging from 0% to nearly 100% coverage among the watersheds. Developed land cover ranged from 0% to 85%, whereas developed open space and agriculture showed smaller ranges of variability (0% to 25%) among the watersheds.

A principal components analysis showed that 93% of the variance in land cover could be explained by three axes. The first axis accounted for 47% of the variance, and it separated watersheds based on forest cover and development, with the developed and developed open space variables loading strongly with one another (Figure 1). The second axis (26% of variance) reflected differences in the watersheds based on the portion of wetlands and agriculture (positive weights on axis) and forest (negative weights). The third axis explained 19% of the variance and captured distinctions between wetland and agricultural land cover.

A cluster analysis using the same five land cover types grouped the watersheds into dominant categories. These clusters represented watersheds with: 1) high development and low agriculture, forest, and wetlands; 2) moderate development and agriculture but low forest and wetlands; 3) high forest and low to moderate levels of other land covers; 4) low development, moderate forest, and moderate to high wetlands; and 5) moderate development, low forest, and high agriculture and wetlands (Figure 2).

Further interpretation of the cluster analysis was supported by a trellis plot that relates ranked spawning prevalence to developed, agricultural, and forest land covers, with unique symbols representing each of the five clusters (Figure 3). The dominant pattern detectable in the trellis plot showed that highly developed watersheds did not support rainbow smelt spawning. The plot also indicated that sites that support higher levels of smelt spawning (ranks 3 and 4) generally had higher forest cover than those ranked lower (ranks 1 and 2).

Land cover variables that were associated with the prevalence of rainbow smelt spawning at a site were identified more formally using an ordered logistic regression. When all four land cover variables (developed, agriculture, forest, wetland) were included in the model, only agriculture showed a significant relationship with the prevalence of smelt spawning ( $p=0.006$ ). Since the land cover variables were log-transformed, the interpretation of the regression output suggests that for every 2.7 unit increase in the proportion of agricultural land cover, we expect a 0.19 unit increase in the ranked prevalence of rainbow smelt spawning at a site on the ordered logit scale when other variables in the model are held constant. The model has a likelihood ratio chi-square of 14.16 with four degrees of freedom and is statistically significant ( $p=0.0068$ ), but its predictive capacity is weak (pseudo  $R^2=0.088$ ,  $C=0.6$ ).

## Discussion

Rainbow smelt currently spawn in many rivers along the coast of Maine, and watersheds adjacent to these rivers represent a wide variety of land cover types. Rainbow smelt spawning was observed at 67% of the historical spawning sites surveyed, but the level of activity was low at most of these sites. Watersheds with high

percentages of developed land appear to no longer support spawning activity. Although some of the small watersheds analyzed as part of this study were over 85% developed, evidence of rainbow smelt spawning was not observed in streams draining watersheds that were over 41% developed.

It is important to recognize, however, that lower levels of watershed development may influence rainbow smelt spawning. Highly ranked rainbow smelt spawning sites (i.e., ranks equal to 4 or 5) only existed in watersheds with less than 10% developed cover. This 10% development threshold is consistent with observations of curtailed spawning activity of alewife in the Hudson River (Limburg and Schmidt 1990). It is likely that watersheds with higher percentages of developed land are less able to support spawning runs of anadromous fish; however, other factors influencing habitat (e.g., discharge, stream gradient, drainage size) may be as or more important in determining the strength of a spawning run at a given site.

Although graphical interpretations of the data indicated that high levels of development were associated with a lack of rainbow smelt spawning, a statistical analysis also detected a relationship between smelt spawning prevalence and the portion of agricultural land cover in the watershed. Positive associations between agricultural land cover and fish habitat quality, community structure, and biotic integrity have been reported for freshwater streams (Wang *et al.* 2002). However, other studies have found agricultural land cover to be detrimental to spawning populations of anadromous species, including rainbow smelt (Pess *et al.* 2002, Regetz 2003, Trencia *et al.* 2005).

The relationship between agricultural land cover and rainbow smelt spawning in Maine coastal rivers requires further study. It is possible that an interaction between development and agriculture exists such that the

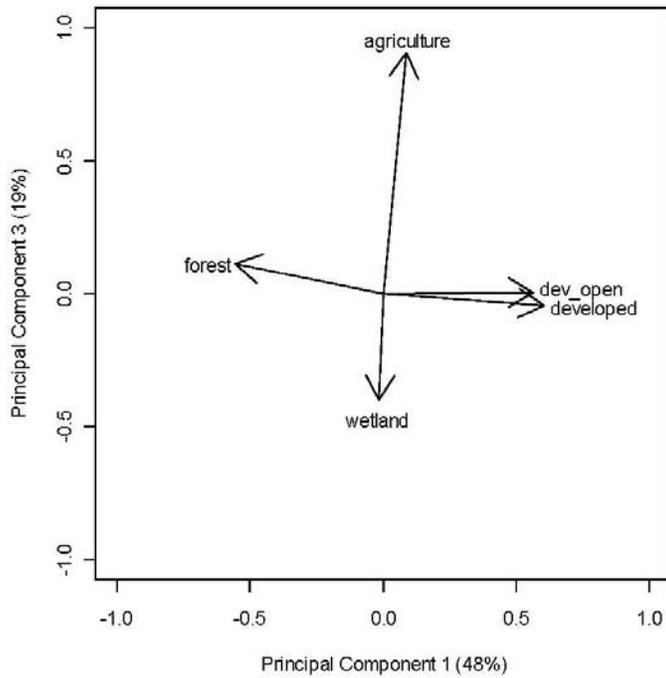
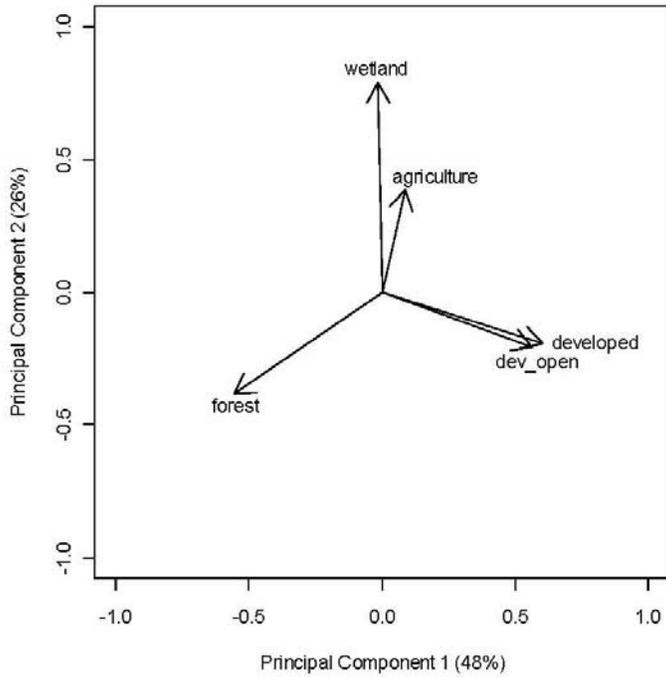
positive influence of agricultural land cover may be driven by an association with lower levels of development. However, it may also be possible that agricultural land cover in the watershed benefits rainbow smelt; the effect of agricultural activities may be buffered by streamside vegetation, or the use of best management practices may enhance the quality of streams in other ways during the rainbow smelt spawning season.

The results of these analyses do not find a strong relationship between rainbow smelt spawning abundance and land cover for small watersheds along the coast of Maine. Further analyses will be conducted to investigate the importance of land cover within a narrow buffer along the stream corridor, as other studies have shown near-stream land cover to have a stronger influence on aquatic ecosystems than land cover at the watershed scale (Wang *et al.* 2002). In addition, the qualitative nature of the rainbow smelt spawning data (i.e., ranked summaries of various numbers of point-in-time observations) used in this study may have affected our ability to discern relationships to land cover. Land cover influences on spawning rainbow smelt will be further assessed using quantitative observations over the full spawning season of the numbers returning to spawn at index stations from Massachusetts to Maine.

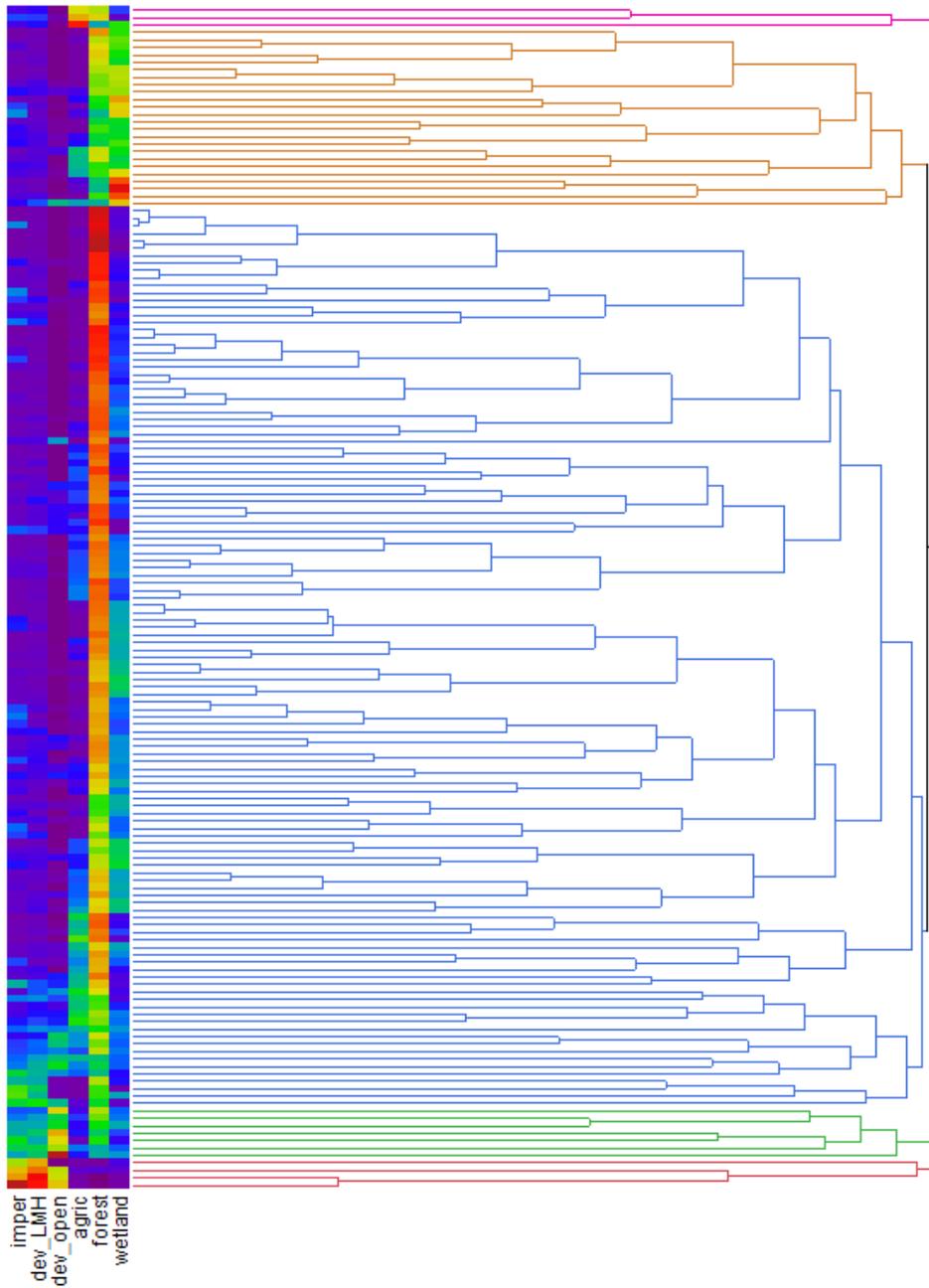
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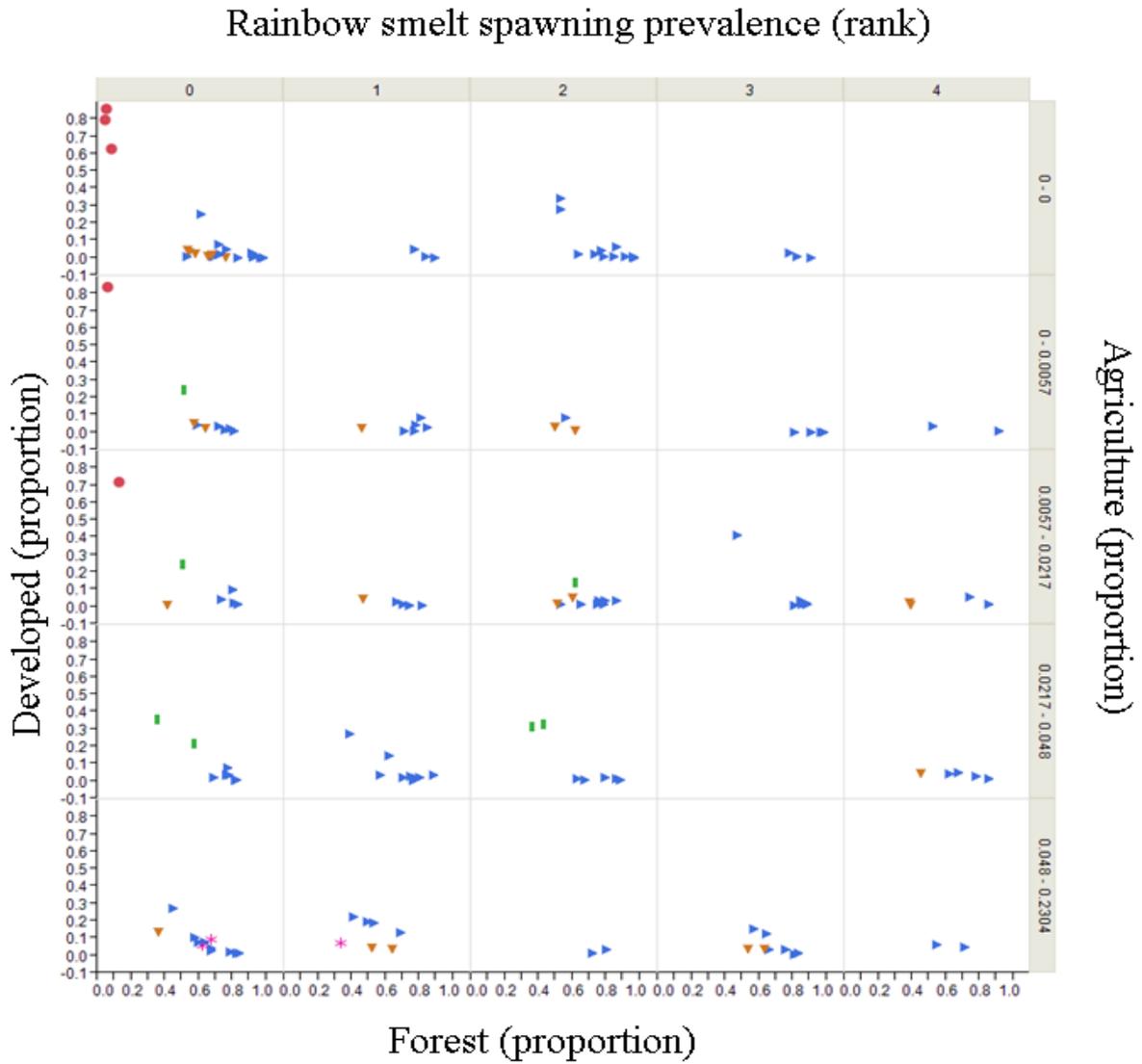
**Figure 1.** Loadings of land cover types in small surveyed watersheds as defined by the first three axes of a principal components analysis.



**Figure 2.** Hierarchical clusters of small surveyed watersheds based on land cover types. The proportion of land cover of each type (e.g., developed, developed open space, agriculture, forest, wetland) is indicated by the colored bars on the left, with red identifying a high proportion of a certain land cover and dark purples associated with low proportions. Five clusters used for further interpretation are shown in unique colors on the dendrogram.



**Figure 3.** Trellis graph of sites with their smelt spawning rank plotted against development, forest, and agriculture in the watershed. Points are coded so that the marker color corresponds to the cluster to which they were assigned in Figure 2.



# Monitoring within-season spawning behavior by rainbow smelt *Osmerus mordax* using passive integrated transponder (PIT) systems

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Rainbow smelt (*Osmerus mordax*) are small anadromous fish that live in near-shore coastal waters and spawn in the spring in coastal rivers immediately above the head of tide in freshwater. During the spawning run, a skewed sex ratio is observed with more males visiting the spawning grounds compared to females (Marcotte and Tremblay 1948; Murawski et al. 1980, a behavior which has been found to increase fertilization success (Purchase et al. 2007). Sampling large groups of smelt during non-breeding seasons has found a balanced sex ratio; on the Parker River, Massachusetts, age-2+ females composed only 11.4% of the sample during one spawning survey compared to 47.4% of the winter fishery catch within the same year (Murawski et al. 1980).

While repeat spawning behavior has been described, the frequency of recurrence according to sex and age has not been quantified. Because most population surveys are conducted during the spawning season, the catch data are biased by repeatedly counting the same males; mortality rates calculated by tracking age classes through time also carry the bias. Previous mortality estimates have been based on total catch during the spawning season. Murawski and Cole (1978) estimated a higher mortality rate for males compared to females in the Parker River,

Massachusetts using a frequency at age model based on spawning survey catches. Because a larger number of age-2 males may repeatedly visit the spawning grounds compared to older males, the data would falsely indicate that age-2 males compose a larger proportion of the population, leading to a higher mortality rate calculation. Quantifying the rate of repeat spawning by age and sex allows the frequency at age to be corrected and accurate mortality estimates calculated.

This study was aimed at characterizing smelt movement patterns during the spawning season using PIT tags in concert with RFID systems to improve population statistics generated from spawning run monitoring. Small passive integrated transponder (PIT) tags can be placed internally in small fish with little mortality and tag loss (Bruyndocx et al. 2002) and be detected using continuously running in-stream radio frequency identification (RFID) systems. The methods and results are reported from the first two years of a four year study to quantify within-season spawning behavior. The results are preliminary and will be compared to data collected in the last two years of the study and will also be compared to results from a similar study in the Fore River, Massachusetts.

## Methods

Within-season spawning behavior by rainbow smelt was examined on Mill Creek at the head of tide of the Harraseeket River in Freeport, Maine. A historically known strong spawning run, the site continues to support annual spawning comparable with the larger spawning runs in the state. Smelt were collected using fyke nets during annual spring spawning runs (March to June) in 2009 and 2010. No more than 60 smelt were tagged each week for ten weeks with 23mm PIT tags and monitored with in-stream continuously running RFID systems (Figure 1). Tagging was based on sex and age class as determined by length.

In addition to the field study, a laboratory retention and mortality study was completed at the Annisquam River Marine Fisheries Field Station in Gloucester, MA. In 2010, 90 smelt were tagged according to sex and length. Daily mortalities were removed and sex, length, and PIT tag number recorded. The holding tank was monitored daily for any expelled tags. The laboratory study will be repeated in 2011.

## Results

In 2009, 143 smelt were tagged (95 male; 48 female) and in 2010, 111 smelt were tagged (70 male; 41 female). The average size for both genders remained similar between the years, however, a wider range of sizes for both genders was tagged in 2010. A proportion of both males and females were never detected by the system. While mortality due to tagging may be responsible, it is also possible that the system failed to detect the downstream movement of these fish. All females either exited the spawning area immediately after tagging or returned once, however, males

returned to the spawning area up to eight times within a season. In both years and combining 2009 and 2010 data, males returned significantly more times than females (Pearson Chi Square;  $\alpha = 0.05$ ; 2009 = 0.0230; 2010 = 0.0004; 2009 + 2010 < 0.0001). When comparing the number of returns by males to length, a bimodal pattern results with one peak in returns at 13-14cm and another peak at 17-19cm.

## Discussion

Quantifying repeat spawning behavior by sex and age will enable us to tune annual smelt fyke net monitoring data to reflect the number of individual smelt visiting the spawning grounds. Because mortality estimates are based on the size of age cohorts over time, previous estimates using spawning survey data have over estimated mortality. Preliminary results show that males return to the spawning grounds at a significantly higher rate than females. Comparing the number of returns to length, we found a peak in the number of returns lengths corresponding to age-1 and age-2 males. Individual ages will be confirmed before final conclusions are made. The results of this study and a similar study at the Fore River, MA will be used to adjust frequency at age tables and mortality estimates.

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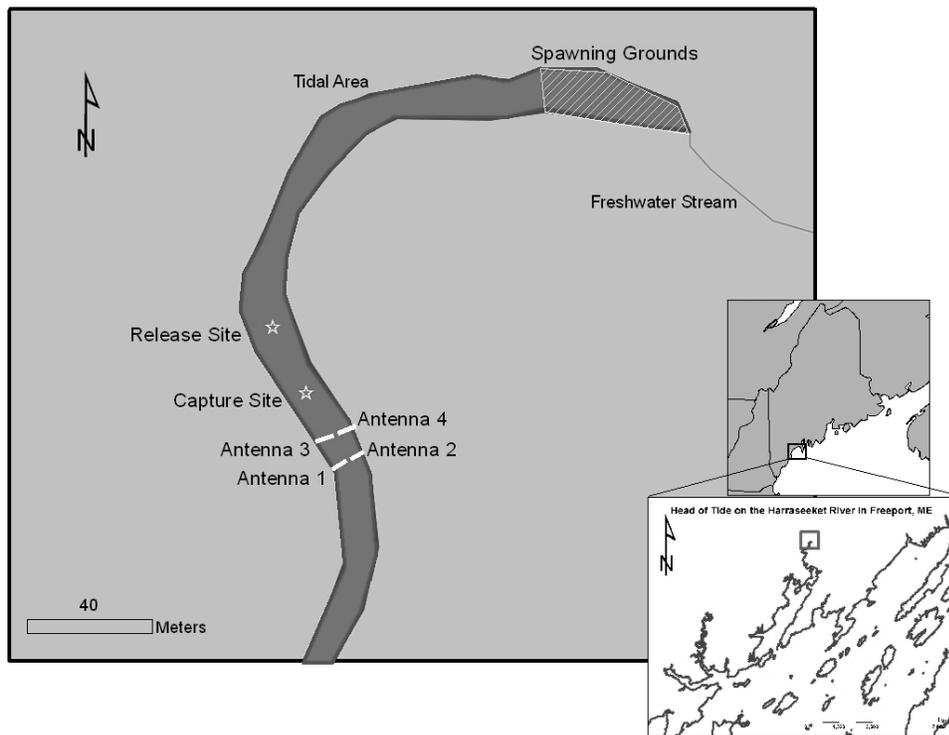
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**Figure 1.** A half duplex (HDX) radio frequency identification (RFID) system was used to monitor repeat spawning behavior of rainbow smelt in the Harraseeket River, Maine for the duration of the spawning season (March to early June) in 2009 and 2010. Two sets of antennas (downstream set: Antenna 1 and Antenna 2; upstream set Antenna 3 and Antenna 4) were placed downstream of the spawning grounds but close to the head of tide. Smelt were captured using a large fyke net placed directly above the antenna arrays.



## Rainbow smelt culture in recirculating systems

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A series of experiments were conducted to evaluate the growth and survival of juvenile rainbow smelt reared in recirculating systems under different environmental conditions (16, 20 and 24°C; 0, 5 and 15 mg/L salinity) and fed 3 dietary protein levels (35, 42 and 50%). Larval smelt were initially fed live prey (rotifers and *Artemia*) and gradually weaned to commercial formulated diets over a 2 or 3-day period. Growth was significantly greater in juveniles fed

the highest protein diet (50%) and reared at the lowest temperature (16 °C), although survival did not differ among treatment groups. Growth and survival were high and did not differ among salinity treatment groups. While smelt are highly adaptable to culture in recirculating systems, high levels of cannibalism will need to be addressed before commercial-scale production can be realized.

## Spawning strategies and dynamics among anadromous smelts, are we aware of only the tip of the iceberg?

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### Introduction

While diadromy is known among less than 1% of fish species (≈227 species; including 15 Anguilliforms, 28 Salmoniforms and 35 Osmeriforms: Nelson, 2006; McDowall, 1987), the phenomenon is nevertheless well documented thanks to the relative importance of such fishes. However, the many studies based on salmon and eels suggest the prevalence of strict models for the anadromy and catadromy that are not necessarily representative of the variability known amongst diadromous species (See Dodson *et al.* 2009; McDowall, 1987). The latter case is typified by smelts (family Osmeridae) which exhibit nearly the entire range of

spawning strategies known for diadromous species (Dodson *et al.*, 2009; McDowall, 2010).

Recent studies recognize 13 families of Osmeriforms (Dodson *et al.*, 2009; Iles & Taylor 2009; Waters *et al.*, 2002; Froese & Pauly, 2011) that include southern smelts (Retropinnids: 6 sp), northern smelts (Osmerids; 13-15 sp), plecoglossids (1 sp) and galaxiids (40 sp). The present study is primarily aimed at describing spawning strategies of the rainbow smelt (*Osmerus mordax*), for which a wider perspective reveals the hidden part of the iceberg! From truly marine to pure freshwater species, a symphony of variation has been composed upon the basic diadromous life-history strategy (Table 1).

Variability in spawning dynamics among *Osmerus*. Although the genus *Osmerus* includes only 3 species, their combined distribution range covers much of the temperate region of the northern hemisphere; *O. mordax* is native to coastal Northeast North America, *O. eperlanus* is found in Europe, and *O. dentex* exploits the Arctic basin and the North Pacific Rim (Kottelat & Freyhof, 2007; Nellbring, 1989; Scott & Crossman, 1973). Throughout these areas *Osmerus* appears highly adaptable, both in terms of genetic origins and their capacity to recolonize post-glaciated habitat (e.g. glacial races) or biological entities (e.g. dwarfs, giants, anadromous, landlocked, planktivorous, benthivorous, etc.). Again, the plasticity typical of this group pertains to most aspects of their history and biology.

For anadromous populations of *Osmerus* spp., the “typical” spawning strategy may be simply illustrated as follows: adults gather early in spring in tributary streams, spawning occurs at night at the margin of the tidal influence, and the sex-ratio is skewed in favour of males, except during the peak of spawning activities when sex-ratio is more equal and large females are present. After 10-14 days of incubation (or more when water temperature remains low, a total of 164°C\*days is required for hatching: McKenzie, 1964; Akielaszek *et al.*, 1985; see Bouchard 1993 for an extensive discussion on the topic) eggs hatch and buoyant larvae drift toward the sea (Ouellet & Dodson, 1985a,b; Chase, 2006). The only identified differences among anadromous populations are related to the duration of spawning activities: about 1-2 weeks in the northernmost part of the range (Pettigrew, 1997; Trencia & Langevin, 2008) and nearly 2 months in the south (Chase, 2009).

Surprisingly, while such typical behaviour is assumed for anadromous populations, the landlocked forms of *Osmerus* spp. exhibit a wide variety of strategies. Among these

populations, alternative spawning habitats are documented and, even for a specific population, the spawning strategy may change year to year. There are also some cases where the anadromous populations are exploiting the brackish estuarine environment as a replacement of the sea (Table 2).

#### What constrains spawning in *Osmerus*?

Many factors are known to influence the survival of eggs (oxygen, pH, salinity, etc.), or simply affect the capacity for adults to use a tributary for spawning (e.g. water velocity, obstacles, etc.) (Rupp, 1959a/b; McKenzie, 1964; and Chase, 2006). Few studies are available that quantify the relative importance of each factor (Brassard & Verreault, 1997; Fuda *et al.*, 2007). We present in Table 3 some values typically reported, and corresponding references, it must be noted that these values are only indicative and should not be used as definitive references. Among all factors known (Table 3), the values for salinity appear somewhat surprising considering that all spawning runs studied to date are located within the freshwater portion of rivers.

Thus, the presumption that salinity may hinder spawning success appears somewhat exaggerated when considering laboratory studies showing that *O. mordax* egg survival is nearly 100% in brackish water of 15 ‰ (Fuda *et al.*, 2007); survival declines only when salinity increases to 20 ‰ (Ayer *et al.*, 2005; Fuda *et al.*, 2007) in laboratory experiments. The spawning of rainbow smelt in semi-diurnal estuarine systems may thus be far less constrained by salinity than previously suspected. This latter observation must be further put in the context of the spring freshet that is likely to push further downstream the saline front creating a freshwater plume outside the river itself.

The St. Lawrence Middle Estuary smelt populations as a case study. Early work within the St. Lawrence estuary suggested the existence

of a single smelt population spawning in tributary streams along the south shore of the Middle Estuary, while larvae were advected in the deep channels of the estuary where they recruited to the adult population. The exploitation of smelt in the whole region was based upon this supposition of a single stock. The single stock hypothesis and other notions of smelt life history in the region had to be abandoned in 1996 when smelt in the St. Lawrence estuary was found to be composed of two genetically-distinct “cryptic” sympatric populations (Bernatchez & Martin, 1996). The two populations identified were named according to the location of the adults during the summer: the North Shore Population (NSP) and the South Shore Population (SSP). With this realization, it appeared that all known spawning sites belonged to the SSP. Only larvae of the NSP were collected in the deep waters of the estuary. Following several studies aimed at quantifying the degree of isolation between these two populations, it appeared that many aspects of their biology were distinct: they originated from two distinct glacial races, they are ecologically distinct, they exploit distinct feeding habitats, they possess distinct morphological features, their larvae exploit different nursery grounds, and they spawn in distinct habitats (Bernatchez & Martin 1996; Lecomte & Dodson 2004; Lecomte & Dodson 2005; Lecomte 2005).

The quest for the identification of the spawning sites. Among the many aspects of the biology that required clarification, the undiscovered location of the spawning sites of the NSP was the most intriguing. The middle estuary harbors high densities of larvae belonging to the NSP population (Pigeon *et al.*, 1997, Lecomte & Dodson, 2004); however, surveys aimed at the various tributaries of the St. Lawrence never found smelt spawning grounds other than those exploited by the SSP (Pigeon *et*

*al.*, 1997; Bernatchez & Martin, 1996). At that moment, it was hypothesized that the NSP spawning ground may be located upstream of the Middle Estuary, as young larvae belonging to the NSP were collected around the Orleans Island. (larvae aged less than 4-7 days).

To better localize the spawning area, ichthyoplankton surveys were conducted in early May between the Middle Estuary (Orleans Is.) and Trois-Rivières, located nearly 260 km upstream of the Middle Estuary. From these surveys it was possible to infer that the NSP was exploiting shallow shoals nearly 90 km upstream of the Middle Estuary (Lecomte & Dodson, 2004). In this area, called Neuville Shoals, no important tributaries were known and no eggs were found in the small brooks and rivers, despite the very large number of larvae found nearby in the St. Lawrence mainstem (Lecomte & Dodson, 2004). The larvae were found to be less than 1 day old in the vicinity of Neuville and 4-5 days old in the vicinity of Québec City. Moreover, drifting eggs were also observed.

Based on the pattern of drifting larvae, we determined the exploitation of the Neuville shoal as a spawning ground, but we were unable to define the precise location of the spawning run or to delimit the zone exploited. In the following years (2005-2006), studies aimed at quantifying the contribution of the smelt egg incubator located on the de l'Église Brook (to supplement the SSP; Fig.1) revealed that larvae belonging to the SSP were quite rare (all larvae from the incubator were marked using Alizarin-red) (Cleary *et al.*, 2007). The percentage was estimated to be 4.5% and 1% for 2005 and 2006 respectively; however, more than 80% of all larvae collected in the vicinity of the incubator were aged of 0-1 day, implying that they could not have originated from the Neuville shoals (Cleary *et al.*, 2007). The following year (2007), a more exhaustive survey stretching from Neuville to the vicinity of de l'Église Br.

confirmed that larvae belonging to the NSP found nearby the incubator were originating from another NSP spawning site located downstream of Québec City (Martin, 2008; for the genetic identification see Côté & Bernatchez 2009).

As we were aware of the potential use of shoal areas as spawning habitat for smelt, the quantification of the use of such spawning habitat became an essential investigation related to smelt fisheries exploitation in the region. Research was undertaken to identify other shallow areas exploited by smelt as spawning habitat. As was observed during spring 2007 and 2008 by the private consultant firm GENIVAR (2007, 2008) and further confirmed in 2010 and 2011 by our own survey (see Legault & Lecomte this volume), large numbers of eggs were found on a shallow shoal close to Beaumont (See Fig.1; Legault & Lecomte, this volume). The genetic identification revealed that they belong to the SSP (see Legault & Lecomte, this volume). Although we still did not succeed in identifying NSP spawning sites, we nevertheless realized that both the NSP and SSP populations exploited shallow shoals to spawn. The extent of the previously unknown shallow shoal spawning areas in the main stem St. Lawrence River in close proximity of de l'Église Brook suggest that spawning activities previously observed within the little brook may be in fact only the tip of the 'reproductive' iceberg!

## Conclusions

We suggest that such alternative spawning strategies (tributary vs. coastal) must be common in rivers with large freshwater plumes or in the low salinity zones of estuaries. Although it is difficult to locate and retrieve eggs laid in such habitat because of environmental conditions, the egg collectors (see

Legault and Lecomte, this volume) are relatively easy to build and can apply to study designs in watersheds where questions remain over the location of spawning habitat. For the SSP, such findings clearly helped us to better understand several conflicting observations and helped us to redirect future research.

It appears that past surveys based on the stereotypical tributary-spawning strategy for the de l'Église Brook run provided a biased evaluation of spawning, as it was based on the proverbial tip of the iceberg. The alternative, shoal-spawning dynamic is rarely documented among anadromous smelt populations, but we do not know whether this is because it is truly rare, or due to the difficulty of detection. We suggest that this alternative spawning strategy may be more widespread than acknowledged and the phenomenon should be quantified, particularly as the strategy may become increasingly important when habitat quality in spawning tributaries declines. I will conclude by presenting a phrase Rupp wrote more than 60 years ago (1959a): "Studies in Maine indicate that shore spawning may be considerably more important in the biology of smelts than has previously been recognized."

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**Table 1.** Example of species and their life-history strategies relative to spawning dynamics

<b>Species</b>	<b>Group</b>	<b>Location of spawning</b>	<b>Larval nursery</b>	<b>Juveniles</b>	<b>Adults</b>	<b>Landlocked populations</b>
<i>Plecoglossus altivelis</i>	Plecoglossidae - Amphidromous	<b>River</b>	<b>Sea</b>	<b>River - Estuaries</b>	<b>Estuaries-Sea</b>	Yes
<i>Mallotus villosus</i>	Osmeridae – Marine	<b>Marine (Beach or Deep-water)</b>	<b>Sea</b>	<b>Sea</b>	<b>Sea</b>	No
<i>Osmerus mordax</i>	Osmeridae – Anadromous	<b>River</b>	<b>Sea (Estuaries)</b>	<b>Sea (Estuaries)</b>	<b>Sea (Estuaries)</b>	Yes
<i>Retropinna retropinna</i>	Retropinnidae – Anadromous	<b>River</b>	<b>Sea</b>	<b>Sea</b>	<b>Sea</b>	Yes
<i>Galaxias olidus</i>	Galaxiidae – Freshwater	<b>River</b>	<b>River</b>	<b>River</b>	<b>River</b>	---
<i>Hypomesus olidus</i>	Osmeridae - Anadromous	<b>River</b>	<b>Sea (Estuaries)</b>	<b>Sea (Estuaries)</b>	<b>Sea (Estuaries)</b>	Yes
<i>Galaxias maculatus</i>	Galaxiidae - Amphidromous	<b>Estuaries</b>	<b>Sea</b>	<b>Sea, after River</b>	<b>River</b>	Yes
<i>Lovettia sealii</i>	Galaxiidae- Anadromous	<b>River</b>	<b>Sea</b>	<b>Sea</b>	<b>Sea</b>	

**Table 2.** Example of alternate spawning dynamics among *Osmerus* spp.

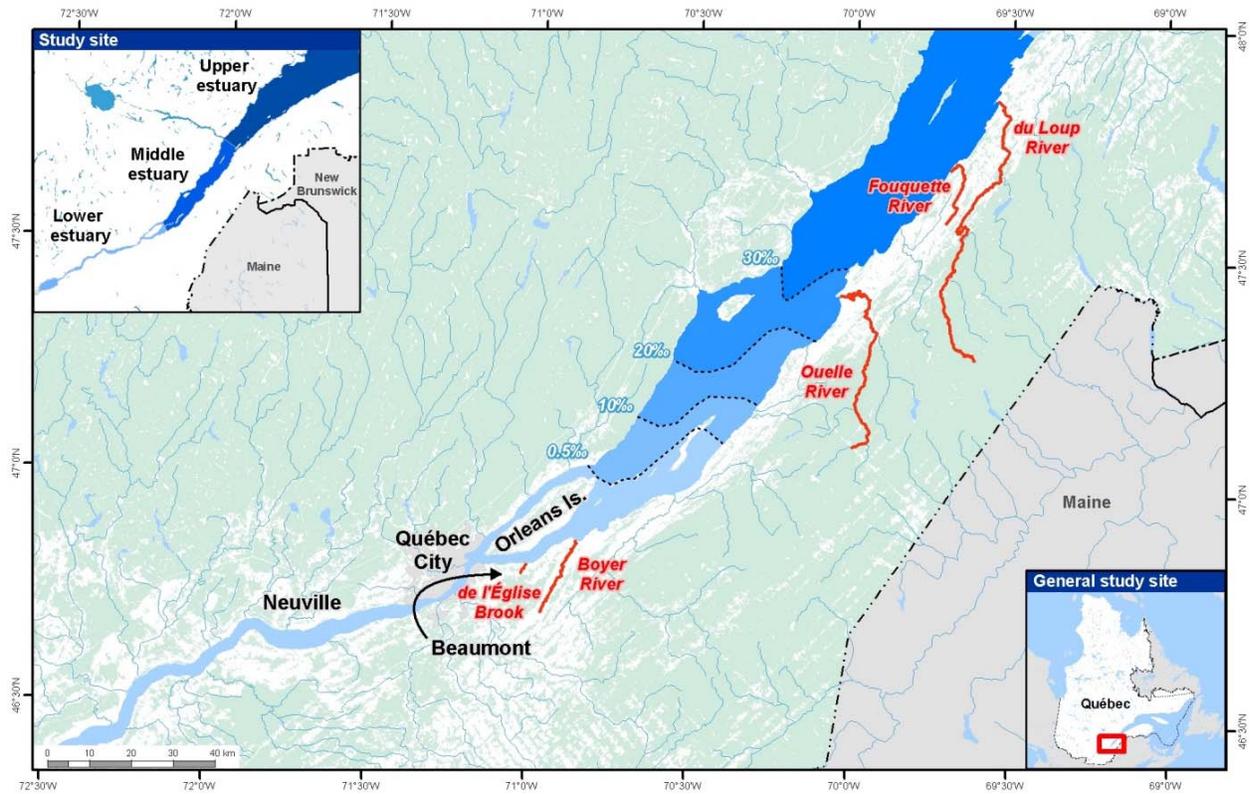
	<b>Species</b>	<b>Spawning site</b>	<b>Location</b>	<b>References</b>
<i>Lacustrine</i>	<i>O. mordax</i>	Shoreline	Some lakes in Maine	Rupp, 1959a,b
	<i>O. mordax</i>	Deepwater	Lake Heney, Quebec	Legault & Delisle, 1968
	<i>O. mordax</i>	Shoreline	Crystal lake, Michigan	Lievense, 1954
	<i>O. mordax</i>	Shoreline	Lake Michigan	Lievense, 1954
	<i>O. mordax</i>	Deepwater	Lake Champlain	Plosila, 1984
<i>Estuarine / Brackish</i>	<i>O. eperlanus</i>	Estuarine portion	Gulf of Bothnia	Hudd & Urho, 1985
	<i>O. eperlanus</i>	Coastal lagoon	Various sites in the Baltic	Shpilev <i>et al.</i> , 2005
	<i>O. mordax</i>	Shallow shoal, upstream of the middle estuary (freshwater)	St-Lawrence, Québec	Lecomte & Dodson, 2004
	<i>O. mordax</i>	Shallow area, upstream of the saline intrusion	Saguenay Fjord	Lesueur, 1998
	<i>O. mordax</i>	Extended migration (>10 km) above tide to riffle	Charles River, Mass.	Chase, 2006

**Table 3.** Factors affecting the spawning dynamics and hatching success.

<b>Variable</b>	<b>Min. value</b>	<b>Optimal value</b>	<b>Max. value</b>	<b>References</b>
Salinity (‰)	0	0-15	< 20	Ayer <i>et al.</i> , 2005 Fuda <i>et al.</i> , 2007
Oxygen (%)	20	100	100	Fuda <i>et al.</i> , 2007
pH	> 5	6.5-9.0	n/a	Fuda <i>et al.</i> , 2007 Geffen, 1990 Brassard & Verreault, 1997
Suspended material (mg/l)	0	n/a	20-25	Brassard & Verreault, 1997
Nitrates (mg/l)	0	0-29.2*		Fuda <i>et al.</i> , 2007
Phosphates (mg/l)	0	0-4.2*		Fuda <i>et al.</i> , 2007
T (°C)	4-5	10-15	n/a	Ayer <i>et al.</i> , 2005 Chase, 2006 (average start)
Water velocities (m/s)	<0.3	0.3-1	< 2	Brassard & Verreault, 1997 Chase, 2006

\* = These values were the most elevated tested and allowed nearly 100% hatching success

**Figure 1.** Study site. Tributaries sustaining smelt spawning runs are illustrated. The Boyer River population is actually extirpated. The isohalines indicate average positions measured at high tide (‰).



# Ghost hunting: quantifying and localizing alternative spawning grounds used by anadromous rainbow smelt (*Osmerus mordax*)

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## Introduction

Within the St. Lawrence Middle Estuary, two sympatric smelt populations are known to co-exist (Bernatchez & Martin 1996; Lecomte & Dodson 2004). The two populations are known to be ecologically distinct and to utilize distinct feeding habitats. They possess distinct morphological features, their larvae exploit different nursery grounds and they spawn in distinct habitats (Bernatchez & Martin 1996; Lecomte & Dodson 2004; Lecomte & Dodson 2005; Lecomte 2005).

The existence of an alternative spawning strategy among St. Lawrence Middle Estuary sympatric smelt populations was first detected in the late 1990s (Lecomte & Dodson 2004; Lecomte 2005). The South Shore Population (SSP) spawns in “traditional” tributary streams close to the nursery habitat (shallow shoals and embayments along the south shore). Previously, nothing was known about the NSP spawning activities. We found that the NSP was exploiting the shallow shoals located in Neuville, which is located 90 km upstream of the population’s estuarine nursery habitat (Lecomte & Dodson 2004).

The observation of recently hatched larvae (1-2 days) belonging to the NSP in the vicinity of Orleans Is. (Fig.1), 70-80 km downstream of

the Neuville area, suggested that major spawning sites used by the NSP were to be found downstream of the site identified in the late 90s (Cleary *et al.* 2007; Martin 2008; Legault *et al.* 2009). As pointed out by Lecomte (this volume), many studies (Pigeon *et al.* 1998; Lecomte & Dodson 2004; Côté & Bernatchez 2009, 2010) provided observations relative to the contribution of SSP larvae in this area, despite the elevated number of SSP larvae produced by the incubator (spawning facility) located nearby in the village of Beaumont (de l'Église Brook) (Trencia & Langevin 2006, 2007, 2008): despite the production of 20-40 millions larvae of the SSP in the incubator, they went almost totally undetected in surveys (e.g. larvae contribution <1% SSP; Pigeon *et al.*, 1998; Lecomte & Dodson, 2004; Côté & Bernatchez, 2009, 2010; Lecomte *et al.*, *In press*) because of the abundance of NSP larvae found in the St. Lawrence directly in front of the de l'Église brook.

During a study aimed at evaluating the use of shallow shoals by smelt in the vicinity of the Beaumont shoals (Fig. 1), GENIVAR, a consulting firm, found egg deposition during two consecutive years (GENIVAR 2007, 2008). The origin of the eggs was a matter of debate as they were not genetically identified. We proceeded to quantify the smelt spawning

habitat in the shallow shoals near Orleans Island. This became a research priority and a prerequisite for an environmental evaluation required for future development of this region.

The objectives of the present study were to (1) assess the importance of egg deposition in this area and (2) genetically identify the origin of eggs.

## Materials and Methods

Egg deposition surveys were conducted in 2010 during the St. Lawrence middle estuary smelt spawning season, which corresponds roughly to the time when water temperature warms from 4°C to 6°C (Trencia *et al.* 2005). As water velocities in the St. Lawrence may reach up to 3.0 m/s in this area due to the combined effect of freshwater outflows and tidal currents, traditional egg collectors cannot be used (e.g., ceramic plates). Thus, we modified the technique initially developed by GENIVAR some years ago. Rows of ten 1/8" (3.2 mm) "satin" steel plates of 30 cm x 30 cm (12" x 12") linked together with wires were used (Photo 1). The "satin" steel is a grade between true galvanized steel and raw steel; it is not as rough as the raw type but it does show greater rust resistance and is less expensive than galvanized steel. We used a combination of 10 plates spaced by approximately 6' (2 m) of wire. Two anchors were used to maintain the rows on the riverbed. Buoys attached to each anchor were used to locate and recover the plates.

Since smelt eggs are adhesive, plates could be left underwater for several days. Because we wanted to quantify the chronology of the spawning activities, and because elevated sedimentation coupled with the strong currents in the area could displace and/or bury the plates, we limited the time span between sampling to every 2-3 days when weather condition allowed. On each occasion, we removed the plate lines

and collected all attached eggs. The number of plates on which eggs were found was recorded. Eggs were kept in 95% ethanol.

The genetic identification technique used followed the technique developed by Pigeon *et al.* (1998) and updated by Lecomte *et al.* (*In press*) to reflect the recent studies specifying population genetic structure (Lecomte & Dodson 2004; Côté & Bernatchez 2009, 2010).

The NSP is composed of 79% allele "B" and 21% allele "A" whereas the SSP is composed of 81 % allele "A" and 19% allele "B" (see Lecomte and Dodson 2004, Lecomte *et al.* *in press*). Thus, alleles are not diagnostic, requiring the use of a mixed-stock technique analysis. The egg contribution (%) of each population for each plate row was calculated using the mixed-stock analysis of Lane *et al.* (1990) for a two population – two allele setup. The use of a mitochondrial marker implies that only matriarchal lineages are detected. Technically, eggs will bear exactly the same genetic signature as that of their mother. Moreover, as females lay several eggs (clutch) during each spawning act, the analysis of eggs attached physically close to each other may over-represent the contribution of a population as they may belong to the same female. Thus, we limited the number of eggs analyzed per row by fixing a limit of an equivalent of 5 eggs per plate. As plates are spaced by 2 m, distinct plates are unlikely to be covered by eggs deposited by the same female. By this procedure, we thus restrained theoretically the over-representation of an individual female (e.g. if 55 eggs are retrieved in a string where only 1 plate was covered by eggs, only 5 eggs will be analyzed). In addition to the eggs collected in 2010, a sample of eggs collected in 2008 was kindly provided by GENIVAR (see GENIVAR 2008).

The egg densities were calculated using the number of eggs deposited over the surface area of plates used within each area sampled. The

number of eggs deposited on each row was summed as they represent a physical location where normally the eggs would accumulate following individual spawning acts. Error estimates were calculated using bootstrap resampling to represent the variability associated with individual rows (e.g. if a female did spawn directly over one plate). The egg deposition areas were calculated using georeferenced bathymetry maps.

## Results

The use of metal plates to locate spawning grounds proved an efficient method, as it was possible to retrieve eggs despite harsh conditions. Although the plates were anchored some rows moved several meters from their initial position. Moreover, in some areas, plates were rapidly covered by sediment (less than 2 days). Thus, all estimates of egg deposition probably underestimate the true egg deposition. Nevertheless, large numbers of eggs were detected, indicating that the area sampled in 2010 is an important spawning area.

The broad distribution of eggs (Fig. 1) reveals that smelt utilize large expanses of shallow shoals. The distribution also indicates that eggs are not only restricted to the downstream river plume, but also are not influenced by any neighboring rivers.

For the 2010 season, the genetic identification shows that egg depositions all belonged to the SSP (Table 1). Contribution above 100% of a population is an artifact created by the method used to calculate the contribution (see Lane et al. 1990). This artifact is accentuated for samples of small size. The over-representation does, however, fall always within the 95% error estimates (1.96 SD).

Samples of the 2008 season revealed that the three sites analyzed were dominated by SSP eggs: two in the “Hot-Spot”, H53 ( $n_{\text{genetic}}=22$ ;

64.9% SSP; SD=15.2%) + H58 ( $n_{\text{genetic}}=26$ ; 102.1% SSP; SD=11.3%), and one in the de l'Église Brook (H66) freshwater plume ( $n_{\text{genetic}}=9$ ; 129.4% SSP; SD=19.6%). In the case of the 2008 samples, it was impossible to correct for over-representation of individual females, hence the elevated estimates for H53 (*i.e.* too many eggs from the same female bearing the same allele).

Assuming that the average egg densities estimated for the three areas surveyed in 2010 (75.7 eggs / m<sup>2</sup>) are representative of the whole shallow area (<2 m depth at low tide along 2.5 km of coastline; 5.5 km<sup>2</sup>) where egg depositions were reported either on the shallow areas (GENIVAR 2008, 2007) or in some brooks (Trencia & Fournier 1999; Trencia 1991), a total of 417 (SE= 251 ) million eggs may be spawned in this region.

## Discussion

The exploitation of shallow shoal was already known for several populations (see Lecomte, this volume), including the NSP (Lecomte & Dodson 2004). We showed that the SSP did also exploit spawning sites located on shallow shoals. The present study revealed that this alternative spawning strategy may be more important than previously thought and is currently used in concert with the “traditional” strategy of spawning in tributaries. For the SSP, the extent and importance of the egg deposition on these shoals needs to be documented to protect the spawning area exploited by the SSP. As the area surveyed was relatively limited in 2010, a more extensive study must be conducted in the near future to better quantify the contribution of this “alternative” spawning strategy. The density of egg deposition appears quite variable in space and time (Fig. 1, Table 1), but considering the limited area sampled by our plates (0.9 m<sup>2</sup> per string) and all limitations

associated with the method (i.e. plates buried under the sand, problems related to the fixation of eggs on iron) the number of eggs sampled is quite elevated.

We suggest that such an alternative spawning strategy may be beneficial in rivers with large plumes of low salinity. Although it is difficult to monitor egg deposition in such habitat because of environmental conditions, the egg collectors are relatively easy to build and should be considered in such environmental situations.

*... and a little update from the 2011 eggs deposition survey along the south shore*

During spring 2011, we extended our survey (see Fig. 1). Eggs were found outside the area surveyed in 2010, nearly 2.5 km downstream of the de l'Église Brook, validating the hypothesis that smelt were exploiting the large stretch of shallow habitat < 2m (at low tide). Moreover, a few eggs were found 12.2 km downstream of the de l'Église Brook, on shallow shoals located adjacent to the Boyer River where an important smelt run was extirpated in the 1980's (Fig.1). The genetic identification has not been completed, but it is likely that they belong to the SSP. However see Dodson *et al.* (this proceeding) for a more precise figure of the genetic definition of the SSP and the populations utilizing the Middle Estuary.

### Acknowledgements

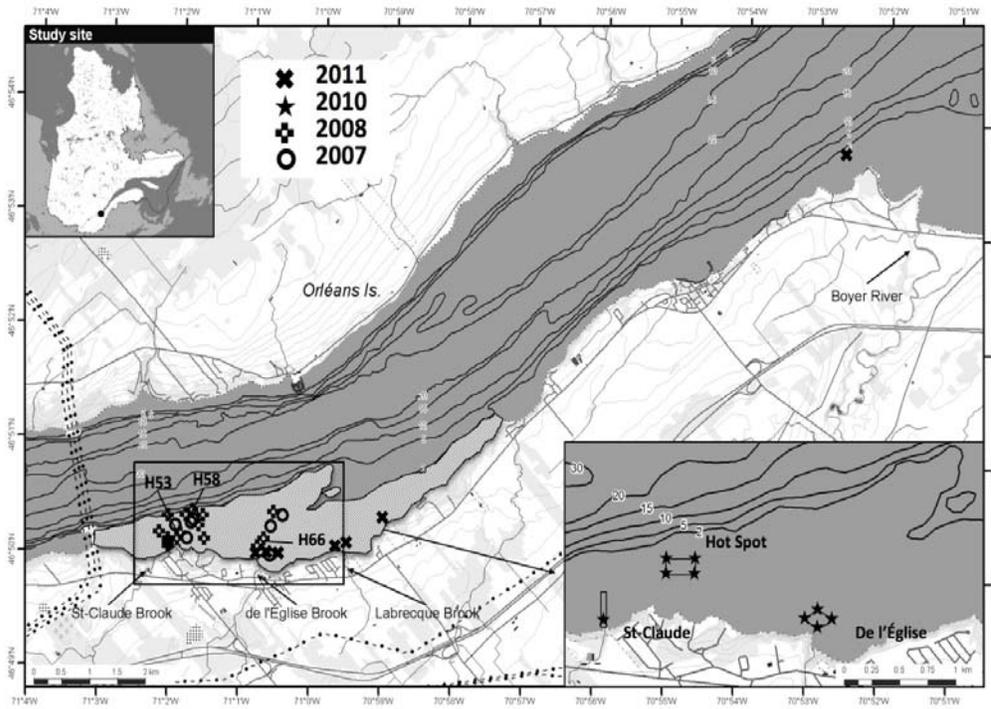
We thank the technicians who faced sometimes harsh conditions to make these surveys a real success: N. Harnois, D. Deschamps, D. Fournier, F. Hudon, O. Thusy, R. Dostie, W. Cayer-Blais, J. Bédard, Y. Souldard, J. Bédard, P.-L. Daigle, E. Henri. Y. Plourde. GENIVAR provided us eggs sampled in 2008 and lent us their plate rows.

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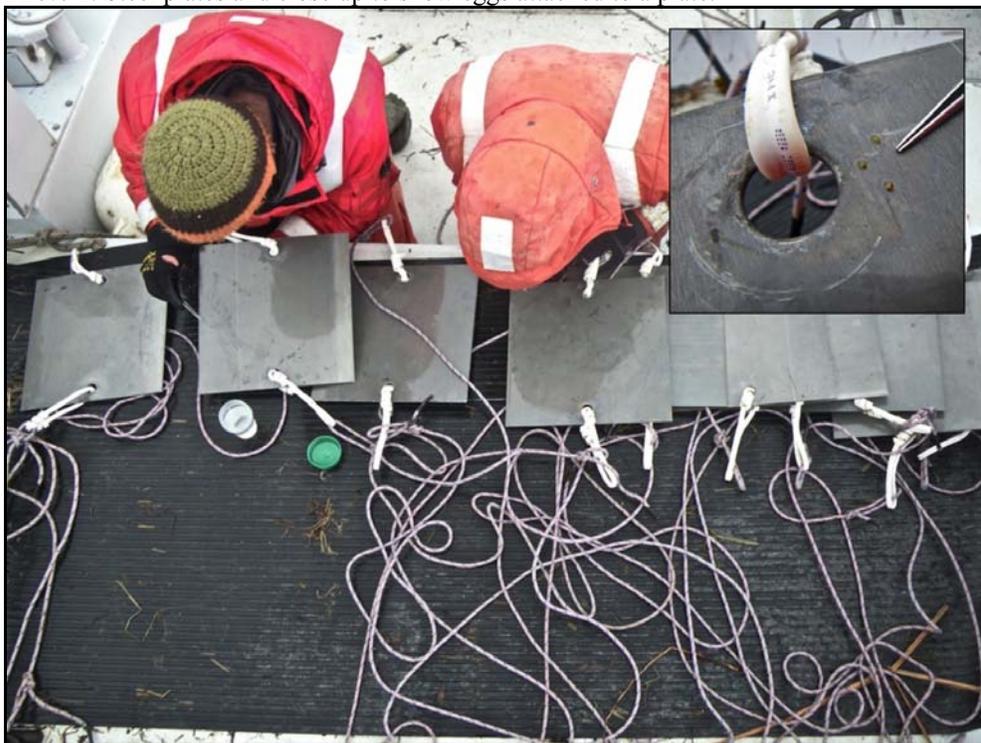
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**Figure 1.** Study site and features are discussed in the text; the shaded area represents the area of egg deposition (Beaumont shoals). Symbols show where eggs were found.



**Photo 1.** Steel plates and close up to show eggs attached to a plate.



**Table 1.** Densities and genetic identification of eggs collected on the shallow shoals in 2010.

	Line <sup>1</sup>	Date <sup>2</sup>	Eggs <sup>3</sup> n (# plates)	Density /100m <sup>2</sup>	Gen. <sup>4</sup> n	SSP %	NSP %	SD %	Estimates for the Areas of interest <sup>5</sup>						
									Eggs n (# plates)	Surface <sup>6</sup> Area (m <sup>2</sup> )	Mean Density eggs/m <sup>2</sup>	Total eggs deposits	SSP %	NSP %	SD %
Claude Br.	P11	06/04/2010	71 (2)	12.2	10	129.4	-29.4	18.0	72 (3)	6 000	26.7	160 000	116.5	-16.5	8.5
	P11	19/04/2010	1 (1)	1.1	1	-12.5	112.5	43.1							
Hot-Spot	P01	14/04/2010	1 (1)	1.1	1	129.4	-29.4	59.5	193 (7)	20 200	42.9	868 000	107.5	-7.5	8.0
	P02	14/04/2010	177 (1)	6.7	5	72.6	27.4	31.1							
	P03	14/04/2010	1 (1)	1.1	0										
	P03	22/04/2010	2 (2)	2.2	1	129.4	-29.4	59.5							
	P04	14/04/2010	11 (1)	5.6	5	129.4	-29.4	26.0							
	P04	19/04/2010	1 (1)	1.1	1	129.4	-29.4	59.5							
de l'Église Br.	P21	16/04/2010	9 (3)	10.0	9	82.1	17.9	22.2	553 (33)	15 900	153.6	2 445 000	103.4	-3.4	2.6
	P22	06/04/2010	1 (1)	1.1	1	129.4	-29.4	59.5							
	P22	16/04/2010	442 (10)	27.8	25	101.0	-1.0	11.0							
	P22	19/04/2010	46 (9)	28.9	25	97.1	2.9	12.5							
	P23	16/04/2010	1 (1)	1.1	1	129.4	-29.4	59.5							
	P23	19/04/2010	51 (6)	27.8	25	122.9	-22.9	12.5							
	P23	22/04/2010	1 (1)	1.1	0										
	P25	16/04/2010	2 (2)	2.2	2	58.4	41.6	50.3							

1 the line correspond to the sites indicated on Fig.1

2 the date indicate the moment when the lines were installed

3 the # plates refers to the number of plates on which eggs were found

4 the number of eggs analyzed according to the methodology indicated in the text

5 The areas of interest are either the Claude br., the Hot-Spot, the de l'Église br.

6 the surface area are the minimal area covered by the lines installed in the various areas (see Fig.1)

## **Nuclear (AFLP) population genetic structure of the St. Lawrence estuary smelt complex**

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In order to better understand nuclear gene flow amongst contemporary populations of the St. Lawrence estuary smelt complex, we employed AFLPs on 16 sample sites (N=315) across the estuary, with multiple sites representing each of the two historical lineages. In preliminary results, 4 primer combinations yielded 154 loci, 67 (43%) of which were polymorphic. We find two distinct genetic clusters, but they do not conform to the classic north/south dichotomy. One cluster is found primarily upstream on the south shore, while a second cluster is found on the north shore, as well as in downstream populations of the south shore (i.e. from Riviere Ouelle to Rimouski).

While most individuals are distinctly one genetic population or the other, genetic intermediates (i.e., hybrids) exist, suggesting gene flow between the clusters. In terms of the historical lineages, these results suggest nuclear introgression from the north shore ecotype to the south shore ecotype in populations from Ouelle to Rimouski, since these sites putatively have 'south shore' mtDNA (further mtDNA sequencing results pending). On the other hand, upstream south shore ecotype populations appear to remain relatively distinct. Firm conclusions await additional samples and analyses.

## **Quantifying zooplankton consumption by larval and juvenile rainbow smelt using a mercury mass balance model**

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### **Introduction**

Consumption rates have seldom been directly estimated in the field for larval and juvenile fish. However, assessing food ingestion rates for early life stages may be as important as adult stages. Because bioenergetics models developed

for adult fish are not applicable to early life stages (Karjalainen et al. 1997), and given that it is difficult to directly assess directly food ingestion rate by larvae and juvenile planktivorous fish because they feed on small particles (Wuenschel and Werner 2004), mercury mass balance models appear to be an

appropriate method to assess food ingestion rates during early life stages of fishes.

Rainbow smelt (*Osmerus mordax*) is an important prey for piscivore species in many lakes (Lantry and Stewart 2000) including Lake Saint-Jean. Lake Saint-Jean is a large reservoir (1053 km<sup>2</sup>) located in the Canadian boreal zone. This lake supports 28 freshwater species including walleye (*Sander vitreum*), northern pike (*Esox lucius*) and the most important sport fish in this region, landlocked Atlantic salmon (*Salmo salar* ouananiche).

In this lake, landlocked Atlantic salmon feed almost exclusively on rainbow smelt. The aim of this study is to assess food ingestion rates by young rainbow smelt in Lake Saint-Jean, Quebec, Canada, using a mass balance model with mercury.

## Materials and methods

Larval, juvenile rainbow smelts and zooplankton were collected seven times during the ice-free season in 2009 from June to October. Determination of mercury in the sample was made for at least five larval fish and five juvenile fish on each date. We used a mercury mass balance model developed by Trudel et al. (2000) to assess food ingestion rate:

$$I = \frac{C_{t+\Delta t} - C_t \cdot e^{-(E+G)\Delta t}}{\alpha \cdot C_d \left[ 1 - e^{-(E+G)\Delta t} \right]} (E+G)$$

where  $I$  is the food ingestion rate (g·g<sup>-1</sup>·day<sup>-1</sup> or day<sup>-1</sup>),  $C_t$  and  $C_{t+\Delta t}$  are the concentration of Hg in fish at time  $t$  and  $t + \Delta t$  (ng·g<sup>-1</sup>), respectively,  $\Delta t$  is the time interval (days) between sampling dates,  $E$  is the elimination rate of Hg by the fish (day<sup>-1</sup>),  $G$  is the specific growth rate (day<sup>-1</sup>),  $\alpha$  is the assimilation efficiency of Hg from food and,  $C_d$  is the concentration of Hg in the prey (ng·g<sup>-1</sup>).

## Results

Based on stomach content analysis, young rainbow smelt fed almost exclusively on three

groups of planktonic prey: *Bosmina* spp., *Daphnia* spp. and calanoid copepods (primarily *Epischura lacustris* and *Leptodiaptomus aschlandi*). The cyclopoid copepod *Diacyclops bicuspidatus thomasi* was also important for first-feeding larvae. MeHg concentrations in fish increased during the ice-free season in the two age classes. MeHg concentrations were between 2 to 5 fold higher in yearling than in young-of-the-year.

Food ingestion rates were expressed as body weight percentage (Table 1). In early summer, young-of-the-year smelt ingested nearly 70% of their body weight per day and then this value decreased to reach less than 25 % per day for the rest of the season. In fall, ingestion rate was at its lowest with less than 5% of body weight per day. On the other hand, yearling consumption was at its highest in early summer with nearly 30% of body weight per day, and declined until the end of October to reach between 0 and 5% of body weight per day.

## Discussion

Food ingestion rates were assessed for early life stages of rainbow smelt using a Hg mass balance model. These fish fed almost exclusively on pelagic zooplankton. Food ingestion rates were closely linked with growth and were variable depending of the age of the fish and the time of the season. Our results suggest that young-of-the-year rainbow smelt had a high energetic demand during their larval stage as measured by food ingestion rate which declined as the summer progressed and remained nearly constant into the fall. The same pattern was observed for young-of-the-year yellow perch, beginning with a high consumption in early summer and declining to roughly 20% of their body weight per day later in the season (Post 1990).

Yearling rainbow smelt food ingestion rates in Lake Saint-Jean was at their highest in June

and July. This generally coincides with zooplankton peak abundance in the lake. Because water and air temperature and daylight duration are at maximum during these two months, zooplankton egg development time decreases during this period and new cohorts can be produced quickly. With high zooplankton abundance during the summer months, less energy is required to search for prey. Hence, rainbow smelt can achieve faster growth in early summer compared to fall when there is less zooplankton abundance.

In conclusion, a mass balance model using mercury provided food ingestion rates for larval and juvenile rainbow smelt in agreement with previous studies. These results provided new data on zooplankton predation by rainbow smelt in lake Saint-Jean and will contribute to evaluate the carrying capacity of the lake for future enhancement programs.

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**Table 1.** Larval and juvenile rainbow smelt food ingestion rates (percent body weight per day) assessed with a mercury mass balance model during summer and fall 2009.

	Rainbow smelt	
	Larval	Juvenile
Early summer	60-70 %	25-30%
Summer	20-25 %	20-30 %
End summer	10-20 %	0-15 %
Early fall	5-20 %	0-5 %

## Carrying capacity of Lake Saint-Jean for rainbow smelt

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Rainbow smelt (*Osmerus mordax*) is a key forage species in several inland aquatic ecosystems such as Lake Saint-Jean, a large reservoir located in the Boreal Shield ecozone in Quebec, Canada. For instance, it is now well established that the production of landlocked Atlantic salmon (*Salmo salar*) is related to abundance of its preferred prey, the rainbow smelt (Havey & Warner 1970). Hence, in Lake Saint-Jean, fishery managers are considering to increase the production of smelt population with a large scale stocking project of larval smelt and/or with the creation of artificial reproduction sites. However, it is important to assess the feeding demand and the food supply for rainbow smelt in Lake Saint-Jean. The general objective of this study was to assess the carrying capacity of Lake Saint-Jean for rainbow smelt. To reach this objective, we first described the diet of larval and juvenile rainbow smelt and we used a mercury mass-balance model to estimate the feeding demand (Jérôme Plourde et al. this issue). Then, we estimated the production of the four main prey items to evaluate the food supply and we finally compared the latter to the total ingestion of larval and juvenile rainbow smelt in Lake Saint-Jean during the ice-free season (May to October).

Results showed that larval (0+) and juvenile (1+) rainbow smelt fed almost exclusively on zooplankton and had almost no other fish competitor for this resource in the pelagic zone. More specifically, they primarily consumed four prey items, the calanoid *Leptodiatomus ashlandi*, the cyclopoid *Diacyclops bicuspidatus thomasi* and the cladocera *Bosmina* spp. and *Daphnia* spp. An individual smelt ingested from 0 to 0.753 g wet of zooplankton per day (Jérôme Plourde et al. this issue). Based on a scenario of high abundance of rainbow smelt in Lake Saint-Jean, the total ingestion was 2.48 g wet per m<sup>-2</sup> from June 15<sup>th</sup> to October 15<sup>th</sup>. Due to their abundance, the consumption of 0+ smelt represented 93.9% of the total ingestion.

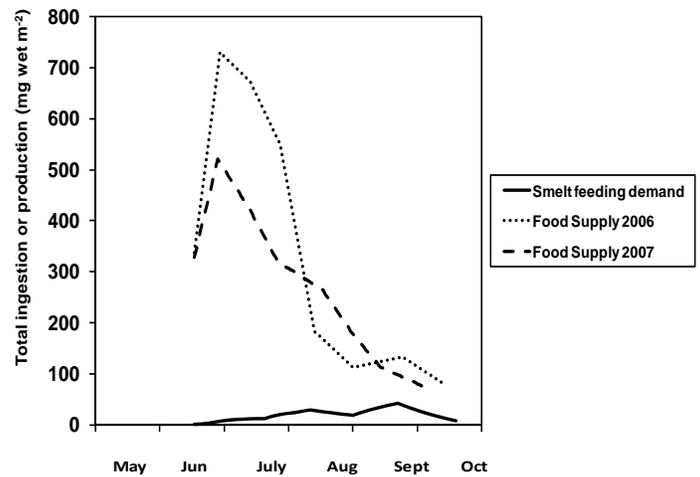
To calculate the food supply, zooplankton was sampled at 12 stations in Lake Saint-Jean every 2-3 weeks from mid-May to early October in 2006 and 2007. In the laboratory, zooplankton was sorted, enumerated and the developmental stage was determined on more than 81,144 individuals from 228 samples. To estimate the secondary productivity of each of the four main preys, the increment summation method was used. All together, the four main preys produce between 32.7g (2007) to 42.8g (2006) wet per m<sup>-2</sup> from May 17<sup>th</sup> to October 10<sup>th</sup>. This production is largely sufficient to meet the

feeding demand of abundant cohorts of larval and juvenile rainbow smelt in Lake Saint-Jean (Fig. 1).

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**Figure 1.** Comparison of the zooplankton food supply and the feeding demand of rainbow smelt in Lake Saint-Jean during the ice-free season.



## Early life history dynamics and recruitment of rainbow smelt in Lake Huron

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Rainbow smelt are an important prey species for native and introduced piscivores in the Great Lakes, but also function as a predator on native fish and zooplankton species. Rainbow smelt abundance in Lake Huron is currently at historic low levels with most of the population comprised of age-0 and age-1 fish. To determine sources of recruitment variability and understand long term population decline, we studied larval stages of rainbow smelt during 2008-2009 and modeled stock recruitment relationships based on bottom trawl catches during 1976-2009. Peak larval rainbow smelt densities in 2008 were double densities observed in 2009. Length frequency analysis revealed a second cohort of lake spawned larvae appeared in late June and early July of both years concurrent with an increase in larval density. Growth rates of larvae were significantly higher

during 2009. Early hatching cohorts during 2008 suffered high mortality, whereas early and late hatching cohorts had relatively high survival during 2009. Stock-recruit models for 1976-1991 appeared asymptotic and recruitment variability was best explained by a combination of lake trout abundance, rainbow smelt stock size, and Lake Huron water levels. During 1994-2009, compensatory processes were evident at high stock sizes and recruitment variability was explained largely by lake trout abundance. These results indicate that variable growth rates and survival of early hatching cohorts strongly influence year class formation of rainbow smelt in Lake Huron. Furthermore, lake trout abundance had a substantial influence on rainbow smelt recruitment dynamics in Lake Huron during 1976-2009, presumably through predation on larval and/or adult life stages.

## History and status of landlocked rainbow smelt (*Osmerus mordax*) in Maine

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Rainbow smelt are anadromous fish which are indigenous to most coastal Maine estuarine waters. They also occur as native and introduced landlocked populations in numerous fresh water inland lakes and ponds statewide. Rainbow smelt are the primary forage fish species for landlocked Atlantic salmon in Maine and elsewhere, and they are thought to have originated in the same four river basins – St. Croix (West Grand Lake, Washington Co.), Union (Green Lake, Hancock Co.), Penobscot (Sebec Lake, Piscataquis Co.) and Presumpscot (Sebago Lake, Cumberland Co.) – as well as associated free-flowing waters within these river basins. Late 1800's fishery records indicate that landlocked Atlantic salmon were primarily found to occur in the presence of landlocked populations of rainbow smelt, and the two species were widely disseminated for the

purpose of generating sport fisheries as early as 1868. After more than a century of stocking and active management, Maine's Department of Inland Fisheries and Wildlife reported in 2006 that 176 Maine lakes (about 485,000 acres) provided a significant fishery for landlocked Atlantic salmon (and rainbow smelt) – nearly 50% of Maine's total freshwater acreage. Rainbow smelt are also exclusively sold as a primary bait fish in Maine for salmonid fisheries statewide, particularly during the winter fishing season. Big Reed Pond (a 94-acre pond in the St. John River drainage) – home to the regionally endemic Arctic char (*Salvelinus alpinus*) and surrounded by old growth forest – is currently being reclaimed in an attempt to extirpate a population of illegally introduced rainbow smelt.

## **Water quality and habitat assessment of rainbow smelt (*Osmerus mordax*) spawning locations in rivers on the Gulf of Maine coast**

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Anadromous fish migrate between marine and freshwater habitats to complete essential life history stages. Widespread reductions have occurred in populations of several species of anadromous fish in New England in recent years. Population trends are not well documented, and factors causing the declines are subject to speculation. Spawning habitat degradation has long been a suspected contributor to the declining health of sea-run fish. The reproductive strategy of depositing a demersal, adhesive egg in freshwater habitats may be challenged by the influence of watershed alterations, particularly in urban areas. Coinciding with declining populations is a growing public interest in restoring these traditional and popular fish runs. Restoration efforts have focused on structural solutions to migration impediments, with less guidance available on the role of water and habitat quality in restoring anadromous fish habitat and populations.

Rainbow smelt populations at the southern end of their range have undergone sharp declines in recent decades. Evidence of extirpation in former runs, low abundance in existing runs, northward movement of the southern range boundary, and truncated age structure allows the supposition that natural mortality, habitat alteration and climate change could be influential in recent trends. Within the category

of habitat alteration, passage obstruction and channel alteration are obvious negative influences. More recently, concern has been raised over water quality and substrate degradation caused by eutrophication, sedimentation, and water supply management, particularly in urban areas (Chase 2006). Overall, there is little specific information on smelt spawning habitat requirements and casual relationships for degradation.

The states of Maine, New Hampshire and Massachusetts are collaborating to create a conservation plan for rainbow smelt in the Gulf of Maine. The effort is funded by the NOAA Office of Protected Resources in the interest of improving knowledge and conservation for Species of Concern, an Endangered Species Act designation given to smelt in 2004. The project includes the development of a Quality Assurance Program Plan (QAPP) for monitoring water and habitat quality at smelt spawning habitats in coastal rivers on the Gulf of Maine coast. The monitoring relates species life history requirements to state and federal water quality criteria and habitat thresholds. Project goals include developing a standardized process to classify the suitability of smelt spawning habitat and contributing to water quality and habitat restoration efforts in New England. Preliminary results and exploratory analyses of smelt

spawning habitat monitoring from 2008-2010 were presented at the workshop.

The QAPP for monitoring diadromous fish habitat includes a Standard Operating Procedure (SOP) for rainbow smelt spawning habitat. The QAPP was adopted by the Species of Concern project and approved by the Massachusetts Department of Environmental Protection (MassDEP) in 2009 and finalized by the Massachusetts Division of Marine Fisheries as a technical report (Chase 2010). The SOP has four primary objectives:

1. Delineate and document river and stream locations where smelt spawning occurs.
2. Measure biotic and abiotic parameters at smelt spawning sites. Identify water and habitat quality deficiencies at each site using physical, chemical and biotic criteria.
3. Develop reference condition thresholds and relationships between abiotic and biotic habitat conditions.
4. Incorporate monitoring results into Clean Water Act (CWA) processes for protecting designated habitat uses, and make recommendations for improving and protecting smelt habitats.

Each state selected spawning sites for water quality monitoring and for fyke net sampling of adult smelt. The fyke nets were maintained during the smelt spawning period (March-May and June at northern Maine stations) and hauled three times per week for typically 10-11 week seasons. With each haul date, the following parameters were measured: water temperature (°C), dissolved oxygen (DO; mg/L), specific conductivity (mS/cm), water pH, and turbidity (NTU). In addition, during the 2008-2009 spawning period ceramic tiles were deployed in riffle habitat where smelt deposit eggs. Periphyton growth on the tiles was collected

biweekly to quantify daily growth and describe algal species composition. The tile deployment sites were also visited weekly to collect total phosphorus (TP) and total nitrogen (TN) samples and record water depth and velocity. Additional physical and local climate data were recorded including air temperature, precipitation, continuous water temperature, percent open canopy, and discharge. A detailed description of sampling methods is provided in the QAPP (Chase 2010).

Summary statistics were generated for sampled parameters by site and for the entire study area and then classified by SOP thresholds assembled from existing water quality criteria (Table 1). The U.S. Environmental Protection Agency (US EPA) has developed criteria for turbidity, TN and TP that are based on the 25<sup>th</sup> percentile of the distribution of observed values across a population of rivers in an ecoregion (US EPA 2000). The 25<sup>th</sup> percentile is adopted as the threshold between degraded conditions and minimally impacted reference locations. The MassDEP has established Surface Water Quality Standards (SWQS) for water temperature, pH and DO as part of their CWA waterbody assessment process (MassDEP 2007). The thresholds are designed to protect designated uses for aquatic life which includes fish habitat. Other potentially important physical and chemical parameters for smelt spawning habitat lack water quality criteria established by regulatory agencies or the scientific literature. For these parameters, the Species of Concern project will evaluate the distribution of data collected during 2008-2010 to identify potential thresholds for water quality and habitat impairment.

Water chemistry was monitored during 2008-2010 spring spawning runs at smelt fyke net stations in each state. The sampling stations were selected to measure freshwater flows near the downstream limit of spawning habitat. Eight

stations were monitored in Massachusetts during an eleven week sampling period that coincided with the smelt spawning period of early March to mid-May. Two stations were sampled in New Hampshire during the same period, and a third was added in 2010. Seven stations were monitored in Maine for a similar duration, but starting later to account for later spawning migrations, with the northernmost stations continuing into June. The preliminary results from the 2008-2010 sampling are provided as summary statistics at each station, and include the classification of each station as *Suitable* (minimally impacted) or *Impaired* based on established criteria for each parameter (Table 2). In addition, water quality data distributions are evaluated to explore the potential of establishing new thresholds that are specifically derived from smelt spawning habitat measurements.

## Results

Water Temperature. The water temperature criterion of  $\leq 28.3$  °C is provided by the MassDEP SWQS to protect aquatic life. The relatively high temperature has limited relevance for smelt spawning that occurs in the cool water of the spring freshet. No individual measurements at any station exceeded this threshold. The median and 75<sup>th</sup> percentiles of water temperature calculated from all stations' median values were 10.2 °C and 11.4 °C, respectively. The scientific literature on smelt provides no direction on potential water temperature thresholds. Despite the apparent lack of application for a water temperature reference condition, there is value in documenting baseline conditions. The onset of smelt spawning and the duration of egg incubation are influenced by water temperature. The southern end of rainbow smelt's range changed dramatically in the 20<sup>th</sup> century with climate warming a possible influence.

Reference values such as the 75<sup>th</sup> percentile or those derived from continuous temperature loggers could become useful thresholds or time series baselines as more data are collected.

Dissolved Oxygen. The dissolved oxygen criterion of  $\geq 6.0$  mg/L is provided by the MassDEP SWQS to protect aquatic life. Similar to water temperature, the DO threshold may have limited relevance because of the high concentrations of DO found in turbulent riffles during the spring freshet. No individual measurements at any station exceeded this threshold. The median DO concentration calculated across all stations' medians was 11.1 mg/L, and the median DO saturation was 98%. Baseline DO data should be recorded for smelt spawning habitat in order to identify acute alterations to expected conditions. However, it is not expected that a DO threshold that relates to life history risks will be routinely applicable for smelt spawning during the spring freshet.

Specific Conductivity. Neither the MassDEP, US EPA, nor the scientific literature provides potential thresholds for specific conductivity. Because of this lack of guidance, specific conductivity was not evaluated in terms of exceedances to an existing or derived threshold. High specific conductance in freshwater can indicate contributions of natural alkaline compounds or ionic composition from pollution sources. For this reason, conductivity has been discussed as a potential proxy for pollution sources, urbanization, and eutrophication. The median specific conductivity does appear to increase with urbanization among project sites, demonstrating potential for evaluating baseline specific conductivity as related to watershed characteristics and smelt life history.

Water pH. The water pH criterion of  $\geq 6.5$  to  $\leq 8.3$  standard units is provided by the MassDEP SWQS to protect aquatic life. Impacts to early life stages of fish have been demonstrated in

acidified waters below 6.5. Laboratory experiments and field observations on rainbow smelt egg incubation indicated little threat from exposure to water with pH near 6.5 and that evidence of mortality does not manifest until pH drops below 6.0 (Geffen 1990). The median and 25<sup>th</sup> percentile calculated from all stations' medians was 7.14 and 6.84, respectively, well above the 6.5 threshold. In this case, the project's estimated threshold between minimally impacted and impaired pH conditions is more conservative than the MassDEP threshold. Adopting mortality thresholds from the scientific literature is another approach to consider. Of the 18 rivers sampled, only five were classified as *Impaired* based on having >10% of individual measurements below pH 6.5. The three southernmost Massachusetts stations were prone to acidification following precipitation and snow melt, and two Maine stations exceeded the threshold but had median values not far below neutral.

Turbidity. The turbidity criterion of  $\leq 1.7$  Nephelometric Turbidity Units (NTU) was adopted from the US EPA Northeast Coastal Zone ecoregion as the threshold for minimally impacted rivers (25<sup>th</sup> percentile). All rivers were classified as *Impaired* based on this criterion due to having greater than 10% of individual measurements exceed 1.7 NTU. The river with the least turbid water, the Westport River, had a median of 1.5 NTU, yet still had 39% of the measurements exceed 1.7 NTU. In this case, the distribution of turbidity data among sample stations may provide a more applicable threshold than the existing criteria. Turbidity at smelt spawning riffles near the tidal zone during spring could be expected to have higher values than the distribution of river data over a wide geographical region used by US EPA. However, even applying the 25<sup>th</sup> and 50<sup>th</sup> percentiles (2.1 and 2.4 NTU) resulted in all rivers exceeding these thresholds, with the Westport River the

closest to a *Suitable* classification with 15% of measurements exceeding 2.4 NTU. The difficulty with establishing a turbidity benchmark may lie in the variable response of streams to precipitation and differences in precipitation occurring at each station. A relevant turbidity threshold for smelt spawning habitat may be realized by separating precipitation effects through an analysis of base flow data.

Total Nitrogen. The TN criterion of  $\leq 0.57$  mg/L was adopted from the US EPA Northeast Coastal Zone ecoregion as the threshold for minimally impacted rivers (25<sup>th</sup> percentile). All Massachusetts rivers exceeded this threshold, with a trend of higher TN in urban watersheds. Four of the ten stations in Maine and New Hampshire exceeded the TN criterion, with a similar urbanization trend. Most median values among river stations were below the 0.57 mg/L threshold, with the exception of a few in more developed watersheds. This resulted in a 25<sup>th</sup> percentile of 0.34 mg/L across all sample station medians. The selection of a TN threshold will be important to the process of developing water quality criteria for smelt spawning habitat. Observations and preliminary analyses from this project indicate a relationship between TN concentrations and substrate quality: the stations with the lowest TN typically had clean gravel and native aquatic moss in spawning riffles, and the stations with the highest TN typically had dense periphyton growth that degrades spawning habitat.

Total Phosphorus. The TP criterion of  $\leq 23.75$  ug/L was adopted from the US EPA Northeast Coastal Zone ecoregion as the threshold for minimally impacted rivers (25<sup>th</sup> percentile). All project rivers exceeded this threshold except for the Jones River in Massachusetts and the two easternmost rivers in Maine. For most rivers, the median TP values were below, yet close to, the US EPA threshold.

Overall, the distribution did not show the variability and urban association found with TN. The 25<sup>th</sup> percentile of the river medians is 19.1 ug/L. The distribution of TP data observed in this study is similar to the US EPA ecoregion data and appears to be suitable for deriving benchmarks for protecting smelt spawning habitat.

Periphyton. Periphyton samples were collected from tiles deployed at sampling stations during the 2008-2009 spawning period. Ash-free dry weight (AFDW, g/m<sup>2</sup>/day) was calculated as a measure of periphyton biomass. No algal biomass thresholds are available specifically for smelt spawning habitat, and most reported river substrate data on periphyton measured benthic chlorophyll. Therefore, the 25<sup>th</sup> percentile of 0.0143 g/m<sup>2</sup>/day was calculated from the AFDW medians and compared to all values. All river stations exceeded this threshold and were classified as *Impaired* for periphyton except for three of the four easternmost stations in Maine where the least watershed development has occurred in the study area. The periphyton data suffers from high variability and low sample sizes at some sites. However, there appears to be potential value in using the 50<sup>th</sup> percentile (0.0605 g/m<sup>2</sup>/day) as a threshold for moderately impacted rivers. At the stations with medians above this potential threshold (Table 1), the periphyton could be characterized as nuisance or excessive growth and appears to be associated with higher TN and urbanization.

## Discussion

The Species of Concern project has provided baseline data on rainbow smelt spawning habitat conditions, and work has begun on establishing thresholds between suitable and impacted water and habitat quality. Exploratory analyses are

underway to develop relationships between abiotic water quality conditions and biotic conditions of smelt spawning habitat. The project has documented measurements of high TN and periphyton growth and observations of degraded spawning substrate. Previous efforts to demonstrate streambed relationships between water quality and habitat quality have been challenged by natural variability and data limitations. Changes in the trophic state of rivers due to nutrient enrichment is an expected response; however the development of empirical relationships and classification systems have been limited by the high variability in substrata responses to nutrient dynamics (Dodds and Welch 2000; Dodds 2007). Recent laboratory experiments have demonstrated lower smelt egg survival exposed to substrate conditions of high sedimentation and periphyton (Wyatt et al. 2010). Reduced smelt egg survival may be associated with restricted oxygen delivery through advection and substrate respiration. These conditions may not be typical during the spring freshet at natural riffles adopted by rainbow smelt for spawning habitat but could become a negative influence on egg survival and population recruitment in streams where channel and hydrologic alterations are severe and water quality is degraded.

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**Table 1.** Water chemistry criteria related to smelt spawning habitat. The water chemistry parameters were adopted from Massachusetts SWQS for protecting *Aquatic Life* at Class B Inland Waters (MassDEP 2007), and US EPA reference conditions (25th percentile) for the Northeast Coastal Zone sub-Ecoregion (US EPA 2000b). Potential criteria are presented based on 25th and 50th percentiles from 2008-2010 project data. Blank cells indicate either that no criterion exists or the derived percentile has limited relevance for smelt habitat.

<b>Parameters</b>	<b>Suitable</b> (MassDEP 2007)	<b>Minimally Impacted</b> 25 <sup>th</sup> Percentile (US EPA 2000b)	<b>Minimally Impacted</b> 25 <sup>th</sup> Percentile (2008-2010 data)	<b>Moderately Impacted</b> 50 <sup>th</sup> Percentile (2008-2010 data)
Temperature (°C)	≤ 28.3			
Sp. Conductivity (mS/cm)			≤ 0.133	≤ 0.195
pH	≥ 6.5 to ≤ 8.3		≤ 6.84	
DO (mg/L)	≥ 6.0			
Turbidity (NTU)		≤ 1.7	≤ 2.1	≤ 2.4
TN (mg/L)		≤ 0.57	≤ 0.34	≤ 0.44
TP (ug/L)		≤ 23.75	≤ 19.1	≤ 20.8
Periphyton Biomass (g/m <sup>2</sup> /d)			≤ 0.0143	≤ 0.0605

**Table 2.** Median water chemistry values from smelt Species of Concern project stations, 2008-2010. The percentage of samples that exceed the parameter criteria from Table 1 are reported under Exceedance (%) where rivers shaded in gray are classified as Impaired (>10% exceedance) and Suitable (≤ 10% exceedance) are without shading. Rivers are listed from South to North.

River	State	Sp. Cond.	Water Exceed.		D.O. Exceed.		pH Exceed.		Turbidity Exceed.		TP Exceed.		TN Exceed.		AFDW Exceed.	
		(mS/cm)	Temp. (C°)	(%)	(mg/L)	(%)	(%)	(%)	(NTU)	(%)	(ug/L)	(%)	(mg/L)	(%)	(g/m <sup>2</sup> /day)	(%)
Westport	MA	0.124	9.39	0%	11.0	0%	5.98	99%	1.5	39%	20.8	14%	0.39	29%		
Weweantic	MA	0.091	11.05	0%	10.5	0%	6.31	89%	2.3	81%	39.3	93%	0.14	20%		
Jones	MA	0.195	9.93	0%	11.8	0%	6.48	55%	2.9	94%	17.0	8%	0.59	49%	0.0240	57%
Fore	MA	0.558	10.43	0%	11.9	0%	7.09	1%	2.2	74%	21.5	39%	0.53	27%	0.0154	50%
Saugus	MA	0.664	9.06	0%	11.9	0%	7.28	0%	2.8	89%						
North	MA	0.946	9.84	0%	12.5	0%	7.23	0%	2.0	77%	21.4	33%	1.45	100%	0.0828	71%
Crane	MA	0.996	9.54	0%	11.8	0%	7.20	1%	3.4	99%	21.0	38%	1.28	100%	0.1198	88%
Parker	MA	0.256	9.11	0%	11.8	0%	7.02	0%	1.8	66%	22.1	36%	0.67	73%	0.0685	63%
Squamscott	NH	0.143	13.22	0%	10.1	0%	6.92	0%			17.4	22%	0.44	16%	0.0598	89%
Oyster	NH	0.183	9.41	0%	11.1	0%	7.40	0%	4.3	100%	21.1	25%	0.36	0%		
Winnicut	NH	0.317	11.55	0%	11.2	0%	7.30	0%	2.7	100%	20.6	36%	0.54	42%	0.0888	100%
Long Creek	ME	0.444	11.35	0%	10.7	0%	7.24	0%	7.0	100%	20.5	24%	0.46	29%	0.0625	100%
Mast Landing	ME	0.133	9.42	0%	11.4	0%	7.14	10%	9.0	100%	19.5	25%	0.27	0%		
Deer Meadow	ME	0.031	11.37	0%	11.0	0%	6.84	16%	2.5	92%	19.1	15%	0.28	0%	0.0068	25%
Tannery	ME	0.142	14.07	0%	10.2	0%	7.68	9%	2.1	54%	21.3	44%	0.35	0%	0.0611	100%
Schoppee	ME	0.197	11.32	0%	9.9	0%			2.1	73%	21.8	33%	0.50	22%		
Chandler	ME		13.22	0%	9.6	0%	6.73	33%			15.0	0%	0.34	11%	0.0111	25%
East Bay	ME	0.044	9.88	0%	10.7	0%	7.34	4%	2.0	65%	11.4	4%	0.22	0%	0.0055	25%
<b>25th percentile</b>		0.133	9.45		10.6		6.84		2.1		19.1		0.34		0.0143	
<b>50th percentile</b>		0.195	10.18		11.1		7.14		2.4		20.8		0.44		0.0605	

## **Pleasant River estuary, smelt fisheries from past to present - a ray of hope for a declining species?**

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The Pleasant River and other nearby estuaries are home to some of the most highly productive smelt populations remaining along the US Atlantic seaboard. The surrounding watersheds are among the least developed in the Gulf of Maine. A small number of commercial fishermen continue to harvest smelt and tomcod using gill nets and bag nets in a locally important and unique heritage fishery - a fishery which once extended well beyond the region into the southern range of the species. Fishermen have worked closely with staff and

volunteers of the Downeast Salmon Federation and with multiple agencies and municipalities to protect, study, and maintain this fishery over many years. We have been working to document the history of the fishery and also to study the status of the population to help us better maintain the commercial fishery. Water quality and fish health monitoring, habitat restoration and protection and other conservation measures are underway. Results of some of the past efforts and descriptions of the ongoing work will be summarized.

## **Evaluation of rainbow smelt passage in a nature-like fishway**

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Fishways are currently being designed, constructed or modified to remediate habitat fragmentation in streams and to accommodate flow limitations. These structures have been principally designed to ensure anadromous salmonid passage using a conventional engineering approach (Larinier 1998). Recently, however, there has been increasing interest in multi-species passage (Lucas and Baras 2001, Haro et al. 1999). In many countries, there is currently a shift from the more conventional fishway's conception toward nature-like fishways which simulate natural streams (Schmutz et al. 1998, Katopodis et al. 2001,

Santos et al. 2005, Calles and Greenberg 2007). However, the efficiency of these structures remains largely unknown (Calles and Greenberg 2005).

To allow multi-species passage, the design and hydraulic characteristics of fishways should be determined according to the most demanding species (Larinier 2002). Rainbow smelt (*Osmerus mordax*) is an anadromous species with reduced swimming capacity (Katopodis and Gervais 1991). It is commonly assumed that smelts cannot successfully ascend fishways and that even small obstacles (e.g. woody debris, cascades) can impede their migration to

spawning grounds (Moring 2005). Because of its poor swimming performance, rainbow smelt is a good indicator species to determine the passage efficiency of fishways.

In recent years, the ascent of smelts has been observed in some fishways in Prince Edward Island (Canada). To confirm these observations, we initiated a research project to scientifically quantify the efficiency of smelt passage in a nature-like fishway (pool-and-weir type) constructed in the Pisquid River (Fig. 1a). The fishway was designed for passage of salmonids and was composed of five weirs (Fig. 1b), with a slope of 2.3%. The drop in elevation between weirs ranged from 0.113 m to 0.258 m (Table 1). The length of the fishway was 53.5 m and the average bankfull width was 10 m.

In 2009, a stationary half-duplex Passive Integrated Transponder (PIT) system (Prentice et al. 1990) composed of four antennas was installed in the fishway (Fig. 1b). These four antennas were custom made and tuned specifically for the dimensions and flow conditions of the fishway (detection range of approximately 0.95 m). Each antenna was laid on the riverbed and positioned across the river in order to span the entire wetted width. One antenna was located downstream of the fishway to record tagged-fish that were approaching the fishway (Antenna 1; Fig. 1b). A second antenna was installed upstream of the first weir to determine if tagged fish successfully entered the fishway. The third antenna was located upstream of the last weir to determine whether tagged fish successfully navigated the entire fishway length. The fourth antenna was located upstream of a riffle (30 m long) above the fishway to determine if the riffle (slope of 1.7%) was itself an impassable barrier. Transponders (23 mm, 0.6 g) were surgically implanted in the abdominal cavity of 465 smelts (230 males, 235 females) captured and released downstream of the fishway. To determine if the fishway was

size selective, these 465 tagged smelts were distributed within 3 size categories: 1) small, fork length < 155 mm (n = 181); 2) medium, 155 mm ≤ fork length < 180 mm (n = 164); and 3) large, fork length ≥ 180 mm (n = 180).

The smelt migration was initiated when water temperature reached 4.5°C (April 15, 2009) in the Pisquid River. The migration was bimodal and the second peak occurred when water temperature reached 8°C (April 23, 2009). Overall, 74% of smelts randomly sampled downstream of the fishway were male and 26% were female. Age was determined on a random sub-sample of 138 individuals and was composed of four age-groups (age 2 to 5) with the majority (86%) of the fish being age 2 and 3 years.

During the experiment, 60 smelts were PIT tagged and retained in a live-box to estimate tag retention and tagging mortality. Sixty unmarked smelts were retained in a similar live-box as a control. From April 15 to April 28, 2009, tag retention was 100%. However, 9 of the 60 (15%) tagged fish did not survive, whereas no mortality was observed in the control group. At least 6 of these mortalities were females. Due to an error in data processing, it was not possible to determine the sex of the remaining 3 mortalities. We believe that the mortality rate (15%) observed in the controlled experiment is of concern, but it does not compromise the overall reliability of this study as most of the tagged fish survived the surgical manipulations. Nonetheless, the effects of tagging on swimming performance remain unknown.

A large number of detections (n = 1042) were observed on the antennas of the PIT-system installed on the fishway. Of these detections, some were consecutive detections of the same fish at a specific antenna. After removing the consecutive detections, 539 detections, corresponding to 213 different individuals, were recorded between April 16 and

30. High spawning activities were observed downstream of the fishway and tagged smelt may have spawned downstream of the structure without attempting to migrate upstream. Therefore, calculations related to the percent of fish that navigated the fishway were based on the number of fish that approached the fishway and were detected at Antenna 1 (i.e.,  $n = 213$  smelts). Of the smelts that approached the fishway, 50% crossed over the first weir and entered the structure (detected at Antenna 2). Six percent of these smelts successfully navigated the entire structure and the riffle located upstream of the fishway (detected at Antenna 4). The highest number of detection (109 detections) was recorded when water discharge decreased to  $1.3 \text{ m}^3/\text{s}$  (April 21, 2011; Fig. 2). Smelt movements nearly ceased (6 detections) when water discharge increased above  $2.5 \text{ m}^3/\text{s}$  (April 23, 2009).

Males were the predominant sex that approached (60%), entered (69%) and navigated the entire fishway (83%; Fig. 3a). However, these results are slightly influenced by the fact that females experienced a higher tagging mortality. There was also a tendency for larger fish to be detected more often than smaller ones on all four antennas (Fig. 3b). Four of the 6 fish that successfully negotiated the entire fishway were from the large size group ( $\geq 180 \text{ mm}$ ).

Field observations suggested that smelts progressed through the fishway by a sequence of “sprints” that were followed by resting behind large rocks. When possible, the smelts selected slower flow paths located near the river banks. Smelt progression within the fishway seemed to almost come to a halt downstream from Weir B (Fig. 1b). This observation suggests that Weir B was especially difficult to pass for most fish, presumably due to the difference in elevation (0.258m; Table 1) and higher water velocities. In comparison to Weir B, the downstream weir (Weir A) offered more slow flowing water paths

along the stream edge which appeared to facilitate smelts passage.

In contrary to the general belief that smelt cannot navigate through fishways, we confirmed that a portion of the tagged smelts can successfully navigate through a nature-like fishway which was designed to accommodate stronger swimmers, (i.e. salmonids). Considering the large number of fish during the migration period, this small percentage still represents a significant number of individuals able to navigate through the fishway. Visual observations of smelts above the fishway suggest that they were able to reach additional spawning grounds, which could reduce egg mortality due to overcrowding (McKenzie 1964).

The effects of tagging on swimming performance remain unknown and further research is required. For example, the use of smaller PITs (13 mm) will most likely reduce potential effects of tagging on migratory behaviour of smelts. Although only a small percentage of smelts successfully navigated the entire fishway, it is anticipated that conducting small modifications to the structure along the stream edge would considerably increase smelt passage. Tracking marked smelts with a mobile PIT detection unit would allow specific passage locations to be determined. Measuring hydraulic conditions at locations of passage is necessary to better understand the passage requirements of smelts in fishways.

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Figure 1: a) Location of the fishway constructed in the Pisquid River (Prince Edward Island, Canada); b) plan view of the fishway (A to E) and location of the antennas (1 to 4).

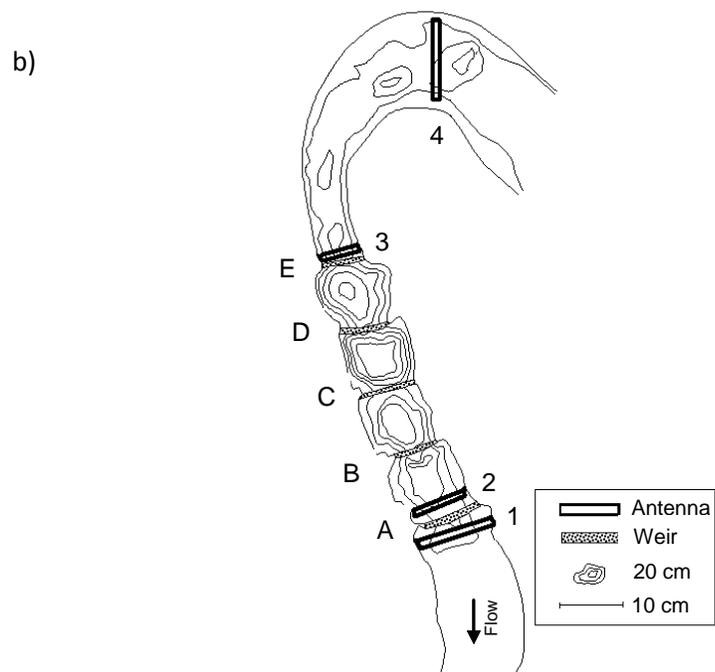
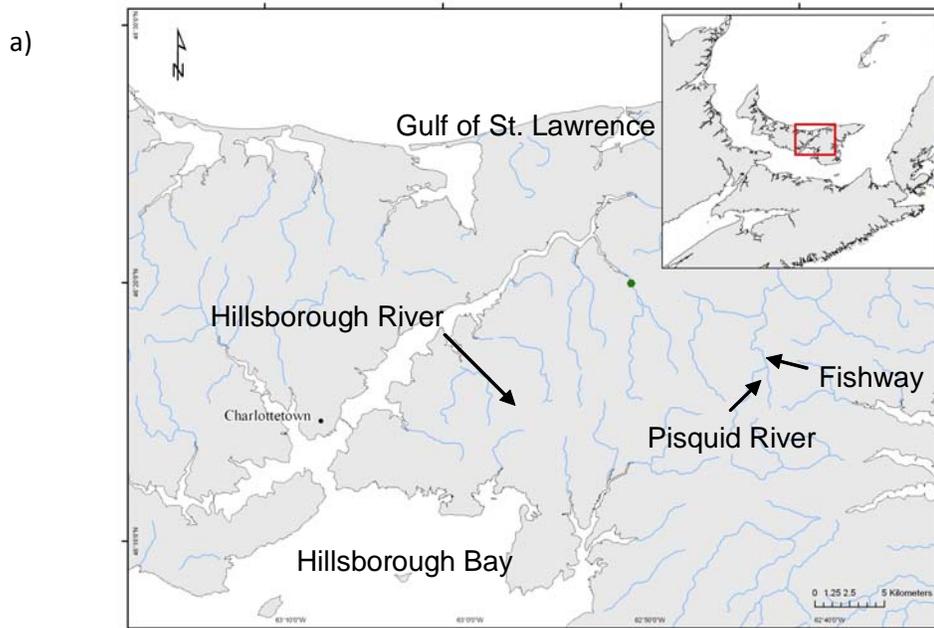


Table 1: Drop in elevation at each weir of the fishway.

Weir	Drop in elevation (m)
E	0.255
D	0.113
C	0.258
B	0.258
A	0.124

Figure 2: Temporal variations of the daily number of detections (n = 539) (a) and river discharge in Pisquid River (b).

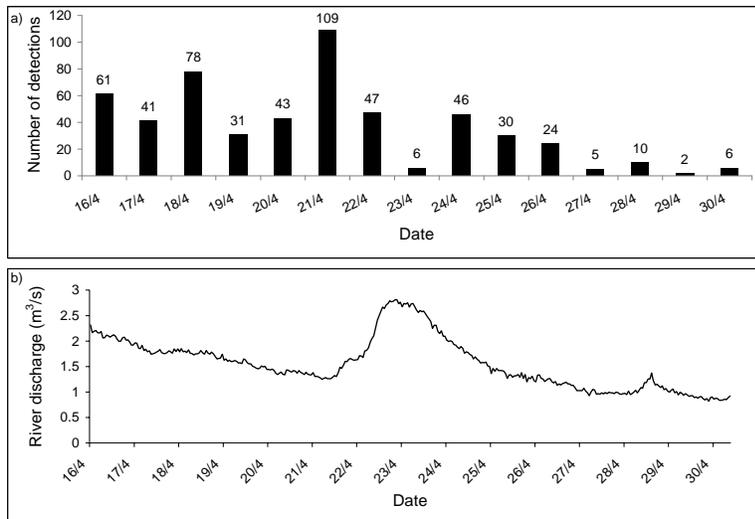
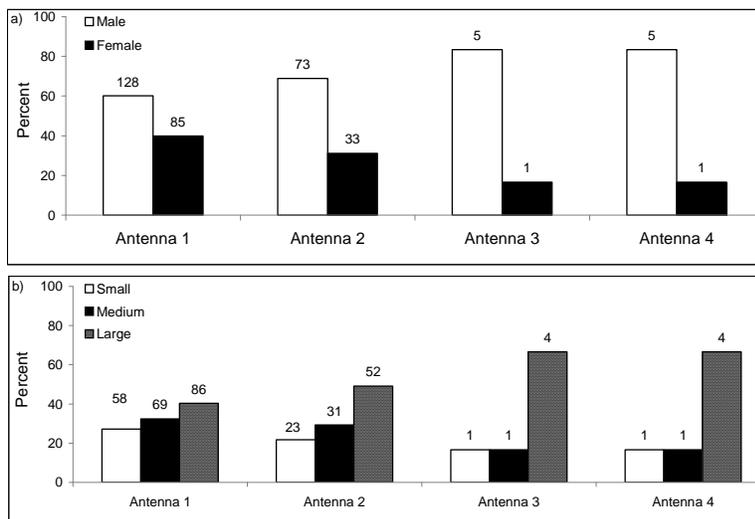


Figure 3: Percent of male and female (a) and size-groups (b) detected at each antenna installed in the Pisquid River.



# Laboratory marking of anadromous rainbow smelt embryos and larvae and the implications for restoration

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The decline of anadromous rainbow smelt (*Osmerus mordax*) in Massachusetts throughout the last few decades has increased the need to develop practical restoration practices. The decrease in populations has been linked to declining water quality, overfishing, and habitat alteration. Recent improvements to water quality and spawning habitat of individual coastal rivers and streams have prompted the development of restoration practices for rainbow smelt. Successful marking and subsequent recapture of hatchery stocked smelt is critical to quantifying effectiveness of restoration efforts. Utilizing recent advances in smelt culture techniques, this study developed methods for marking otoliths in rainbow smelt. The first experiments examined the marking of smelt embryos with buffered oxytetracycline hydrochloride (OTC), while subsequent experiments examined the use of OTC for marking larvae.

Adult smelt in spawning condition were captured from a coastal Massachusetts river and transported to a Division of Marine Fisheries (DMF) facility and were strip-spawned in the laboratory (Ayer et. al 2005). There were two experiments in 2006 to mark eyed rainbow smelt embryos: the first experiment used concentrations of 500 and 1000 mg/L OTC with controls while the second used 1500 and 2000 mg/L OTC with controls. Each experiment used a 24 hour immersion of embryos in one of the specified treatments.

In 2007, two experiments were conducted using larvae 2-3 days post hatch. The first experiment used concentrations of 250 and 500 mg/L OTC with controls and the second used

750 and 1000 mg/L OTC with controls. Each experiment used a four hour immersion of larvae in one of the specified treatments. Hatched fish were enumerated for the embryo experiments, and mortality of the larval experiments was recorded. Fish were retained and reared in a recirculating water system in the laboratory. At regular intervals, fish were sampled for up to 12 months to examine otoliths for the persistence of an OTC mark under ultraviolet light.

The survival of embryos and larvae for all experiments was not significantly different for all treatments as compared to controls. The 2006 experiment that examined higher concentrations (1500 and 2000 mg/L) of OTC on embryos was discontinued after 24 hours, once it was observed that some of the OTC came out of solution and coated the embryos. Otoliths from larvae marked as eyed-embryos with 500 and 1000 mg/L OTC displayed distinct fluorescent marks when compared to controls for up to six months after marking. Upon examination one year after marking, no discernible marks could be found. The otoliths from larvae marked with 250, 500, 750 and 1,000 mg/L OTC all displayed visible fluorescent OTC marks upon initial examination. At the six-month examination, the 250 mg/L OTC marked otoliths had no discernible marks while the 500, 750, and 1000 mg/L OTC marked otoliths had clearly visible marks. Upon examination of the 500, 750, and 1000 mg/L OTC marked otoliths after one year, all treatments displayed visible fluorescent marks.

After determining the most effective method for marking smelt otoliths, batches of 50,000-

200,000 smelt larvae were marked and released into the Crane River, Danvers, MA, at a documented smelt spawning site during spring months from 2007-2011. Returning adult rainbow smelt were captured during the spawning run in the Crane River during fyke net sampling beginning in the spring of 2008. All captured adult smelt were retained and their otoliths were removed and examined under ultraviolet light for the presence of an OTC mark. There were 94 adult age-1 smelt captured in the spring of 2008, and of those, there were 14 fish with OTC marked otoliths (~15%). During the spring of 2009, there were 52 age-1 and age-2 fish captured; of those only three were marked and all three were age-2 fish.

Although there has been little success in marking finfish embryos, it has been successful for both Ayu (*Plecoglossus altivelis*) (Tsukamoto 1987) and coregonid fishes (Dabrowski et. al 1986). The possibility of marking eyed embryos would allow for a greater ease of working with non-motile individuals compared to hatched and free-swimming larvae. Although larvae hatched from embryos marked with OTC retained a fluorescent mark for over six months, when the otoliths were examined after one year, no marks were discernible. The retention and subsequent detection of the OTC mark after at least one year is essential for the monitoring of restoration practices with rainbow smelt.

The mass marking of larval fish with OTC has been successful for many different species including striped bass (*Morone saxatilis*) (Secor et. al 1991) and walleye (*Sander vitreus*) (Brooks et. al 1994). These experiments also found marking larval smelt with OTC to be successful in producing long-term marks on otoliths. The decision was made to use the lowest concentration of OTC (500 mg/L) that produced a clearly visible mark on otoliths after one year to keep costs to a minimum and to limit

exposure of large numbers of smelt larvae to higher OTC concentrations.

The small numbers of returning marked smelt are viewed as a success by researchers. Recapture of spawning adult smelt with OTC marked otoliths at the restoration river has expanded the project and spurred researchers to continue stocking and monitoring at the river. Over 10 million marked rainbow smelt larvae have been stocked into the Crane River since 2007, and monitoring will continue in hopes of recapturing more marked adults. New restoration sites for rainbow smelt are being examined and sampled for baseline population data before beginning any stocking. Site suitability assessments must be completed at any possible restoration site before large-scale stocking efforts begin and long-term post-stocking monitoring should be performed to demonstrate stocking success.

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## **The rainbow smelt restoration plan for the St. Lawrence estuary:**

### **Where are we eight years later?**

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In the St. Lawrence estuary (Canada), anadromous rainbow smelt (*Osmerus mordax*) colonize almost all coastal salt- and brackish waters in the estuary and the gulf. Up to five genetically distinct populations have been identified so far, and the one restricted to the south shore of the estuary was listed as a vulnerable population in 2005. This listing followed a sharp decline in abundance and abandonment of three major spawning tributaries. At that time, only three spawning tributaries were still active, but water quality and river habitat were deteriorating rapidly.

To reverse the trends, managers and stakeholders developed a restoration plan. A team composed by governmental agencies, universities and non-governmental organizations was set up to increase collaboration, share information, and coordinate efforts among members. To support these objectives and fill information gaps, research was first identified as a major objective, and research activities were implemented to gain specific knowledge on smelt ecology, habitat needs and population dynamics. A total of 25 actions were identified, ranked by priority and order of execution, and shared among members of the team.

Water quality restoration, spawning habitat rehabilitation and fishing mortality reduction were identified as major objectives. Performance measures for every restoration activity were implemented. Specifically, monitoring programs were standardized for spawning run sampling on one tributary (Fouquette River), egg deposit index on all spawning tributaries, larval rainbow

smelt trawling in two nurseries, creel surveys for summer and winter recreational fishery and monthly sampling for water quality on every active and deserted spawning river. Annual assessment for these monitoring activities is routinely done during a statutory annual meeting where progress towards restoration objectives is measured and discussed.

Protection of remaining active spawning rivers and restoration of the ones previously used were considered to be the highest priority restoration goals, and watershed management was implemented on those tributaries to improve water quality. Involvement of landowners, mainly farmers, was a key issue for decreasing nutrient loading. Phosphorus was targeted as the main nutrient of concern because it can cause rapid periphyton growth during the smelt spawning season. New agricultural practices and municipal sewage treatment were successfully implemented. After 12 years, a 20-fold decrease in phosphorous concentration was measured on the Fouquette River, and water quality is now more conducive to rainbow smelt spawning.

Spawning run monitoring enables measurements of adult stock morphological parameters and relative abundance. Standardized sampling procedures performed on a spawning tributary for 19 years give accurate information on one of the most vital stages for recruitment. Data gathered from spawners (CPUE, age, fecundity) were integrated into a Spawning Stock Index (SSI) so that stock composition and abundance could be tracked annually (Fig. 1). The SSI shows that the stock is composed

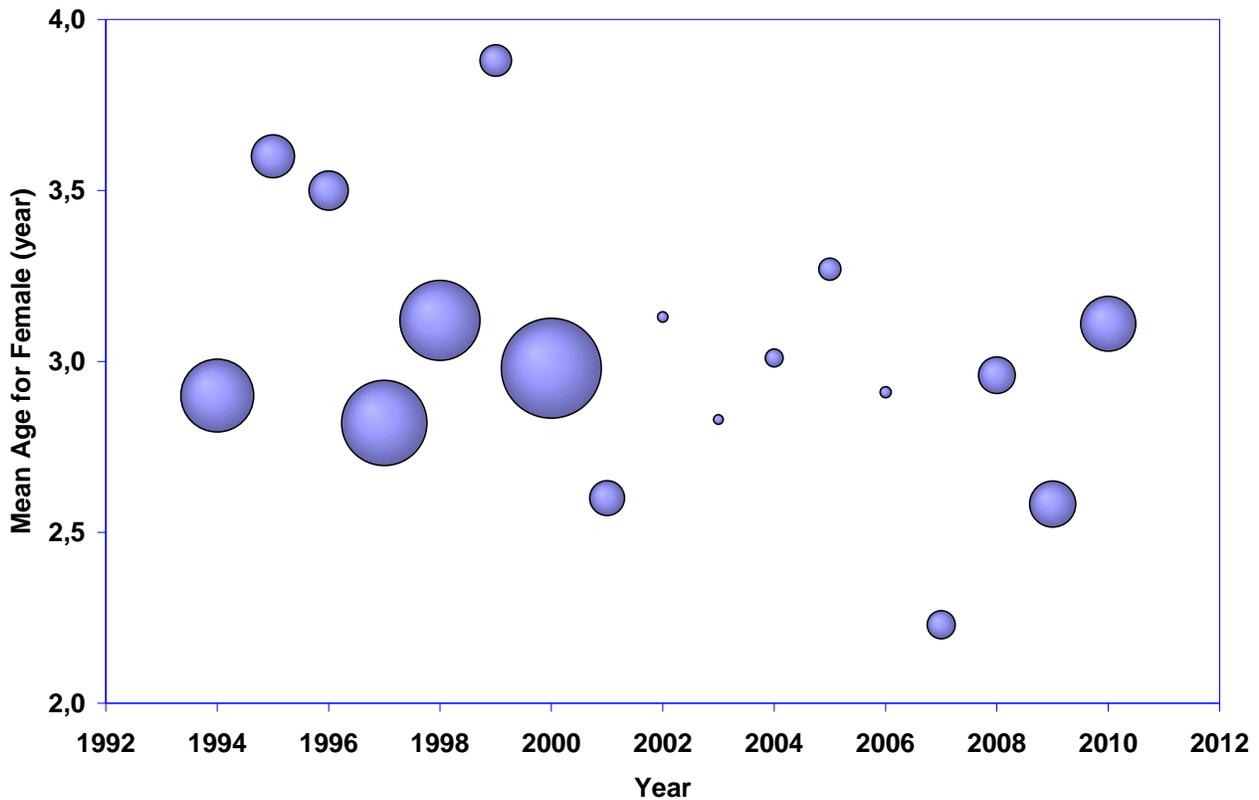
mainly with individuals at their first reproduction, with the mean age at maturity being 3.1 years.

An important decrease in abundance was noted after 2000. This decline is correlated with an increase in popularity of the winter recreational fishery on the St. Lawrence south shore. Further studies on population dynamics estimated that recreational fishing mortality (commercial fishing was already closed) represented approximately  $\frac{3}{4}$  of total mortality ( $Z= 1.33$ ). In 2007, the daily catch limit was

reduced by half, from 120 to 60 rainbow smelts/fisherman/day.

All these efforts have begun to pay off. Water quality increased on active spawning grounds, and two once-deserted spawning tributaries have now been active for a few years and are contributing to recruitment. Adult abundance has increased slightly, and the mean age of the stock is increasing. Although these encouraging observations remain fragile, it proves that restoration objectives can be achieved and declining trends reversed with a team approach.

**Figure 1.** Spawning Stock Index from 1992 to 2010 for the Fouquette River.



## Poster Abstracts

### **A historical view of anadromous rainbow smelt populations and fisheries in the eastern United States**

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Smelt fishing on the Parker River in Newbury, MA (Photo by O. P. Gould in G.O. Shield 1897).



#### **Introduction**

The rainbow smelt (*Osmerus mordax*) is a prized table fish that has cultural, ecological, and economical importance along its native range from Virginia to the Canadian Maritimes. Smelt fisheries were prominent in numerous coastal communities on the East Coast of the U.S. until the latter half of the 20<sup>th</sup> century. Populations in the Southern range have gradually declined and appear to have been recently extirpated. Their occurrence between the Hudson River and

Southern New England has become rare, and the southernmost location of a smelt spawning run is presently known to occur in Massachusetts.

We reviewed available historical information regarding smelt harvests, fishing interest, and market demand for smelt throughout the Eastern U.S., and have provided a summary of these findings by state. The focus is on the mid-Atlantic and Northeastern U.S. smelt stocks where significant changes have occurred to anadromous smelt populations. The following causal factors linked to the historical decline are summarized: 1) coastal river dam creation, 2)

fishing, and 3) industrial water pollution. This summary offers a historical perspective on this important recreational and commercial fish, and discusses present threats and challenges.

### **Historical Fisheries and Present Condition**

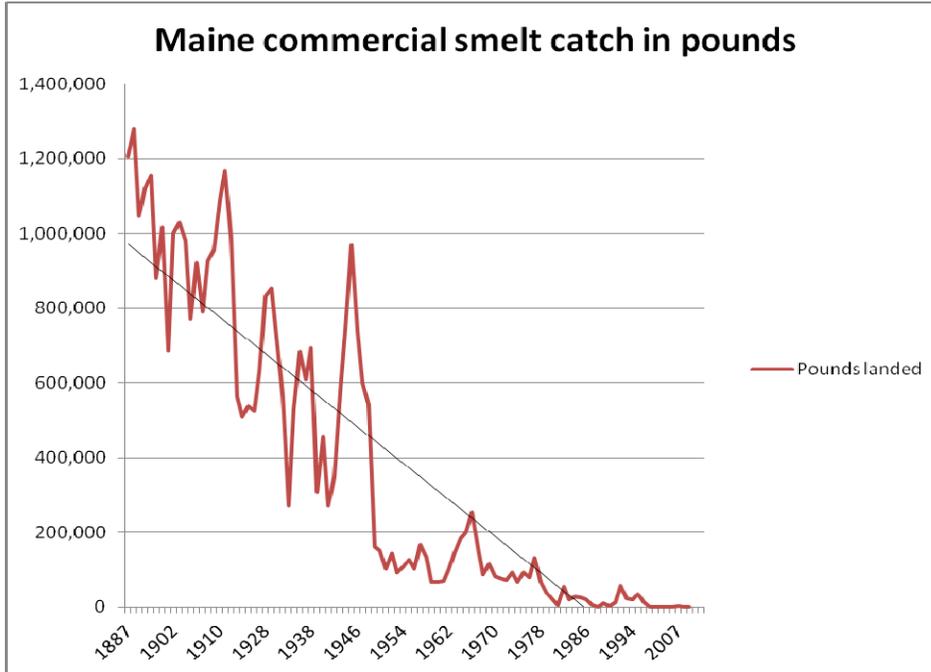
Mid-Atlantic States: Once supporting small fisheries, now extirpated. Numerous historic smelt range estimates include Virginia (Goode 1884 in Kendall 1927; Bigelow & Schroeder 1953), Maryland and Delaware (Robins & Ray in Collette & Klein-MacPhee 2002), however, limited information is available regarding fisheries in these states. In 1833, smelt were plentiful in New Jersey with “wagonloads” of smelt harvested in Newark Bay (NY Times 1881 in Fried and Schultz 2006). Yet by 1849, the New Jersey populations were noticeably declining. The last commercial catch of smelt in New Jersey was reported in 1921 (Fried and Schultz 2006). The once prominent smelt fishery of New York was no longer considered commercially viable as early as 1887 (NY times 1881, Mather 1887, Mather 1889; in Fried and Schultz 2006); the last regular commercial catch of smelt was in 1962 (Fried and Schultz 2006). Survey efforts conducted by New York State Department of Environmental Conservation last detected smelt in the 1980’s (C. Hoffman, New York State DEC, *pers. comm.* Sept. 2010).

New England: Declining populations still support a strong cultural heritage and limited fisheries. The peak catch of smelt in Connecticut was in 1880 with 27,000 lbs; smelt harvest in the state steadily declined with limited harvest since 1930 (Fried and Schultz 2006). In 2003, Fried and Schultz (2006) sampled for smelt in Connecticut using weirs, fyke nets, gill nets and ichthyoplankton tows with no rainbow smelt

detected, but a seine survey yielded only nine adult smelt in one river system. Since 2008, rainbow smelt have been listed as endangered in Connecticut. Rainbow smelt harvests in Rhode Island have also steadily declined since 1880. Since 1965, there has been nearly zero harvest recorded (Fried and Schultz 2006). Massachusetts’ commercial smelt harvests have also declined for at least the past 90 years. Fried and Schultz (2006) summarized federal commercial catch rates and noted three peaks in Massachusetts harvest, 1879 with 35,000 lbs, 1919 with 39,000 lbs, and 1938 with 25,000 lbs. Larger catches were likely during spawning run net fisheries prior to the 1870s but were poorly documented (Kendall 1927). Today in Massachusetts there is limited recreational catch and insignificant commercial harvest.

Peak commercial catch of smelt in New Hampshire was between 1940-1945, with an estimated 150,000 lbs/yr harvested (Fried and Schultz 2006). Commercial harvests in New Hampshire have diminished since 1987 (Fried and Schultz 2006), but an active recreational smelt fishery remains. Like New Hampshire, there was a prominent commercial smelt fishery in Maine, but as early as 1869 it was evident that the Maine smelt populations were declining (Atkins 1869 in Kendall 1927). In the late 1800’s annual catch rates for Maine were over a million lbs/yr, but after the 1940’s, commercial catch for smelt dropped off (Figure 1). Today limited commercial catch is recorded with a total of 3,803 lbs harvested over a 4 year period (2006-2009) (Maine commercial landings data). Recreational smelt fishing remains popular and is an important part of the recreational fishing economy in Maine.

**Figure 1.** Maine commercial smelt catch in pounds (Squiers et al. 1976 and NOAA Fisheries 2011).



### Historical Threats

Causal relationships to smelt population declines have not been easily demonstrated. Establishing causal responses is complicated by the absence of population data and poor early harvest records. The history of land use in the region indicates that the following causal factors are likely primary influences on the historic smelt population decline: dam creation, water pollution in river systems, and overfishing (Limburg & Waldman 2009). These causal factors are believed to have remained a chronic stressor to local populations for at least 100 years. Alterations to urban watershed hydrology and climate change are two potential influences that have received less attention than the listed causal factors yet could exert significant consequences on smelt distribution and recruitment.

Dams. Historic coastal river dam creation was likely the most readily identifiable negative

impact on smelt migrations to their spawning habitat. Dams built near the head of tide have reduced the amount of available spawning habitat. Dams may also impact smelt recruitment by reducing embryo survival through crowding and the effects of increased salinity (Baird 1967 in Collette & Klein-MacPhee 2002). There is poor documentation of when, where, and the number of dams that were installed along the East coast, but were ubiquitous throughout the region. Today, in Massachusetts alone there are at least 99 head of tide dams in place (pers. comm. A. Bilbo-Miles Massachusetts Office of Dam Safety September 2010).

Water pollution. Water pollution was another chronic stressor on smelt populations across the Northeast. As early as 1867, river water pollution in Connecticut was noted as the “principal cause of declining catches of all fish species” (The Connecticut Fish Commissioners 1867 in Fried and Schultz 2006). Squiers et. al. (1976) noted that after 1945 there was an

increase in water pollution in rivers which likely contributed to declines in smelt populations in Maine. Chronic industrial point sources have declined in the US in the decades following the 1972 passage of the Clean Water Act. Recent monitoring in Massachusetts has raised concerns over non-point impacts, particularly related to acidification and eutrophication in urban watersheds (Chase 2006).

Overfishing. The fishing pressure during smelt spawning runs before 1850 may have exerted high mortality on smelt populations. Seine, dip net, and weir fishing were three common highly productive methods of smelt harvest. A single haul of a seine net could yield up to 6,700 pounds of smelt (The report for the Commissioner of Fisheries in Massachusetts 1869 in Kendall 1927). By 1868, dip netting and seine netting was prohibited in Massachusetts (Kendall 1927).

## Conclusion

Major changes have occurred in anadromous populations of smelt throughout the East Coast of the U.S. The southern end of the range moved dramatically northward in the 20<sup>th</sup> century. Total harvests have declined substantially in this period. Overfishing does not seem to be a major influence in the spatial changes or stock conditions of recent decades. Environmental influences on stock recruitment could be important stressors exacerbated by the impact of climate change and the resulting changes in ocean temperature, pH, and circulation. Currently the southernmost viable population of anadromous smelt on the East

Coast appears to be located in Buzzards Bay, south of Cape Cod.

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# **The Penobscot Estuarine Fish Community Survey: An overview with rainbow smelt (*Osmerus mordax*) monitoring components**

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## **Introduction**

The Penobscot ecosystem allows for a unique opportunity to monitor estuarine responses to major upstream river restoration projects including main-stem dam removal, active diadromous species population enhancement, and habitat improvement projects. There is also a growing need for fishery scientists and managers to understand interactions between freshwater, diadromous, and marine species within the complex estuary habitat. Therefore, we are developing the Penobscot Estuarine Fish Community Survey, which is part of the wider Maine Estuaries Diadromous Survey. The primary aim of the former is to describe the spatial and temporal distribution of fish in the Penobscot estuary by implementing a comprehensive fishery-independent survey. The survey will be developed using a combination of fish capture and remote sensing gears with collective environmental and habitat monitoring. We anticipate rainbow smelt (*Osmerus mordax*) to have an integral presence in the Penobscot estuary and that our monitoring will better describe population dynamics and life history variation that is currently poorly documented.

## **Methods**

In 2010, we used 18 and 45 m long beach seines made of 5 mm nylon delta style mesh. The seines were 1.8 m and 2.4 m high respectively with a tapered bag of 5 mm mesh in the mid-point of the net. The shorter (1.8 m)

seines were abandoned early in the sampling season due to low capture rates. Nets were made with a weighted footrope and buoyant floats on the head rope. Wooden poles were lashed to the ends of each seine to aid in net retrieval. Each of 12 sites, (8 upper estuary (Figure 1) and 4 lower estuary (Figure 2)), was sampled weekly at approximately slack low tide from August through November.

The two sized fyke nets, 1 m and a 2 m mouth, were constructed of successively smaller square metal tube frames that were surrounded with mesh net (0.6 cm for small, 1.9 cm for large). Two 9.1 m wings extended from the opening of each fyke at an angle of approximately 30° when set and have an optional central lead net of 9.1 to 18.2 m. The wings and lead had a weighted footrope with buoyant floats on the head-rope and are of the same height as the fyke itself (either 0.91 m or 1.83 m high). Each net had two throats tapering to a semi-rigid opening of 12.7cm for the small net and 45.7 cm for the larger net and emptied into a rearward cod-end. Nets were set in conjunction with beach seine sampling and were fished at approximately 6 hour intervals corresponding with a tidal cycle (flood to ebb or ebb to flood).

## **Results**

We conducted 148 seine hauls and 7 fyke net sets from August 3 through November 2, 2010. This resulted in the capture of over 19,000

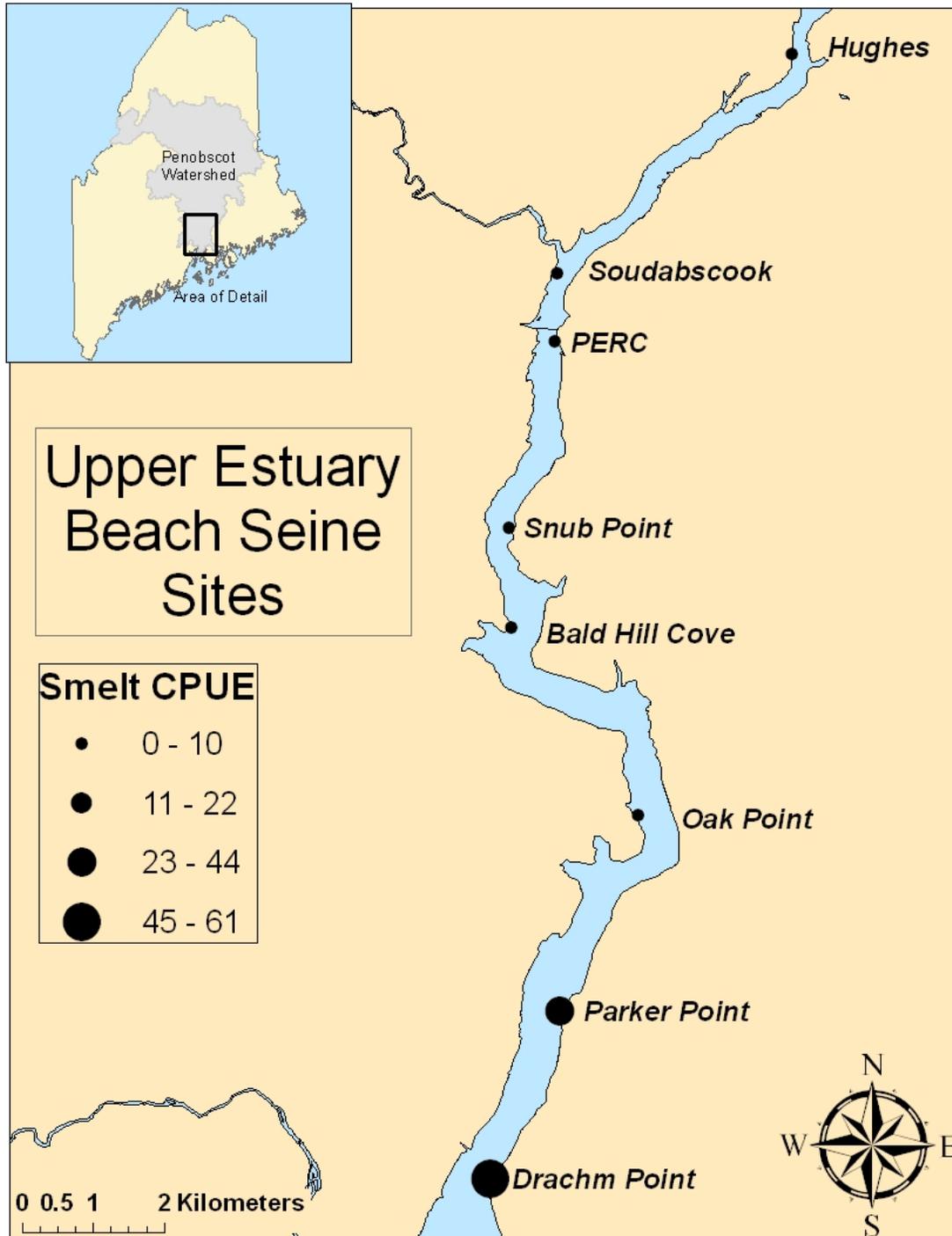
individuals comprised of 45 fish and invertebrate species. Most numerous in our samples were common mummichog (*Fundulus heteroclitus*) and Atlantic silverside (*Menidia menidia*) representing 35% and 30% of the catch, respectively. Invertebrates were numerous in our catches (25% total catch), represented mostly by sand shrimp (*Crangon septemspinosa*). We captured 290 rainbow smelt (*Osmerus mordax*) young-of-year (YOY) and 1 adult, all in the beach seines. Rainbow smelt represented 1.4% of the individuals captured during sampling but were present in 20.9% of beach seine hauls. Catch per unit effort (CPUE) (catch per seine haul) ranged from 1 to 8.4 in the lower estuary, and abundance was greatest on September 15. CPUE ranged from 0 to 18.25 in the upper estuary with a peak on October 28 (Figure 3). Mean total lengths for YOY ranged from 48 to 68 mm in the lower estuary and 46 to 60 mm in the upper estuary (Figure 4).

We also foresee substantial opportunities for collaboration with state and academic entities in continuing efforts to understand diadromous fish populations and their link to the estuarine environment.

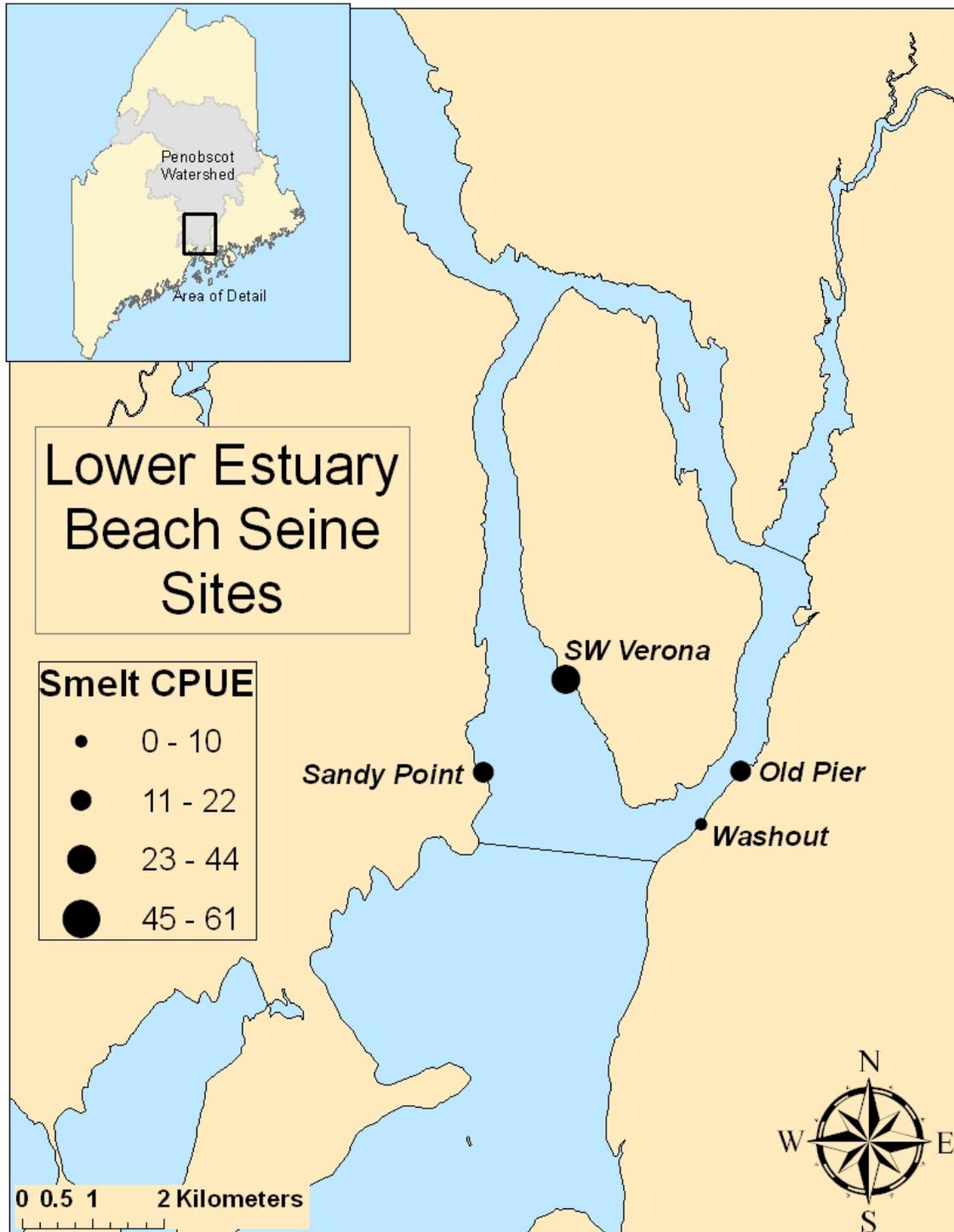
### **Conclusions – Future work**

In 2011, we plan to continue sampling with beach seines at our established index site, expand our fyke net sampling, and add new gears such as pelagic trawls and hydroacoustics. Our 2011 goals are to sample using the various gears from April through November to encompass as much of the diadromous migration season as possible. The 2010 and 2011 scoping effort will allow for the refinement of sampling design to be implemented in 2012 and beyond. We anticipate main-stem fyke net and trawling efforts will document relative abundance and timing of adult rainbow smelt during their spawning migration. Similarly, beach seining efforts will document relative recruitment success and juvenile emigration timing. We envision our efforts to be refined over time in order to create a long-term and robust data set.

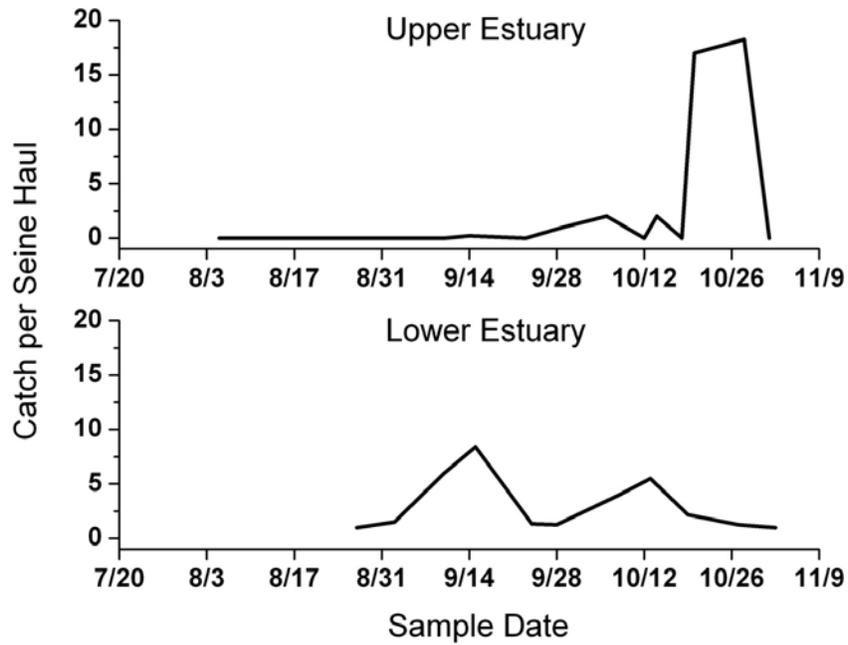
**Figure 1.** Map of beach seine sites sampled within the upper Penobscot estuary during surveys conducted in fall 2010. Markers represent maximum relative smelt abundance observed.



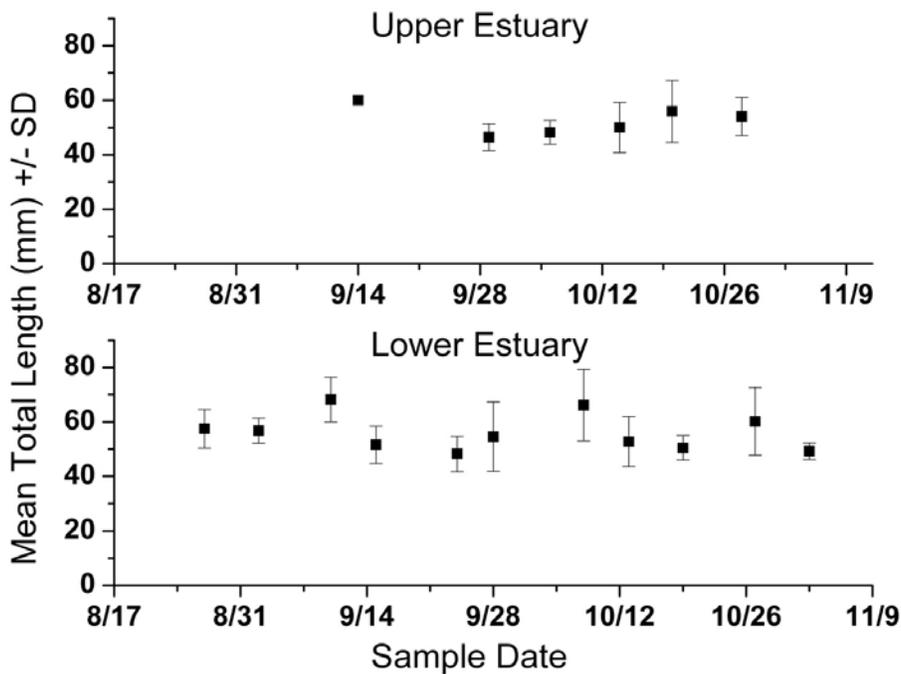
**Figure 2.** Map of beach seine sites sampled within the lower Penobscot estuary during surveys conducted in fall 2010. Markers represent maximum relative smelt abundance observed.



**Figure 3.** Catch per unit effort for Rainbow smelt (*Osmerus mordax*) in the upper and lower Penobscot estuary during preliminary beach seine surveys conducted in Fall 2010. Note sampling did not take place in lower estuary until 8/27/2010 and smelt were not detected in upper estuary until 9/14/2010.



**Figure 4.** Mean total length (mm) +/- SE for rainbow smelt (*Osmerus mordax*) in the upper and lower Penobscot estuary during preliminary beach seine surveys conducted in fall 2010.



# **Out on the ice – sampling Maine’s recreational winter smelt fishery on the Kennebec River and Merrymeeting Bay**

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Ice fishing on frozen tidal rivers is a long standing tradition in New England. In Maine, industrious entrepreneurs have made a seasonal business by setting up and renting out hundreds of small ice-fishing shacks for recreational smelt fishing on the Kennebec River and Merrymeeting Bay. These groups of shacks (camps) make up a large proportion of fishing pressure on the river during winter months. Although camps must acquire a state license each season, fishermen and camp owners are not required to report any of their catch information.

While small ice fishing shacks appear on most frozen rivers and streams in the state from late December to March, only the Kennebec River and Merrymeeting Bay have commercially operated camps (Figure 1). A smelt camp may manage anywhere from 15 to 100 shacks each year. At these camps, anglers can rent a shack with all equipment included for one or more tides. Because of the popularity and ease of fishing a single tide, people are fishing for smelt out of the Kennebec River and Merrymeeting Bay waters twenty-four hours a day during the winter months.

Adopting sampling methods currently used by the New Hampshire Fish and Game Department (NHFG; NHFG Progress Report 2009) and methods used in a 1979-1982 study conducted by the Maine Department of Marine Resources (DMR; Flagg 1983), DMR again began conducting creel surveys in 2009. As part of this survey, DMR staff visited participating camps two or three times per week on a rotating basis to collect biological information about the recreational catch. All anglers at the camp who

were fishing during a specific tide were interviewed, although some anglers declined the interview. Staff measured smelt to the nearest millimeter, determined sex, and collected scale and fin clip samples from all retained fish. A sub-sample of adult smelt was collected for toxic contaminants analysis to update intake and health advisories. The number of anglers, fishing hours, and the number of fishing lines used was also recorded.

Comparing data from the Maine DMR 1979-1982 study (Flagg 1983) to preliminary data collected 2009-2011, the recent survey found a slightly lower catch-per-unit-effort (CPUE), however inter-annual variability was significantly larger than the comparison between the two study periods (Table 1). CPUE was calculated as total number of smelt caught per line-hour of fishing. Annual fluctuations in CPUE occurred in both surveys, but the recent survey had the lowest CPUE recorded (0.1662) during the two time series.

The mean length differed significantly between males and females within each year of the 2009-2011 survey (t-test  $p < 0.0001 < 0.05$  in all cases) as well as between the years for each sex (ANOVA  $p < 0.0001 < 0.05$  in all cases) (Figure 2). Mean length increased more than 10 cm for both males and females from 2009 to 2010 while the CPUE decreased from 0.7278 to 0.1662. This indicated a potential problem with a younger age class in 2010. In all years, the mean sex ratio (M:F) was fairly even and did not differ significantly between years (2009=1.61; 2010=1.53; 2011=1.52).

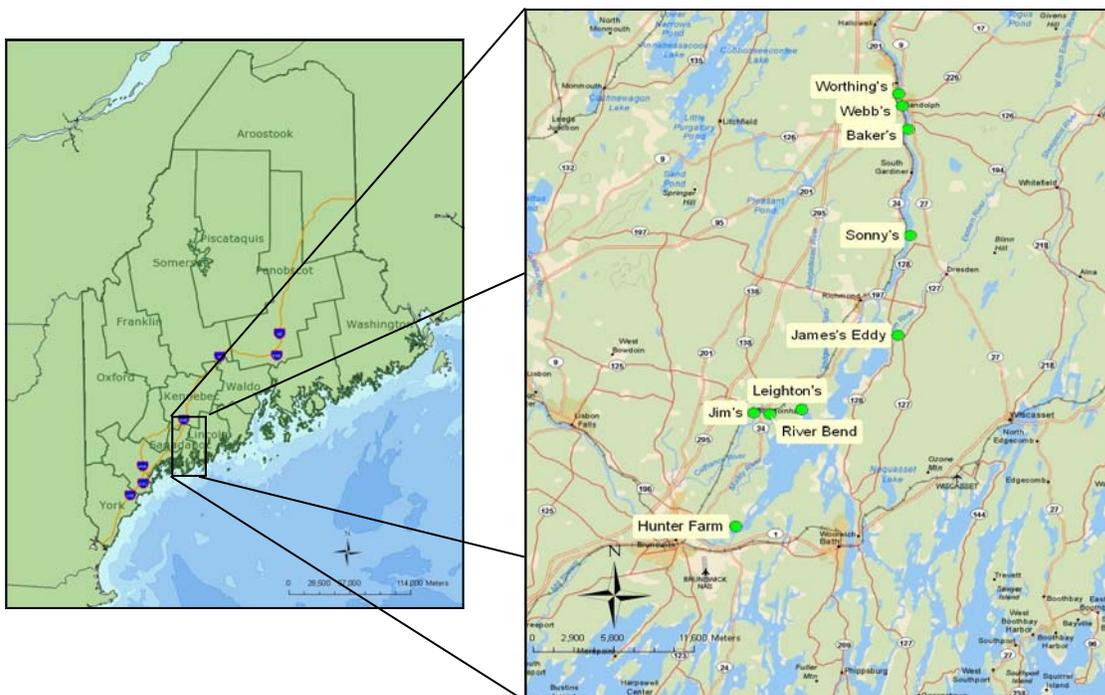
Catch card boxes were also posted at each camp for fishermen to voluntarily report information about their total smelt catch and any bycatch; responses varied widely between sites and between years. There were 122 responses in 2009, 6 in 2010, and 37 in 2011 from catch cards for all camps combined. The low response in 2010 could be attributed to negative response by anglers to the impending recreational Salt Water Fishing License, which was undergoing state public hearings during the fishing season. It is our hope that with continued interaction with anglers and camp owners that the number of responses will increase. Despite the low number of responses in 2010, the catch cards still reflected a decline in catch from 2009 to 2010, and increase again in 2011 (mean catch 2009 = 116; 2010 = 45; 2011 = 139). This trend was also evident in the creel survey data.

An age-at-length key is currently being developed to compare the age composition of the current population to that of the 1979-1982 survey and the NHFG surveys. Monitoring will continue at the smelt camps to develop a long-term dataset to understand more about inter-annual variability and changes in the population.

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**Figure 1.** Commercial ice fishing camps sampled during the winter smelt creel survey on the Kennebec River and Merrymeeting Bay, Maine (2009-2011).

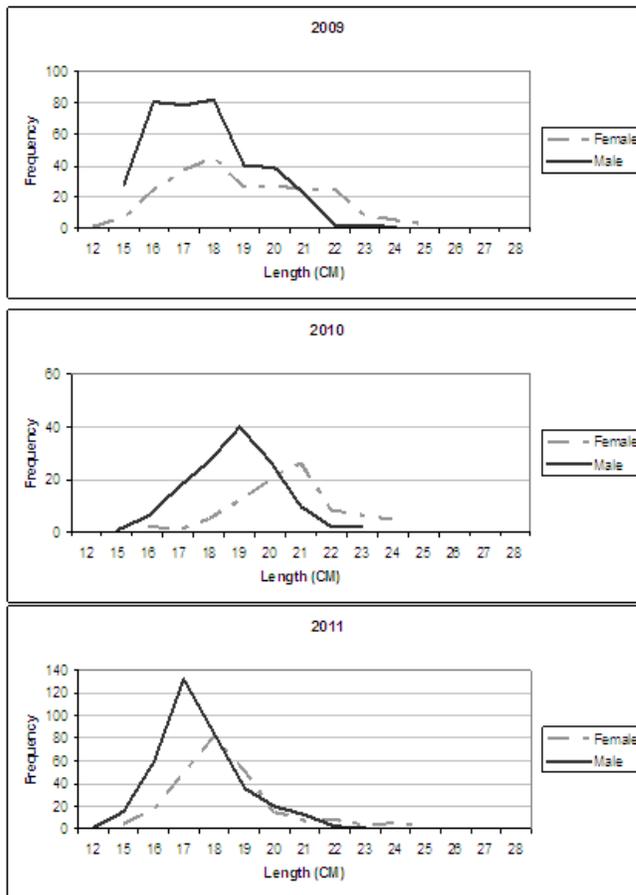


**Table 1.** Catch per unit effort (CPUE) observed during the rainbow smelt winter creel survey in Maine (1979-1982, 2009-2011).

Year	Total Catch # of Smelt	Total Effort as Line-Hours	CPUE (Smelt per Line-Hour)
1979	452,904	576,434	0.7857
1980	197,853	355,850	0.5560
1981	245,189	566,780	0.4326
1982	405,593	550,205	0.7372
2009*	1353	1859	0.7278
2010*	372	2238	0.1662
2011*	2305	4274	0.5393
1979-1982 Combined	1,301,539	2,049,269	0.6351
2009-2011 Combined	4030	8371	0.4814

\*Preliminary data not to be reported without permission by DMR

**Figure 2.** Frequency at length in centimeters of males and females observed during the rainbow smelt winter creel survey in Maine (2009-2011). Plots show the frequency at length in centimeters by year. Frequency by sex is separated in each year plot, males are shown by a solid line and females by a gray dashed line. The mean length differed significantly by sex within each year as well as between years.



Year	Average Length (mm)		Min Length (mm)		Max Length (mm)		Standard Deviation	
	Female	Male	Female	Male	Female	Male	Female	Male
2009	194.2	181.4	125	150	284	275	23.62	17.58
2010	210.0	192.4	163	159	263	234	17.08	14.15
2011	188.1	179.6	155	121	250	238	17.11	14.12

## Using otolith microchemistry to distinguish rainbow smelt larvae from different natal rivers

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The St. Lawrence south shore population of rainbow smelt (*Osmerus mordax*) spawn in four known locations (Rivière du Loup, Rivière Fouquette, Rivière Ouelle and Ruisseau de l'Église). It is important to distinguish the origin of larval smelt in order to evaluate the contribution of each river to the natural population in the St. Lawrence estuary. The general objective of this study was to evaluate the use of the otolith microchemistry method for

distinguishing the origin of rainbow smelt larvae from different natal rivers. The otolith core from rainbow smelt larvae from four spawning sites were analysed with a solution-based inductively coupled plasma-mass spectrometry (ICP-MS). A discriminant function analysis based on the concentrations of the most important trace elements in the otolith will be used to separate the larval smelt into their natal river.

## Gene flow between anadromous and freshwater rainbow smelt (*Osmerus mordax*)

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Five microsatellite loci were used to estimate gene flow between anadromous rainbow smelt (*Osmerus mordax*) from Casco Bay, Maine and Sebago Lake, Maine. Sebago Lake is drained by the Presumpscot River which flows into Casco Bay. The river has been dammed since the early 18<sup>th</sup> century. A stock enhancement project transferred 10,000,000 anadromous smelt eggs taken from Casco Bay fish into Sebago Lake

over a five year period from 2002 to 2006. Gene diversity indices calculated using Fstat software are consistent with limited gene flow between populations (  $F_{st} = 0.035$ ,  $G_{st} = 0.017$ ,  $R_{st} = 0.079$ ). Cluster analysis indicates that the direction of gene flow was from Casco Bay to Sebago Lake.

# Microsatellite analysis & MHC 2a of rainbow smelt, *Osmerus mordax*, in the Pleasant River Watershed

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Rainbow smelt, *Osmerus mordax*, is an economically important fish for the Downeast fishing industry as well as being a major food source for many other species in the area, including *Salmo salar*. We will use microsatellites and other genetic markers to determine if population structure exists in the Pleasant River watershed. We will use the tetranucleotide microsatellite primers that were found in existing literature. Samples were collected from four rivers during the 2010

season: Pleasant River (47 individuals), Narraguagus River (54 individuals), Harrington River (30 individuals), and East Machias River (2 individuals). Although microsatellites are polymorphic among our samples, a preliminary survey using a major histocompatibility complex (MHC) marker suggests that our samples are monomorphic. In contrast, a sample of 24 Atlantic salmon from the Pleasant River had four alleles at (we believe) the same locus.

## Improving methods to accurately age rainbow smelt (*Osmerus mordax*)

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### Introduction

Regional collaborations to study the decline of anadromous rainbow smelt (*Osmerus mordax*) have prompted state fisheries managers in Massachusetts, New Hampshire, and Maine to standardize ageing methods. The development of digital imaging using video-mounted dissecting microscopes enables us to archive images, share reference collections, cross-train staff, and compare ages. We compared scales, otoliths, and finrays and refined cleaning methods to develop a standard protocol. We also began to test validation methods that can be easily transferred between states.

Rainbow smelt have large scales that can be easily collected with no mortality. Smelt have a relatively short life span, living up to six years but more commonly to three years. Annuli on smelt scales are visible as bright scars and can be easily differentiated from inter-annual circuli (McKenzie 1958). Previous efforts to age anadromous rainbow smelt and develop mortality estimates have exclusively used scales (Bailey 1964, Ivanova 1982, McKenzie 1958). A study of freshwater smelt found higher ageing precision using finrays compared to otoliths (Walsh *et al.* 2008).

## Protocol methods

We sample anadromous rainbow smelt annually as part of two studies to monitor the species in the Gulf of Maine. In Massachusetts, New Hampshire and Maine, we collect smelt at 18 spawning sites annually. In New Hampshire and Maine, we also collect scale samples 1-3 times a week as part of annual creel surveys of the winter recreational fisheries. All smelt caught are sexed and measured to the nearest millimeter and the total catch recorded. We collect scales from up to 20 fish per sex per centimeter size bin (10cm, 11cm, 12cm, etc.) over the course of the season. Because the age at length may change over the run, we typically collect 5 scale samples per sex per size bin per week. Scales are taken from the side of the fish midway between the lateral line and the base of the dorsal fin.

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. Although the annuli on smelt scales can sometimes be easily visible through this mucous, it can make false annuli and older ages difficult to distinguish. To remove the mucous membrane, scales are first placed in small brass or stainless steel screen baskets and then immersed in a solution of pancreatin and agitated. Whaley (1991) found cleaning scales in 5.0% pancreatin solution using a high-frequency sonicator for 15 minutes yielded the highest percentage of usable scales compared to agitation in water alone for 15 minutes or in 5.0% pancreatin solution for 48 minutes. We have found that 2.0% pancreatin solution adequately cleans smelt scales. A higher pancreatin concentration removes too much of the surface and causes scales to curl under cover slips. A mounting medium can keep the scales flush, but because we mount a large number of scales, we prefer the lowest cost and most

efficient method. Simply reducing the concentration of pancreatin negates the need for a mounting medium. The cleaned scales are then placed on individually labeled microscope slides with a drop of water and covered with a thin glass slip.

Otoliths and fin rays are collected from a subset of fish from which scales were taken during the spawning run surveys. These are embedded in a two part epoxy. Transverse sections encompassing the core of the feature are cut on a low speed saw equipped with two diamond blades separated with a 0.4 mm spacer. These sections are affixed to slides using Flo-texx mounting medium.

Scales, otoliths, and fin rays are viewed using the image analysis program Image Pro (V6.2) which drives a digital video camera mounted atop a dissecting microscope with transmitted lighting. Image Pro software and transmitted lighting improves image quality, which emphasizes true and false annuli. Two individuals with no prior information about the length or sex of the fish age each structure, and there is no communication between readers. If there is a discrepancy, age is assigned by a third more experienced reader or by consensus.

## Results

We compared ageing precision between scales and otoliths. There was no significant difference between ages given by two readers using scales (Chi Square  $p = 0.34 > 0.05$ ), but there was a significant difference using otoliths ( $0.02 < 0.05$ ). We also compared ages given using scales and otoliths from the fish where the reader had no prior knowledge about which scales and otoliths belonged to the same fish; there was a significant difference between ages (2 trials by 2 readers, Chi Square  $p = 0.00001 < 0.05$  and  $p = 0.0001 < 0.05$ ) (Figure 1). Age assignments using otoliths overestimated

especially young ages because smelt otoliths repeatedly have a large number of sub-annual checks, a finding that is consistent with other studies (Walsh *et al.* 2008).

We have not yet collected enough fin rays to make a valid conclusion about ageing precision using this structure. However, we have found that collecting fin rays requires more training and time in the field compared to scale collection.

Age Validation. We are currently refining methods to validate ages given using scales. We use oxytetracycline hydrochloride (OTC) to mark yolk-sac larvae reared in the lab. The OTC leaves a small permanent mark on the otolith. Larvae are released at an annually sampled spawning site. A subset of the catch in following years is taken and each otolith examined for an OTC mark. The scale is then independently aged. Each release year can be differentiated by varying the number of times larvae are exposed to OTC. In 2010 we recaptured 14 OTC marked smelt that had been released as larvae in 2009; all readers independently correctly assigned scales from these fish age 1 with no prior knowledge.

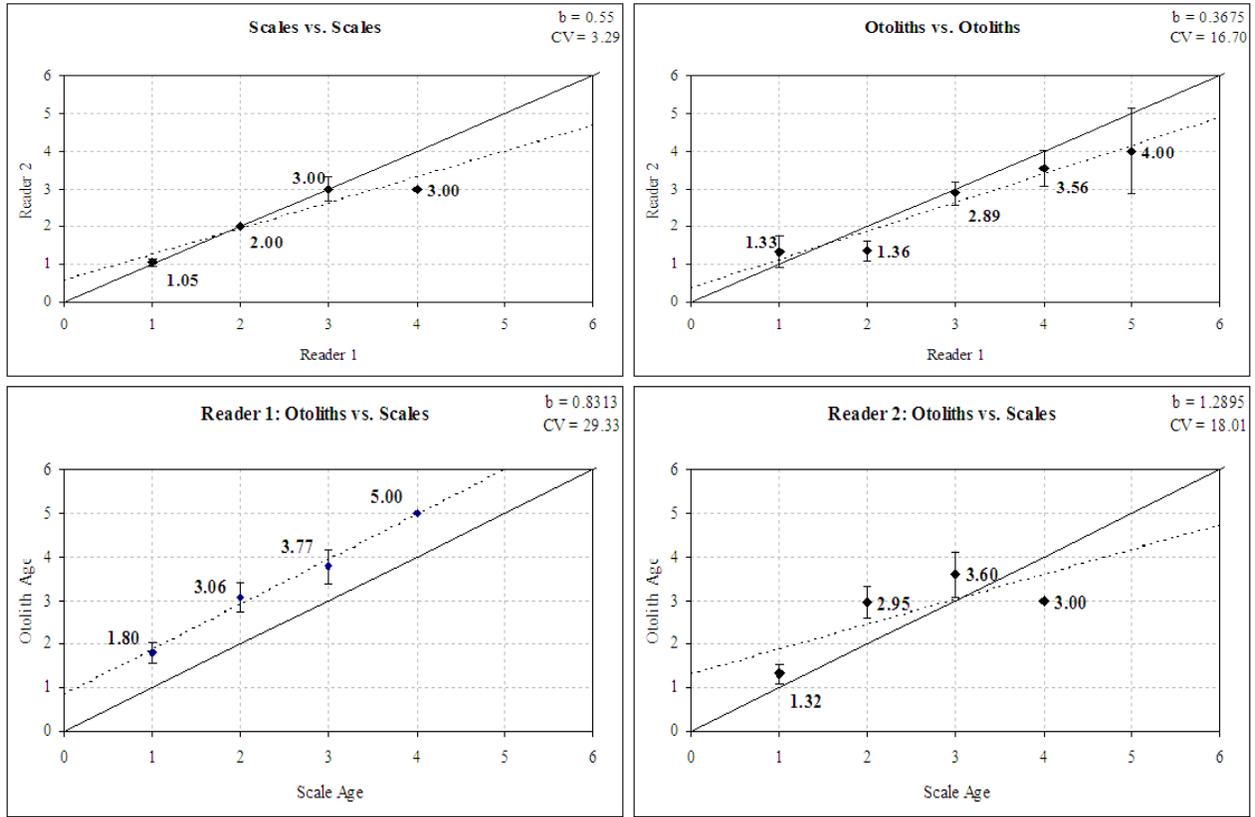
The ability to take high resolution digital images of consistent quality enables us to build reference collections that can be used between multiple agencies. Reference collections of scale images are being developed to determine

accurate age-length keys for the species, as existing keys have been applicable only to small geographic areas or short time periods.

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**Figure 1.** Rainbow smelt age bias plots between readers using scales and otoliths (top panel) and between structures (bottom panel). The solid line in each plot indicates the 1:1 age line. Dashed lines show the mean age (95% CI) estimated on the y-axis for each age on the x-axis. The intercept and CV are given for each comparison.



## **Are common smelt (*Retropinna retropinna*) a sustainable food source for rainbow trout (*Oncorhynchus mykiss*) in Lake Rotoiti, New Zealand?**

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Lake Rotoiti is a warm, monomictic, eutrophic lake in the North Island of New Zealand. It is home to a popular rainbow trout fishery (*Oncorhynchus mykiss*), which is supplemented by hatchery-raised yearlings. Native common smelt (*Retropinna retropinna*) constitute 83% of the diet of rainbow trout over 200 mm long. Despite their importance as a prey species, the life history of smelt in Lake Rotoiti is poorly understood, and the capacity of the smelt population to support increased levels of trout stocking is unknown.

In 2008, the Ohau Channel Diversion Wall was installed to improve water quality in Lake Rotoiti. The effect of this wall is to divert nutrient rich water from Lake Rotorua directly down the Kaituna River, rather than into Lake Rotoiti. It is possible that the diversion wall has had a negative impact on spawning migrations of common smelt between Lake Rotorua and

Rotoiti, which could in turn affect food supply for rainbow trout.

Sampling is currently being carried out in order to assess abundance and dynamics of smelt in Lake Rotoiti. In the past year, littoral catch rates of smelt varied diurnally and seasonally, with highest catches of up to 2,000 smelt in an 800 m<sup>2</sup> electric fishing transect in autumn, which was likely due to a migration of smelt into the littoral zone to spawn. Semi-quantitative sampling of smelt eggs, coupled with monitoring of reproductive maturity of spawning adults, has shown that spawning is most prevalent at the lake's more exposed eastern beaches. In addition, numbers of larvae were highest in this area. This suggests that smelt are reproducing locally, providing a sustainable food source for rainbow trout. This study also indicates the importance of exposed beach habitat for sustaining smelt populations.

## **Diadromous Species Restoration Research Network:**

### **A five-year collaborative research effort**

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The goal of the Diadromous Species Restoration Research Network (DSRRN) is to advance the science of diadromous fish restoration and promote state-of-the-art scientific approaches to multiple-species restoration at the ecosystem level. DSRRN

integrates many diverse activities that improve the understanding of ecosystems and enhance restoration outcomes, facilitates the study of questions fundamental to diadromous fish ecology and restoration through scientific meetings, workshops and local networking, and

enhances coordination of diadromous species restoration efforts of academic, government, and watershed stakeholders. The Network, which is funded through the National Science Foundation, provides information and networking on research and restoration funding opportunities, research, and restoration project partnerships, conferences and meetings, the

Penobscot Science Exchange, fisheries and restoration links, and the Gulf of Maine Knowledge Base which provides access to spatially referenced bibliographic information so that users can locate information using text-based and map-based searches by state/province and by watershed.

## **Penobscot River Science Exchange: A consortium for dam removal and diadromous fish restoration research**

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Covering 8,570 square miles, the Penobscot River is Maine's largest and New England's second largest watershed. Unfortunately, centuries of dam construction have blocked the migration of diadromous fish to their upstream spawning and juvenile-rearing habitats. The Penobscot River Restoration Project will restore nearly 1,000 miles of sea-run fish habitat by removing two large hydroelectric dams in the lower part of the river and providing improved fish passage at a third dam upstream. In 2008, the Penobscot River Restoration Trust and agency and academic researchers began conducting studies and environmental monitoring on the river in order to establish pre-dam removal conditions that will allow managers to document restoration outcomes. This group of approximately 30 researchers

makes up the Penobscot Science Exchange, which is a collaboration with the Diadromous Species Restoration Research Network (DSRRN), a five-year, NSF-funded collaborative research effort to advance the science of diadromous fish restoration.

This poster provides descriptions and photographs of research projects currently being conducted on the Penobscot in conjunction with the dam removals and the Penobscot Science Exchange. Projects include shortnose sturgeon movement and spawning, bird assemblages, sea lamprey movement in tributaries, iron-drainage impacts to water quality, alewife population structure and migration, marine-freshwater food web linkages, sea lamprey and Atlantic salmon interactions, and dam removal impacts on fish assemblages.