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Quantifying within-season repeat spawning by rainbow smelt *Osmerus mordax* with implications for comparing sex ratios and survey catches among runs

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Abstract

Anadromous Rainbow Smelt *Osmerus mordax* provide an important near-shore prey base but have experienced severe population declines during the last century. Population estimates from spawning run surveys have not been calculated because within-season repeat spawning, a behavior qualitatively described for this species based on previous studies, has not been quantified. Our objective was to determine repeat spawning behavior at two spawning sites using Passive Integrated Transponder technology. A preliminary laboratory tag retention and mortality study was performed to determine the effect of tagging on study results, showing that tagging did not cause significant mortality and tags were well retained. In our field studies, continuous monitoring of tagged fish showed that Rainbow Smelt made movements to the spawning grounds against the tide and at low tide to allow the fish to be at the spawning grounds during the darkest nighttime hours, this is in contrast to past assumptions that Rainbow Smelt only spawn during nighttime high tide. We found that repeat spawning behavior was a predominantly male behavior, consistent with past studies. The behavior of males did vary between the two sites, though at each site the behavior was consistent among study years. In contrast, the behavior of females was consistent both among study years and between the sites. This finding indicates that future assessments of Rainbow Smelt spawning populations could be improved using female catch numbers, similar to other species displaying skewed sex ratios.

Keywords: repeat spawning; passive integrated transponders; Rainbow Smelt; habitat use; tagging mortality; forage fish

Introduction

Anadromous Rainbow Smelt *Osmerus mordax* live in nearshore coastal waters and spawn during the spring in freshwater rivers and streams immediately above the head of tide. Highly skewed sex ratios (M : F) have been documented for multiple spawning populations (Marcotte and Tremblay 1948; Murawski et al. 1980; Landsman et al. 2018), though fairly even sex ratios have been reported for the same populations during non-breeding seasons (Murawski et al. 1980; Enterline et al. 2012). Similar to other anadromous species displaying skewed sex ratios during the spawning run (Kissil 2011), the sex ratio for Rainbow Smelt evens out as the spawning run progresses (Enterline et al. 2012). Multiple males have been observed attending a single female during spawning (Langlois 1935; Hoover 1936, Clayton 1976; Lischka and Magnuson 2006), a behavior described for other anadromous and non-anadromous species (Nikolsky 1963; Hutchings et al. 1999; Stoner et al. 1999; Byrne and Avise 2009). Fertilization from several males has been shown to increase fertilization success of Rainbow Smelt eggs (Purchase et al. 2007), likely because milt quality differs among males, and because females broadcast spawn in sections of streams with relatively high velocities (Hulbert 1974; Burness et al. 2004) where milt can be dispersed quickly. Previous tag and recapture studies have documented individual male Rainbow Smelt on the same spawning grounds multiple times within a season (Marcotte and Trembly 1948; Rupp 1968; Murawski et al. 1980), while females are thought to ovulate during one spawning event (Marcotte and Tremblay 1948).

Behaviors that influence survey catch data (e.g. skewed sex ratios) must be understood and quantified to accurately model population stocks and manage fisheries (Rowe and Hutchings 2003; Hoenig and Hewitt 2005; Bradshaw et al. 2007; Su et al. 2012). While repeat spawning behavior in Rainbow Smelt has been described, it has not been quantified. Differences in the number of repeat spawning events by sex and age could bias mortality and population estimates. Quantifying the number of repeat spawning events would allow catch numbers and frequency at age distributions to be corrected if necessary and allow accurate morality and population estimates to be calculated (Rooker et al. 2014; Zemeckis et at. 2014; Ward et al. 2019).

This study quantified Rainbow Smelt repeat spawning behavior at two sites in the U.S. Gulf of Maine, Mill Creek, Freeport, Maine (ME) and the Fore River, Braintree, Massachusetts (MA), using internally placed 23 mm Passive integrated transponders (PIT) tags that were continuously monitored by radio frequency identification (RFID) systems. We (1) observed differences in repeat spawning behavior between male and female Rainbow Smelt; (2) determined whether spawning frequency is age dependent; and (3) assessed diel and tidal movement patterns to the spawning grounds. We predicted that repeat spawning behavior was a predominately male behavior based on previous studies. Based on recent state-run spawning survey catches, we also predicted that younger fish made more repeat spawning trips compared with older fish. While some studies have found little mortality and tag loss using internal PIT tags in small fish (Bryondocx et al. 2002), others have found that mortality differed among species and body shape (Brewer et al. 2016); therefore, species specific mortality and retention studies should be performed before relying on tag return data in wild populations. We performed parallel laboratory tag retention and mortality studies to determine whether mortality due to tagging impacted the conclusions of the field study.

Methods

Study Area

Movement of Rainbow Smelt was assessed at spawning areas in Mill Creek, Freeport, ME (Figure 1) in 2010 and 2011, and the Fore River, Braintree, MA (Figure 2) in 2011 and 2012. Mill Creek is located at the head of tide of the Harraseeket River in Freeport, ME and drains into Casco Bay. Historically and currently, this site supports annual spawning comparable with the strongest spawning runs in the state. This unaltered stream flows over a gravel bed at the spawning grounds and opens downstream to an unrestricted salt marsh. The average water velocity at the spawning grounds during the spawning season is 0.468 m/s (Enterline et al. 2012). The surrounding watershed (20.7 km^2) remains relatively undeveloped (3.0% impervious surface cover; 59 people/km^2) and is primarily forested (75.3%), as are most other watersheds supporting smelt spawning in the state (state means = 4.1% impervious surface cover; 67.6% forest cover; 65.7 people/km^2). Although the Mill Creek spawning grounds are located in land protected for wildlife, the spawning run supported a recreational smelt dip-net fishery that was not closed until 2014, and thus some of the study fish may have been subject to harvest mortality.

The Fore River is a coastal river and estuary area with its mouth at Boston Harbor in Quincy, MA. The river has traditionally supported a strong Rainbow Smelt spawning run, with the spawning grounds located directly above the head-of-tide in Braintree, Massachusetts (Figure 2). The river continues to support one of the largest documented spawning runs in the state (Chase and Childs 2001; Chase 2006). Average spring velocity is 0.623 m/s, and average spring discharge is 1.92 m^3/s (Enterline et al. 2012). The surrounding watershed (74.7 km^2) is primarily developed land (47.3% of watershed area) although forested area is the secondary land cover (28.1%). The watershed is densely populated (831 person/km^2), with a high percentage of impervious surface coverage (27.4%).

Fish Collection

Rainbow Smelt were collected as part of a regionally standardized spawning assessment conducted by the MA Division of Marine Fisheries (DMF) and ME Department of Marine Resources (DMR). Fish were collected using fyke nets placed immediately downstream of the spawning grounds (fyke nets: mouth 1.2 x 1.2 m box; 3.4 m approximate length from mouth to cod end; three chambers; first throat tapered to 12.7 cm; second throat tapered to 7.6 cm; 0.8 m diameter hoops; wings 1.2 x 1.2 m box on each side; 1.9 cm mesh size; no leader used). Fyke nets were set at mid-channel in the intertidal zone below the downstream limit of smelt egg deposition. Nets were deployed three days/nights each week, hauled on each of the three days during low tide after overnight sets. The fyke net opening faced downstream to intercept the upstream spawning movements of smelt that occur at night. Fyke net catches were assumed to be representative of the size and sex composition of the spawning run.

All non-target species were counted, a subset (30) measured (total length (TL) mm), and released. All Rainbow Smelt caught as part of the fyke net survey were counted, sexed, and a subset (100 males and 100 females) were measured to the nearest millimeter (TL). Sex was determined by the presence (males) or absence (females) of external nuptial tubercles (Warfel et al. 1943) or by the expression of milt with slight ventral massage if tubercles were not obvious. The latter occurred most often for late-run age-1 males.

Fish Tagging

Fish were tagged internally using 23 mm PIT tags (23 x 3.8 mm, 0.6 g air weight, OregonRFID, Portland, OR). PIT tags were sterilized with commercially available 99% isopropyl rubbing alcohol and gently inserted into a small incision (<1 cm) made with a hooked scalpel in the peritoneal cavity directly posterior to the pectoral fins. A small amount of antibacterial ointment (Neosporin®) was used to cover the wound. No sutures were made to close the incision to minimize handling time. Each fish receiving a PIT tag was also tagged with a VIE mark (Northwest Marine Technology, Inc., WA) in the operculum for visual identification. An anesthetic was not used during the lab trial or field tagging because of the possibility of human consumption of the fish within a short period following tagging at the Mill Creek, ME site.

Recreational fishing was allowed at this site, and therefore regulations for human consumption did not allow the use of anesthetic. The overall likelihood for the tagged fish to be caught, however, was low – a separate study at the fyke net survey sites estimated that the proportion of tagged fish was less than 1% of the nightly catch at each run (unpublished data from S. Turner, B. Chase, and M. Ayer, MA DMF).

Tag Retention and Laboratory Mortality Due to Tagging

Mortality and retention was assessed at the MA DMF Annisquam River Marine Fisheries Field Station, March 22 – May 24, 2012. Rainbow Smelt were collected from the Fore River, MA as part of the annual fyke net survey as described above. Fish were placed in holding tanks with tap water that was previously de-chlorinated by heating the water to steaming point so the chlorine gas evaporated, and brought to 20 PSU using Instant Ocean Synthetic Sea Salt. Water temperature was held constant at 15°C by an in-line chiller. Fish were held overnight and a water change was performed prior to tagging to eliminate the effect of mortality due to capture stress. Tagged fish were selected according to sex and length (TL; approximately 30 from each category: M ≤ 14.9 cm, F ≤ 14.9 cm, M 15-20.9 cm, F 15-20.9 cm, M ≥ 21 cm, F ≥ 21 cm). Sex was determined as described above. Control fish were measured and sex identified, and then placed into the same tanks as tagged fish. Daily mortalities were removed and sex, length, and PIT tag number recorded. The holding tanks were monitored daily for any expelled tags.

We assessed differences in mortality over time between tagged and control fish using non-parametric Kaplan-Meier survival curves followed by a log-rank test to compare the hypothesis that the two mortality curves did not differ throughout the time period. A generalized linear model (GLM) with a binomial distribution (logistic regressions) was fit to the data to determine the effect of length (as an approximation of age; <14.9 cm as age-1, 15-20.9 cm as age-2, >21 cm as age-3 and older) and sex on mortality. For the GLM, mortality was classified as a yes/no response value.

Repeat Spawning

Movement by Rainbow Smelt to and from their spawning grounds was assessed directly downstream of the spawning areas at Mill Creek, ME in 2010 and 2011, and the Fore River, MA in 2011 and 2012. Rainbow Smelt were collected as part of the regionally standardized surveys described above. Scale samples were taken from all tagged fish to confirm age. Scales were cleaned and aged as described by O'Malley et al. (2017).

Half duplex (HDX) RFID systems were placed at the head-of-tide for the duration of the spawning season (March – June). Two sets of antennas were placed in the river approximately ten meters apart to obtain information on the directionality of fish movement. Antennas were placed downstream of the fyke net so that any returning tagged fish would trigger the antennas before encountering the net (Figures 1 and 2). Each set of antennas was made of two side-by-side antennas (Mill Creek: approximately 1.5 m high x 4.2 m wide, channel ~8.5 m, Fore River: 1.75 m high x 6.6 m wide, channel = 13.7 m) to span the width of the stream channel. Antennas in the same span were fixed to A-frames anchored in the middle of the creek channel.

Antennas at Mill Creek, ME were powered continuously using two 125 watt solar panels connected to four deep-cycle 12V batteries. Antennas were made of double wrapped 4-gauge welding cable connected to a multiplexer circuit board which combined the input into the reader control module (OregonRFID). At the Fore River, MA, antennas were made of 4-gauge welding cable connected to a 4-reader system (OregonRFID) running on 14V DC power supply plugged into AC power. At the Fore River, MA, each antenna was wrapped in a way to form three loops within each cross-sectional area to minimize the loop area and increase the detection probability, while also wrapping the wire in a reversing manner to counteract high electrical noise at the site (Figure 2). At each site, the antennas' detection range was tested daily using a 23 mm PIT tag placed by hand at multiple points in the field of each antenna. Antennas were tuned as needed to maximize the detection range. The normal detection range for each antenna reached the entire inner portion of the antenna loop and up to 1.5 m on both the upstream and downstream sides of each antenna.

Rainbow Smelt were tagged as described above for the laboratory trials. In each sampling year at each site, no more than 60 fish were tagged each week for ten weeks. Tagging was based on sex and length (TL for all; 10 individuals from each category: M $\leq$ 16.9 cm, F $\leq$ 16.9 cm, M 17-20.9 cm, F 17-20.9 cm, M $\geq$ 21 cm, F $\geq$ 21 cm). While we aimed to tag equal numbers of fish within each of these bins each week, the actual number of fish tagged was also dependent on fyke net catches. Scales were collected to age in the lab following the methods outlined by O'Malley et al. (2017). Each fish receiving a PIT tag also received a VIE mark in the operculum for visual identification upon recapture. All tagged fish were placed in a recovery tank with aerated river water held at stream temperature for at least ten minutes before being released above the capture site.

Hourly tide data covering the entire sampling periods were obtained using WXTide32 (1.6.2, 2007). Tide data for the Fore River at Weymouth, MA was offset +15 minutes on the ebb tide and +20 minutes on the flood tide, and data from the Harraseeket River, South Freeport, ME was offset +30 minutes on the ebb tide and +40 minutes on flood tide consistent with field observations.

The performance of each RFID system was assessed by examining the proportion of tagged smelt that were detected at least one time by one antenna. The effect of the study variables (study site, year, sex, age, date of tagging) on detection probability was determined using a GLM with a binomial distribution (logistic regression). We chose GLM as an analytical method because it can be applied to response variables with nonnormal distributions (Nelder and Wedderburn 1972; Agresti 2002), and because of the ability to define the best underlying distribution of the data without prior transformation (e.g. arcsine) (Warton and Hui 2011). The best fit model was determined based on AICc values, assessment of overdispersion (Warton and Hui 2011), and a comparison of the residual variance on the degrees of freedom (Faraway 2006). Fish that were never detected were excluded from all further analyses.

We also examined the ability of the RFID system to record every movement made by each detected fish. This "efficiency value" was assigned for each detected fish based on the recorded movements through the antenna array compared with the number of movements at each antenna assumed to be missed. For example, if a fish were observed moving upstream (recorded first by a downstream antenna and then an upstream antenna) and after a period of time (typically over an hour) was then observed by only a downstream antenna, an upstream antenna was assumed to be "missed" because it was not likely for a Rainbow Smelt to remain within the 10 m distance between the arrays for a long period of time. The resulting efficiency value for each fish (a percentage) is the number of recorded passes through the antenna array over the number of assumed total passes through the antenna array. We used a beta regression model (Ferrari and Cribari-Neto 2004) to assess the effect of each study variable (site, year, sex, age, date of tagging) against the non-integer efficiency values. The beta regression model was more appropriate than performing a GLM on logit transformed proportions because there was higher variation around the mean but less variation at the lower and upper limits of the distribution, the distribution was asymmetric, and because the data were beta distributed (Ferrari and Cribari-Neto 2004). The best model was chosen based on the R^2 model fit.

Repeat spawn movements were identified by considering the patterns, timing, and frequency of upstream and downstream movements made by each detected fish. Directional movement was determined by the sequence of detections on upstream and downstream antennas. A spawning movement was assigned when a single fish made a movement upstream into the spawning area, followed by a period of no detections for more than one hour, and then made a downstream movement. We then assessed the effect of the study variables (site, year, sex, age, date of tagging) on the number of repeat spawn events of all individual fish. The best fit distribution family for the number of repeat spawn events was determined before performing subsequent analyses (Faraway 2006). Multiple iterations of GLMs using a negative binomial distribution (found to be the best fit distribution) were performed to determine the impact of the study variables on the number of repeat spawn events for both sexes combined and for each sex separately.

Beta regressions and GLMs were performed using age as a continuous variable and also as age blocks: age-1, age-2, and age-3 and older. While individuals up to age-5 were encountered, the sample sizes of age-4 and age-5 fish were very small; model tests using age blocks considered these together with age-3. Date of tagging (DoT) was found to be circular and some model iterations used sin(DoT) and cos(DoT) as transformed model parameters.

Estimated marginal mean repeat spawning values from the best fit full model GLM (that considering both sexes together) were then used to correct sex ratios at each site for the years of this study. Sex ratios were determined as the total number of females and males caught throughout the spawning season as part of the fyke net survey described above.

Statistical analyses were performed using R Studio (Version 3.6.1). For all tests, statistical significance was considered when $p < 0.05$.

Results

Tag Retention and Laboratory Mortality Due to Tagging

A total of 123 Rainbow Smelt were collected and held at the MA DMF laboratory as part of the tagging mortality study; 63 received PIT tags and 60 served as control fish. Of the 63 Rainbow Smelt tagged, all retained their tags during the study period or until the individual died.

At the end of the study a similar percentage of control and tagged fish remained alive; 75.0% (45/60) of control fish, compared with 69.8% (44/63) of tagged fish. Over the nine-week study period the survival probability for both control and tagged fish declined most markedly during the first ten days, was then relatively stable until mid-way into the sixth week (day 48), and finally began to fall again likely because the fish did not feed well in captivity. Mortality among tagged fish was generally higher than control fish, though there was comparable mortality during the first five days and the last ten days of the study and high overlap in confidence intervals throughout the trial (Figure 3). Overall, the mortality curves did not vary significantly from each other throughout the time period (long-rank test on Kaplan-Meier survival curves, $P = 0.39$).

Sex and length did not significantly influence mortality for either control (GLM, binomial distribution, logit link, sex (male) $\beta = 0.14$; length $\beta = 0.08$; $P > 0.05$ for both) or tagged fish (sex (male) $\beta = -1.01$; length $\beta = -0.10$; $P > 0.05$ for both). Among females, 76.5% (26/34) of control fish remained alive at the end of the study; 61.1% (22/36) of the tagged females remained alive. For males, 73.1% (19/26) of the control fish remained alive; 81.5% (22/27) of the tagged males remained alive (Table 1). Considering length as a proxy for age, 83.3% (5/6) of the smallest length (<15 cm) control fish remained alive, while 50.0% (2/4) of the tagged fish remained alive. For the middle length category (15-20 cm), 77.5% (31/40) control fish remained alive, while 70.5% (31/44) tagged fish remained alive. For the largest fish (>20 cm), 64.3% (9/14) of the control fish remained alive compared with 73.3% (11/15) of the tagged fish (Table 2).

RFID System Performance

Over the course of the field study to monitor Rainbow Smelt repeat spawning behavior 841 fish were tagged, of which 723 were detected at least one time. No smelt were observed above the upstream antennas, between the antennas, or in the spawning grounds during low-tide daytime field observations. Water level at these periods was typically less than 6 inches and the entire area was accessible by the field crew. There was some difference in the proportion of detected fish between the sites and sexes. Overall at Mill Creek, ME, 274/334 (82.0%) fish were detected, while a higher proportion were detected at the Fore River, MA site (449/507, 88.6%). Males were more likely to be detected at least once (429/482, 89.0%) compared with females (294/359, 81.9%). The proportion detected was more consistent among study years at the Fore

River, MA site (2011: 218/247, 88.3%; 2012: 231/260, 88.8%) compared with the Mill Creek, ME site (2010: 96/112, 85.7%, 2011: 178/222, 80.2%; Table 3). Overall, the detection proportions were significantly influenced by site and sex, but not by study year, age, or date of tagging (GLM, binomial distribution, logit link; sex (male) β = 0.59, P = 0.004; site (Mill Creek, ME) β = -0.57, P = 0.030; age β = -0.06, P > 0.05; year (2012) β = -0.14, P > 0.05; DoT β = 0.02, P > 0.05; Table 4).

Of the fish that were detected, the ability of the RFID array to record every pass through each antenna was measured for all detected fish. The resulting efficiency values were not significantly influenced by sex (beta regression, male β = -0.17, P > 0.05), study site (Mill Creek, ME β = 0.21, P > 0.05), study year (2012, β = -0.08, P > 0.05), or date of tagging (β = 0.20, P > 0.05, sinDoT β = 0.35, P > 0.05, cosDoT β = 12.62, P > 0.05), however, efficiency values did decline with increasing age (β = -0.27, P = 0.0001) (Table 4; Figure 4).

Repeat Spawning

Of the Rainbow Smelt that made at least one return to the spawning grounds, most did so the night following tagging (M = 78.5%, F = 76.8%). We considered this behavior an artifact of being caught during their initial spawning attempt, held overnight in a net, and released the following day. It was noted during handling and tagging that the majority of fish retained their milt and eggs. This first return, therefore, was not considered a repeat spawning event for further analyses if it occurred the night following tagging.

Males re-visited the spawning grounds more frequently than females. Females were observed returning to the spawning grounds up to four times, while males returned up to nine times within a single spawning season. This pattern was fairly consistent among study years and between the two sites (Table 5). The mean number of repeat spawning events (RSE) was repeatedly and significantly higher for males (RSE = 1.46 $\pm$ 0.09) compared with females (RSE = 0.41 $\pm$ 0.05; GLM, negative binomial distribution, log link; sex β = 1.18, P < 2e-16).

Male and female Rainbow Smelt made more repeat spawning visits at the Fore River, MA in both years (females: 2011 RSE = 0.28 $\pm$ 0.07, 2012 RSE = 0.78 $\pm$ 0.10; males: 2011 RSE = 1.72 $\pm$ 0.18, 2012 RSE = 1.46 $\pm$ 0.14) compared with Mill Creek, ME (females: 2010 RSE = 0.20 $\pm$ 0.12, 2011 RSE = 0.15 $\pm$ 0.05; males: 2010 RSE = 1.51 $\pm$ 0.20, 2011 RSE = 1.11 $\pm$ 0.14; site β = -0.48, P = 1.40e-03). Fish tagged later in the season were less likely to return as many times as those tagged earlier in the season (sinDoT: β = 6.91, P = 6.88e-04; cosDoT: β = 1.21, P = 0.01). Repeat spawning behavior did not vary by study year or age when both sexes were considered (P > 0.05 for both, Table 6).

Modeling the estimated number of returns based on sex, age, state, year, and date of tagging showed a pattern of first increasing and then decreasing returns as the spawning season progressed, with returns at the Fore River, MA consistently higher than at Mill Creek, ME (Figure 5). After accounting for the effect of the differences between sites, years and date of tagging, males were found to have 3.3 times more returns than females (estimated marginal mean response: male corrected RSE = 1.15, female corrected RSE = 0.35; Figure 6a).

Considering each sex separately, repeat spawning declined significantly by age for females (estimated marginal mean age-1 RSE = 0.44, age-2 RSE = 0.24, age-3 and older RSE = 0.18; β = -0.46, P = 0.01). (Figure 6b). While we noted that the mean number of returns for females at the Fore River, MA was higher than at Mill Creek, ME (Table 5), most of the females at both sites returned two or fewer times (93.6% and 98.2%, respectively) and there was high overlap in the error around each mean for all years except 2012. Assessing all variables together found that site (Mill Creek, ME, β = -0.47), year (2012, β = 0.53), and date of tagging (sinDoT β = 6.43, cosDoT β = 0.49) did not significantly effect female repeat spawning (P > 0.05 for all; Table 6).

When considering only male repeat spawning, age did not significantly influence repeat spawning (β = 0.06, P > 0.05). Males tagged later in the run were less likely to repeat spawn (sinDoT β = 5.01, P = 0.02; cosDoT β = 1.84, P = 6.81e-04). Repeat spawning was significantly lower at Mill Creek, ME compared with the Fore River, MA (β = -0.43, P = 6.90e-03), where repeat spawning also differed among study years (β = -0.59, P = 0.02; Tables 5 and 6). After accounting for the effect of year and date of tagging influences, males were 1.5 times more likely to repeat spawn at the Fore River, MA than at Mill Creek, ME (Figure 6c).

Of the fish that were tagged, 10 males (1.0% of all tagged fish) returned in the year after they were tagged. The number of repeat spawn movements these fish made did not differ between the year of tagging (RSE = 2.3 $\pm$ 0.80) and the following year (RSE = 3.1 $\pm$ 0.66; two-tailed t-test: df = 9, P > 0.05).

Sex ratios for all Rainbow Smelt caught as part of the regional fyke net surveys at the Fore River, MA and Mill Creek, ME during the study years were skewed before and after correcting for repeat spawning behavior. Correcting for repeat spawning using the estimated marginal mean repeat spawning rates determined by the full model (Figure 6a) reduced the sex ratios at Mill Creek, ME from 3.70:1 (M : F) to 2.32:1 in 2010, and from 6.43:1 to 4.04:1 in 2011. At the Fore River, MA, correcting for repeat spawning reduced the sex ratios from 4.64:1 to 2.91:1 in 2011, and from 2.07:1 to 1.31:1 in 2012.

Diel and Tidal Influences

Continuous data collection made it possible to assess how spawning movements correlated to diel and tidal cycles. All spawning movements were made during nighttime hours, where most upstream movements occurred from 1800 hours to 2100 hours, and downstream movements from 0300 hours to 0600 hours. Daytime movements were observed only eight times and were characterized by continuous detections on or near the daytime high tide time. These were not considered spawning movements. While most spawning movements were made with the incoming tide (70.9%, 378/533), active nighttime upstream movements also occurred during the ebb tide. At the Fore River, MA in 2012, 35.0% (91/260) of upstream movements made to the spawning grounds during nighttime hours were made against the tide or when the tide was low. At Mill Creek, ME this pattern was also evident in both study years, where 31.9% (23/72) of movements in 2010 and 20.8% (26/125) of movements in 2011 were made against the outgoing tide (Table 7).

Discussion

This was the first study to compare repeat spawning rates by Rainbow Smelt among sites and during more than one spawning season. Our results were comparable with past studies finding that a higher proportion of males displayed this behavior compared with females, and moreover did so to a greater extent (Langlois 1935; Hoover 1936; McKenzie 1964; Clayton 1976; Murawski et al. 1980). For example, freshwater Rainbow Smelt in Branch Lake, ME participated in spawning up to eight times, while females returned three to four times (Rupp 1968). We found a similar pattern: anadromous male Rainbow Smelt were observed to return up to nine times in the Fore River, MA, and seven times in Mill Creek, ME, while females returned up to four times at both sites.

Understanding what drives catch composition is key to correctly interpreting data from surveys performed during the spawning run (Rooker et al. 2014, Zemeckis et at. 2014). Regional Rainbow Smelt surveys in the Gulf of Maine performed 2008 – 2011 found that sex ratios during the spawning season were biased toward males at all sites, but with high variation from 1.5:1 (M : F) to 9.5:1 (Enterline et al. 2012). It had been assumed that skewed sex ratios were in part attributable to higher rates of repeat spawning by males compared with females (Langlois 1935; Hoover 1936; McKenzie 1964; Rupp 1968; Clayton 1976; Murawski et al. 1980), however, it was unknown whether this was due solely to behavior or whether underlying spawning populations may be female limited. While non-spawning populations of Rainbow Smelt may in fact have even sex ratios (Murawski et al. 1980; Enterline et al. 2012), spawning populations may be female limited because of later age-at-maturity for females (Chase et al. 2019) leading to less participation by age-one females in the spawning runs compared with males (Enterline et al. 2012). Populations dominated by males may be less robust than those containing a higher proportion of females because the limiting factor for population growth is often the abundance of females (Marsden and Robillard 2004).

In the current study, correcting sex ratios by repeat spawn rates did not reduce the skewed sex ratios to 1:1. A pattern emerged at each site where a lower repeat spawning rate by males was accompanied by a higher sex ratio (both uncorrected and corrected ratios). Though this pattern was not evident for females, the repeat spawn rates did not vary significantly for females among sites as they did for males. This pattern may be reflective of male saturation of the spawning grounds. Like many other fish species, the beginning of the spawning run is dominated not only by males but by older fish of both sexes (e.g. Noltie and Keenleyside 1987; Eckmann 1991; Morgan and Trippel 1996; Windle and Rose 2006). Among broadcast spawning species, the early spawning run is characterized by multiple males attending to older and larger females with the highest fecundity; a behavior shown to increase fertility success (Bekkevold et al. 2002; Purchase et al. 2007). It may be that repeat spawning by male Rainbow Smelt is in fact higher when there are fewer males and competition for females is reduced.

We found repeat spawning behavior to be more consistent among female Rainbow Smelt, while the behavior varied for males among sites and study years, suggesting that spawning population comparisons among runs may be more accurate using only female catch numbers after correcting decreasing repeat spawning with age. Population estimates can be inaccurate when species exhibit sexually dimorphic behavior that affects catchability (Fraser et al. 2003, Wilderbuer and Turnock 2008), leading some to derive estimates from only the sex limited portion of the population (Saunders and McFarlane 1993; Au and Smith 1997; Marshall et al. 2011). For Rainbow Smelt, spawning populations have been compared using male and female combined catch-per-unit-effort (CPUE) values uncorrected by repeat spawning behavior (Enterline et al. 2012). Correcting these CPUE values using only female catch numbers corrected by repeat spawning rates by age may provide a better foundation for comparing the size of spawning populations in the Gulf of Maine if sample sizes are large enough. However, it is worth noting that while female repeat spawn rates among the two sites were not significantly different, rates were higher at one site. Further, the rate of returns by females at other sites is unknown. While is it entirely possible repeat spawning behavior by females has a negligible effect on catch numbers because of the low return rates for this sex, it is also possible that using inappropriate rates from the sites in this study could have a significant effect on re-calculated catch numbers at a site where female return rates are different because female catch numbers can be very small. For example, less than 23 female Rainbow Smelt were caught in the North River, MA from 2008 – 2011 (Enterline et al. 2012).

Although monitoring Rainbow Smelt movement with PIT tags and RFID systems provided beneficial and repeatable results, the method was not without its limitations. Half-duplex rather than full-duplex RFID systems were used in this study because of the former's ability to collect data in tidal waters subject to changes in salinity, flow, and antenna shape. While half-duplex RFID systems had high detection rates when tuned properly, they were limited by their read rate (14 scans per second). If large numbers of tagged fish swam through an antenna nearly simultaneously, a likely occurrence for schooling Rainbow Smelt, the system would not have detected all movements (Schmidt et al. 2016). Detection and efficiency rates for the two studies indicated that our systems had a relatively high performance rate at over 80% for all study years (A. Haro, USGS Conte Anadromous Fish Branch, pers. comm).

While lab mortality studies found no differences among control and tagged fish consistent with studies on similar smelt species (Wilder et al. 2016), mortality was higher for females (38.9%) compared to males (18.5%). Though the difference was not significant, it may still have biological relevance in the field study where females were more likely to never be detected. Although we may have seen less mortality using smaller PIT tags, they would not have been appropriate for use in large-scale RFID systems with the antenna dimensions described in this study because the power distribution of the RFID systems would have been too dispersed to detect tags smaller than 23 mm (A. Haro, USGS Conte Anadromous Fish Branch, pers. comm). It is possible, therefore, that we underestimated female returns because of higher initial mortality; however, because the RFID system recorded the movement patterns (efficiency values) of the detected fish at equal rates for males and females, we do not believe that return rates assigned to detected fish were underestimated or display sex bias. Lower detection proportions for females may also be due to the unlikelihood of females to repeat spawn. After the first attempt at spawning and the tagging event, some females may have simply left the system without detection if they moved with a school of tagged fish through the RFID system. Return rates , specifically for females, could also be underestimated for age-2 and age-3 and older based on lower RFID efficiency values with increasing age during the field studies. While age did not seem to be related to trends in male repeat spawning, return trips did decrease with age among females.

Our estimates of repeat spawning rates may also have underestimated the true behavior because any individual fish may have made multiple visits to the spawning grounds before it was tagged. While tagging was stratified by sex and length weekly, striving for an equal number of males and females from each age group, the actual number of fish tagged in each category varied based on fyke net catches and was biased towards males at the beginning of the run. For males, the number of returns did decline with the date of tagging, while it did not for females, indicating that we likely underestimated the male repeat spawning rate. This can also be examined through the behavior of fish that returned in the year following their initial tagging. During this second detection year, the complete spawning behavior was detected without interruption. Though the sample size of the second year returning smelt was very small (10), the number of return trips made by these fish during their tagging year and the following year was consistent.

Results from this study supported the assumed pattern of nighttime spawning (Rupp 1959, McKenzie 1964; Murawski et al. 1980; Lawton et al. 1990) as all movements made to the spawning grounds occurred during dark hours, or in the hours approaching darkness. Daytime observations were consistent with either movements of Rainbow Smelt descending from the spawning grounds after spending nighttime hours on the grounds, or slow passive drifting during daytime high tides. The latter case was observed only eight times, and was differentiated from active swimming movements by the detection patterns. Nighttime spawning events did not always coincide with nighttime high tide, in contrast to previous studies (Clayton 1976; Murawski et al. 1980). The pattern was evidenced at both the Fore River, MA and Mill Creek, ME where 20.8% to 35.0% of upstream movements made to the spawning grounds during dark hours were made against the tide or when the tide was low.

This result may have implications when considering stream connectivity and access to spawning grounds. It has been assumed by some infrastructure managers and permitting agencies that culverts do not present a passage problem if passage is available during at least a portion of the tide. Based on our results, this assumption is unfounded and culverts perched during lower tides may be limiting Rainbow Smelt passage to the spawning grounds. In Maine, where over 75% tidal crossing have been surveyed, it is estimated that 60-80% of these crossings may present passage problems for Rainbow Smelt (based on unpublished data from E. Bartow-Gillies and S. Moore, Maine Coastal Program, and A. Abbott, USFWS). Similarly, a survey of road-stream crossings in Southern Labrabor estimated that 53% crossing presented passage problems for Rainbow Smelt and other anadromous species (Gibson et al. 2011). Given that many Rainbow Smelt spawning sites are small coastal streams that are crossed by at least one road, the impact of reduced passage may be a major factor in population declines and deserves further study.

The ability to effectively manage declining Rainbow Smelt populations is dependent on correctly characterizing spawning population dynamics and factors influencing spawning success (Langton et al. 1996; Lawson and Rose 2000; DFO 2007; Bigford 2013). Our results indicate that skewed sex ratios during Rainbow Smelt spawning runs are not fully explained by males' higher propensity to repeat spawn. The data presented here may be useful to managers when considering sex ratio and catch comparisons among spawning populations. While comparing male catch numbers is more feasible because of larger sample sizes, this should be considered carefully as repeat spawning by males may differ among years and runs. Using the estimated repeat spawn rates determined by this study may under-represent the behavior at some sites, especially if rates are higher for smaller populations. More consistent behavior among females may provide managers the ability to use this portion of the population to derive spawning population assessments. Finally, we found that the use of PIT and RFID technology provided meaningful information about Rainbow Smelt behavior at discrete locations. Our findings about movement timing underscore the need to protect and improve passage to spawning grounds as part of this species management.

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References

Agresti, A. 2002. Categorical data analysis, 2nd edition. Wiley Inter- science, Hoboken, New Jersey.

Anscombe, F. J. 1948. The transformation of Poisson, bionomial, and negative binomial data. Biometrika 35: 246-254.

Au, D. W., and S. E. Smith. 1997. A demographic method with population density compensation for estimating productivity and yield per recruit of the leopard shark (*Triakis semifasciata*). Canadian Journal of Fisheries and Aquatic Sciences 54(2): 415-420.

Ayer, M. H., C. Benton, W. King, J. Kneebone, S. Elzey, M. Toran, K. Grange and D. L. Berlinsky, 2005. Development of practical culture methods for rainbow smelt larvae. North American Journal of Aquaculture 67: 202-209.

Bartlett, M. S. 1947. The use of transformations. Biometrics 3: 39-52.

Bégout Anras M. L., D. Covés, G. Dutto, P. Laffargue, F. Lagardère. 2003. Tagging juvenile seabass and sole with telemetry transmitters: medium-term effects on growth. ICES Journal of Marine Science 60 (6): 1328-1334.

Béguer-Pon, M., J. Benchetrit, M. Castonguay, K. Aarestrup, S. E. Campana, M. J. W. Stokesbury. 2012. Shark Predation on Migrating Adult American Eels (*Anguilla rostrata*) in the Gulf of St. Lawrence. PLoS ONE 7(10): e46830

Bekkevold, D., M. M. Hansen, and V. Loeschcke. 2002. Male reproductive competition in spawning aggregations of cod (*Gadus morhua*, L.). Molecular Ecology 11(1).

Berlinksy. 2005. Development of Practical Culture Methods for Rainbow Smelt Larvae. North American Journal of Aquaculture 67(3): 202-209.

Belyanina, T. N. 1969. Synopsis of biological data on smelt, Osmerus eperlanus (Linnaeus) 1758. FAO Fisheries. Synopsis, Ser. No. 78. 57 pp.

Bigford, T. E. 2013. What Exactly Is Fish Habitat and Why Must We Care? Fisheries Management 38(6): 246

Bigelow, H. B., and W. C. Schroeder. 1953. Fishes of the Gulf of Maine. U.S. Fish and Wildlife Service., Fish Bulletin. No. 74, 53(3).

Bigelow, H. B., and W. C. Schroeder. 1963. Family Osmeridae. Fishes of the western North Atlantic. Mem. Sears Found. Mar. Res. (1) Part 3: 553-597.

Bradshaw, C. J. A., H. F Mollet, and M. G. Meekan. 2007. Inferring population trends for the world's largest fish from mark–recapture estimates of survival. Journal of Animal Ecology 76(3): 480-489.

Brennan N. P., K. M. Leber and B. R. Blackburn. 2007. Use of coded-wire and visible elastomer tags for marine stock enhancement with juvenile red snapper Lutjanus campechanus. Fisheries Research 83: 90-97.

Brewer, M. A., P. J. Rudershausen, B. D. Sterba-Boatwright, J. H. Merrell, and J. A. Buckel. 2016. Survival, Tag Retention, and Growth of Spot and Mummichog following PIT Tag Implantation. North American Journal of Fisheries Management 36:3, 639-651.

Bryondocx L., G. Knaepkens, W. Meefus, L. Bervoets and M. Eens. 2002. The evaluations of passive integrated transponder (PIT) tags and visible implant elastomer (VIE) marks as new marking techniques for the bullhead. Journal of Fish Biology 60: 260-262.

Buckley, J. L. 1989. Species Profiles: Life Histories and Environmental Requirements of Coastal Fishes and Invertebrates (North Atlantic); Rainbow Smelt. US Fish and Wildlife Service Biol. Rep. 82(11.106). US Army Corps of Engineers, TR EL-82-4. 11pp.

Burness, G., Casselman, S. J., Schulte-Hostedde, A. I., Moyes, C. D. & Montgomerie, R. 2004. Sperm swimming speed and energetics vary with sperm competition risk in bluegill (Lepomis macrochirus). Behavioral Ecology and Sociobiology 56: 65–70.

Byrne, R. J., J. C. Avise. Multiple paternity and extra-group fertilizations in a natural population of California grunion (Leuresthes tenuis), a beach-spawning marine fish. Marine Biology 156(8): 1681-1690.

Chase, B. C., and A. R. Childs. 2001. Rainbow smelt (Osmerus mordax) spawning habitat in the Weymouth-Fore River. Massachusetts Division of Marine Fisheries Technical Report TR-5. 34 pp.

Chase, B.C. 2006. Rainbow smelt (Osmerus mordax) spawning habitat on the Gulf of Maine coast of Massachusetts. Massachusetts Division of Marine Fisheries Technical Report TR-30. 173 pp.

Chase, B. C. 2019. Fecundity and reproductive life history of anadromous rainbow smelt (*Osmerus mordax*) in coastal waters of Massachusetts. Fishery Bulletin 117:151–168.

Collette, B. B., and G. Klein-MacPhee, G. 2002. Bigelow and Schroeder's fishes of the Gulf of Maine. Washington and London: Smithsonian Institution Press.

Clayton, G. R. 1976. Reproduction, first year growth, and distribution of anadromous rainbow smelt, Osmerus mordax, in the Parker River and Plum Island Sound Estuary, Massachusetts. M.S. Thesis. University of Massachusetts, Amherst. 102 pp.

Clark, S. R. 2016. Effects of Passive Integrated Transponder Tags on the Physiology and Swimming Performance of a Small-Bodied Stream Fish. Transactions of the American Fisheries Society 145:1179–1192.

Fisheries and Oceans Canada (DFO). 2007. Integrated Smelt Fishery Management Plan, Eastern New Brunswick Area, Gulf Region, 2007-2011. 80pp.

Eckmann, R. 1991. A hydroacoustic study of the pelagic spawning behavior of whitefish (*Coregonus lavaretus*) in Lake Constance. Canadian Journal of Fisheries and Aquatic Sciences 48: 995–1002.

Enterline, C. L., B. C. Chase, J. M. Carloni, and K. E. Mills. 2012. A Regional Conservation Plan For Anadromous Rainbow Smelt in the U.S. Gulf of Maine. Maine Dept. of Marine Resources. 96 pp.

Faraway, J. J. 2006. Extending the linear model with R: generalized linear, mixed effects and nonparametric regression models, volume 124. CRC Press, Boca Raton, Florida.

Ferrari, S. L. P., and F. Cribari-Neto. 2004. Beta Regression for Modelling Rates and Proportions. Journal of Applied Statistics 31(7): 799–815.

Fraser, D. J., C. Lippé, L. Bernatchez. 2003. Consequences of unequal population size, asymmetric gene flow and sex-biased dispersal on population structure in brook charr (*Salvelinus fontinalis*). Molecular Ecology 13(1): 67-80.

Gibson, J., R. L. Haedrich, and C. M. Wernerheim. 2011. Loss of Fish Habitat as a Consequence of Inappropriately Constructed Stream Crossings. Fisheries 30(1): 10-17.

Greenstreet, S. P. R., and R. I. G. Morgan. 1989. The effect of ultrasonic tags on the growth rates of Atlantic salmon, *Salmo salar* L., parr of varying size just prior to smolting. Journal of Fish Biology 35(2): 301-309.

Griffiths, S. P. 2002. Retention of visible implant tags in small rockpool fishes. Marine Ecology Progress Series 236: 307-309.

Haddon, M. 2011. Modelling and Quantitative Methods in Fisheries. Taylor and Francis. 471 p.

Hoenig, J. M., and D. A. Hewitt. 2005. What Can We Learn about Mortality from Sex Ratio Data? A Look at Lumpfish in Newfoundland. Transactions of the American Fisheries Socitey 134: 754-761.

Hoover, E. E. 1936. The Spawning Activities of Fresh Water Smelt, with Special Reference to the Sex Ratio. Copeia 1936(2): 85-91.

Hulbert, P. J. 1974. Factors affecting spawning site selection and hatching success in anadromous rainbow smelt (Osmerus mordax, Mitchill). M.S. Thesis, Univ. Maine, Orono, 44 p.

Hutchings, J. A., T. D. Bishop, C. R. McGregor-Shaw. Spawning behaviour of Atlantic cod, Gadus morhua: evidence of mate competition and mate choice in a broadcast spawner. Canadian Journal of Fisheries and Aquatic Sciences 56(1): 97-104.

Kendall, W. C. 1926. The Smelts. Bulletin of the United States Bureau of Fisheries 42.

Kissil, G. W. 1974. Spawning of the Anadromous Alewife, Alosa pseudoharengus, in Bride Lake, Connecticut. Transactions of the American Fisheries Society 103(2): 312-317.

Kohr, R. L. and P. A. Games. 1974. Robustness of the analysis of variance, the Welch procedure to heterogeneous variances. Journal of Experimental Education 43: 61-69.

Landsman, S. J., N. McLellan, J. Platts, M. R. van den Heuvel. 2018. Nonsalmonid versus Salmonid Passage at Nature-Like and Pool-and-Weir Fishways in Atlantic Canada, with Special Attention to Rainbow Smelt. Transactions of the American Fisheries Society 147(1): 94-110.

Langlois, T. H. 1935. Notes on the spawning habits of the Atlantic smelt. Copeia 3: 141-142.

Langton, R.W., R. S. Steneck , V. Gotceitas , F. Juanes and P. Lawton. 1996. The Interface between Fisheries Research and Habitat Management. North American Journal of Fisheries Management 16(1): 1-7.

Lawton, R., P. Brady, C. Sheehan, S. Correia, and M. Borgatti. 1990. Final Report of Spawning Sea-Run Rainbow Smelt (Osmerus mordax) in the Jones River and Impact Statement of Pilgrim

Station on the Population, 1979-1981. Pilgrim Nuclear Power Station Mar. Environ. Mont. Pro. Rep. Ser. No. 4, Mass. Div. Mar. Fish., Sandwich, MA, 72 pp.

Lawson, G. L., and G. A. Rose. 2000. Small-scale spatial and temporal patterns in spawning of Atlantic cod (Gadus morhua) in coastal newfoundland waters. Canadian Journal of Fisheries and Aquatic Sciences 57(5): 1011-1024.

Lischka, S. A., and J. J. Magnuson. 2006. Timing and site selection of spawning in a landlocked population of rainbow smelt in Wisconsin. Environmental Biology of Fishes 76 (2-4): 413-418.

Marcotte, A., and J. L. Tremblay. 1948. Notes sur la biologie dur l'éperlan (Osmerus mordax Mitchell) de la Province de Québec. Sta. Biol. Saint-Laurent, Que. Contrib. 18: 107 pp.

Mardsen, J. E., and S. R. Robillard. 2004. Decline of Yellow Perch in Southwestern Lake Michigan, 1987–1997. North American Journal of Fisheries Management 24(3): 952-966.

Marshall, C. T., C. L. Needle, A. Thorsen, O. S. Kjesbu, and N. A. Yargina. 2011. Systematic bias in estimates of reproductive potential of an Atlantic cod (*Gadus morhua*) stock: implications for stock recruit theory and management. Canadian Journal of Fisheries and Aquatic Sciences, 2006, 63(5): 980-994.

McKenzie, R. A. 1964. Smelt life history and fishery of the Miramichi River, New Brunswick. Fisheries Research Board of Canada Bulletin 144. 85 pp.

Morgan, M. J. and Trippel, E. A. 1996. Skewed sex ratios in spawning shoals of Atlantic cod (*Gadus morhua*). – ICES Journal of Marine Science, 53: 820–826.

Murawski, S. A., and C. F. Cole. 1978. Population dynamics of anadromous rainbow smelt Osmerus mordax, in a Massachusetts river system. Transactions of the American Fisheries Society 107: 535–542.

Murawski, S. A., G. R. Clayton, R. J. Reed, and C. H. Cole. 1980. Movements of Spawning Rainbow Smelt, Osmerus mordax, in a Massachusetts Estuary. Estuaries 3(4): 308-314.

Nelder, J. A., and R. W. M. Wedderburn. 1972. Generalized linear models. Journal of the Royal Statistical Society Series A 135:370–384.

Nikolsky, G. V. 1963. The ecology of fishes. Academic Press, New York: 352 p.

Noltie, D. B., and M. H. A. Keenleyside. 1987. Breeding ecology, nest characteristics, and nest-site selection of stream- and lake-dwelling rock bass, *Ambloplites rupestris* (Rafinesque). Canadian Journal of Zoology 65: 379–390.

O'Malley. A. J., C. Enterline, J. Zydlewski. 2017. Size and age structure of anadromous and landlocked populations of rainbow smelt. North American Journal of Fisheries Management 37(2): 326-336.

Olsen, E. M. and L. A. Vollestad. 2001. An evaluation of visible implant elastomer for marking Age-0 brown trout. North American Journal of Fisheries Management 21: 967-970.

Purchase, C. F., D. J. Hasselman, and L. K. Weir. 2007. Relationship between fertilization and the number of milt donors in rainbow smelt Osmerus mordax (Mitchell): implications for population growth rates. Journal of Fish Biology 70: 934-946.

Rooker, J. R., H. Arrizabalaga, I. Fraile, D. H. Secor, D. L. Dettman, N. Abid, P. Addis, S. Deguara, F. S. Karakulak, A. Kimoto, O. Sakai, D. Macías, and M. N. Santos. 2014. Crossing the line: migratory and homing behaviors of Atlantic bluefin tuna. Marine Ecology Progress Series 504: 265-276.

Rowe S., and J. A. Hutchings. 2003. Mating systems and the conservation of commercially exploited marine fish. Trends in Ecology and Evolution 18(11): 567-572.

Rupp, R. S. 1959. Variation in the Life History of the American Smelt in Inland Waters of Maine. Transactions of the American Fisheries Society 88: 241-252.

Rupp, R. S. 1968. Life history and ecology of the smelt (Osmerus mordax) in the inland waters of Maine. Final Rep., Fed. Aid. Fish. Proj. F-10-R, Maine Dept. Inland Fish Game. 36 p.

Saunders, M. W., and G. A. McFarlane. 1993. Age and length at maturity of the female spiny dogfish,*Squalus acanthias*, in the Strait of Georgia, British Columbia, Canada. Environmental Biology of Fishes 38(1-3): 49-57.

Schmidt, T., C. Löb, B. Schreiber, and R. Shulz. 2016. A Pitfall with PIT Tags: Reduced Detection Efficiency of Half-Duplex Passive Integrated Transponders in Groups of Marked Fish. North American Journal of Fisheries Management 36:951–957.

Stevens, J. D., R. W. Bradford, and G. J. West. 2010. Satellite tagging of blue sharks (*Prionace glauca*) and other pelagic sharks off eastern Australia: depth behaviour, temperature experience and movements. Marine Biology 157:3 575-591.

Stoner, A.W., A. J. Bejda, J. P. Manderson, B. Phelan, L. L. Stehlik. 1999. Behavior of winter flounder, Pseudopleuronectes americanus, during the reproductive season: laboratory and field observations on spawning, feeding, and locomotion. Fishery Bulletin 97(4): 999-1016.

Su, N., C. Sun, A. E. Punt, S. Yeh, W. Chiang, Y Chang, H. Chang. 2012. Effects of sexual dimorphism on population parameters and exploitation ratios of blue marlin (Makaira nigricans) in the northwest Pacific Ocean. Aquatic Living Resources 26: 19-24.

Tudorache, C., E. Burgerhout, S. Brittijn, G. van den Thillart. 2014. The Effect of Drag and Attachment Site of External Tags on Swimming Eels: Experimental Quantification and Evaluation Tool. PLoS ONE 9(11): e112280.

Ward, E. J., H Delgado-Nordmann, R. E. Brenner, A. H. Beadreau, S. D. Moffitt, A. O. Shelton. 2019. Assessing long-term changes in sex ratios of Pacific herring in Prince William Sound, Alaska. Fisheries Research 211: 300-308.

Warton, D. I., and F. K. C. Hui. 2011. The arcsine is asinine: the analysis of proportions in ecology. Ecology 92(1): 3-10.

Wilder, R.M., J. L. Hassrick, L. F. Grimaldo, M. F. D. Greenwood, S. Acuña, J. M. Burns, D. M. Maniscalco, P. K. Crain, and T. C. Hung. 2016. Feasibility of Passive Integrated Transponder and Acoustic Tagging for Endangered Adult Delta Smelt, North American Journal of Fisheries Management 36:5 1167-1177.

Wilderbuer, T. K., and B. J. Turncok. 2008. Sex-Specific Natural Mortality of Arrowtooth Flounder in Alaska: Implications of a Skewed Sex Ratio on Exploitation and Management. North American Journal of Fisheries Management 29(2): 306-322.

Windle, M. J. S., and G. A. Rose. Do cod form spawning leks? Evidence from a Newfoundland spawning ground. Marine Biology 150(4): 671-680.

Wippelhauser, G. S., G. B. Zydlewski, M. Keiffer, J. Sulikowski, and M. T. Kinnison. 2015. Shortnose Sturgeon in the Gulf of Maine: Use of Spawning Habitat in the Kennebec System and Response to Dam Removal. Transactions of the American Fisheries Society 144:4 742-752.

Winter, J. D. 1983. Underwater biotelemetry. Pages 371–395 in L. A. Nielsen and D. L. Johnson, editors. Fisheries techniques. American Fisheries Society, Bethesda, Maryland.

Zar, J. H. 1999. Biostatistical Analysis: Fourth Edition. Prentice Hall. 663 p.

Zemeckis, D. R., W. S. Hoffman, M. J. Dean, M. P. Armstrong, and S. X. Cadrin. 2014. Spawning site fidelity by Atlantic cod (Gadus morhua) in the Gulf of Maine: implications for population structure and rebuilding. ICES Journal of Marine Science: Journal du Conseil 71(6): 1356-1365.

Tables

Table 1. Proportion of Rainbow Smelt control fish and fish tagged internally with 23mm Passive Integrated Transponders alive at the end of a nine-week mortality study at the Massachusetts Division of Marine Fisheries laboratory. The total percentage of alive/dead is shown for each followed by the numbers of fish in each category are shown in parentheses.

	Control		Tagged	
Sex	Alive	Dead	Alive	Dead
Female	76.5% (26/34)	23.5% (8/34)	61.1% (22/36)	38.9% (14/36)
Male	73.1% (19/26)	26.9% (7/26)	81.5% (22/27)	18.5% (5/27)
Both Sexes	75.0% (45/60)	25.0% (15/60)	69.8% (44/63)	30.2% (19/63)

Table 2. Mortality proportions among length categories for Rainbow Smelt control fish and fish tagged internally with 23mm Passive Integrated Transponders in holding tanks at the Massachusetts Division of Marine Fisheries laboratory. Length categories approximate age breaks reported in Enterline et al. (2012). The numbers of fish in each category are shown in parentheses following each percentage.

	Control		Tagged	
Length Group	Alive	Dead	Alive	Dead
< 15 cm	83.3% (5/6)	16.7% (1/6)	50.0% (2/4)	50.0% (2/4)
15 - 20 cm	77.5% (31/40)	22.5% (9/40)	70.5% (31/44)	29.5% (13/44)
> 20 cm	64.3% (9/14)	35.7% (5/14)	73.3% (11/15)	26.7% (4/15)

Table 3. Percent of tagged Rainbow Smelt that were detected at least one time at each site in each study year, followed by number of fish detected versus number tagged in each category.

	Percent detected at least one time (numbers)

State	Year	Female	Male	Both Sexes
Mill Creek, ME	Both Years	78.1% (114/146)	85.1% (160/188)	82.0% (274/334)
	2010	85.4% (35/41)	85.9% (61/71)	85.7% (96/112)
	2011	75.2% (79/105)	84.6% (99/117)	80.2% (178/222)
Fore River, MA	Both Years	84.5% (180/213)	91.5% (269/294)	88.6% (449/507)
	2011	83.9% (78/93)	90.9% (140/154)	88.3% (218/247)
	2012	85.0% (102/260)	92.1% (129/140)	88.8% (231/260)
All tagged fish		81.9% (294/359)	89.0% (429/482)	-

Table 4. Model results examining the proportion of Rainbow Smelt tagged with 23mm Passive Integrated Responders detected and the efficiency of the RFID readers to record all movements (OR = odds ratio calculated as an exponent of the coefficient β, 95% CI = 95% confidence interval; *$P < 0.05$). Covariates included sex, age, study year, study site, and date of tagging (DoT). Brackets for the categorical variables sex, year, and site (ME = Mill Creek, ME) indicate the reference category.

Response (model)	Variables	β	OR (95% CI)	*P*
Proportion of tagged Rainbow Smelt detected at least one time (binomial GLM with logit link)	Sex (male)	0.59	1.80 (1.21-2.69)	4.00e-3*
	Age	-0.06	0.94 (0.71-1.25)	0.64
	Year (2012)	-0.14	0.87 (0.37-2.05)	0.76
	Site (ME)	-0.57	0.56 (0.33-0.94)	0.03*
	DoT	0.02	1.02 (1.00-1.04)	0.08
RFID recording efficiency (beta regression with logit link)	Sex (male)	-0.17	0.85 (0.70-1.02	0.09
	Site (ME)	0.21	1.23 (0.96-1.59)	0.11
	Age	-0.27	0.76 (0.66-0.87)	1.00e-03*
	Year (2012)	-0.08	0.92 (0.61-1.38)	0.69
	DoT	0.2	1.22 (0.63-2.39)	0.55
	sinDoT	0.35	1.41 (4.80e-04 - 4.18e+03)	0.93
	cosDoT	12.62	3.02e+05 - 2.46e+22)	0.53

Table 5. Mean number and range of within-season spawning movements by Rainbow Smelt at two sites in the U.S. Gulf of Maine as determined by interpretation of Passive Integrated Transponder detections.

Site	Year	Sex	Mean number of returns (±1SE)	Range of repeat spawning movements per fish
Mill Creek, ME	2010	Female	0.20 (±0.12)	0-4
		Male	1.51 (±0.20)	0-7
	2011	Female	0.15 (±0.05)	0-3
		Male	1.11 (±0.14)	0-6
Fore River, MA	2011	Female	0.28 (±0.07)	0-3
		Male	1.72 (±0.18)	0-9
	2012	Female	0.78 (±0.10)	0-4
		Male	1.46 (±0.14)	0-7

Table 6. Repeat spawning analysis assessing the effect of sex, age, study year, study site, and date of tagging (DoT; GLM, negative binomial distribution, log link, OR = odds ratio calculated as an exponent of the coefficient β, 95% CI = 95% confidence interval; $*P < 0.05$). Brackets for the categorical variables sex, year, and site (ME = Mill Creek, ME) indicate the reference category.

Response (model)	Variables	β	OR (95% CI)	P
Repeat spawning by both sexes	Sex (male)	1.18	3.27 (2.56-4.19)	<2e-16*
	Site (ME)	-0.48	0.62 (0.46-0.82)	1.40e-3*
	Age	-0.09	0.91 (0.78-1.06)	0.25
	Year (2012)	-0.30	0.74 (0.47-1.17)	0.19
	sinDoT	6.91	1.00e+03 (1.73e+01-6.10e+04)	6.88e-04*
	cosDoT	1.21	3.36 (1.27-9.01)	0.01*
Repeat spawning	Site (ME)	-0.47	0.62 (0.28-1.32)	0.23
	Age	-0.46	0.63 (0.43-0.90)	0.01*

by females	Year (2012)	0.53	1.70 (0.56-5.53)	0.36
	sinDoT	6.43	6.25e+02(0.09-8.40e+06)	0.15
	cosDoT	0.49	1.63 (0.20-1.19e+01)	0.61
Repeat spawning by males	Site (ME)	-0.43	0.65 (0.47-0.88)	6.80e-03*
	Age	0.06	1.06 (0.89-1.26)	0.52
	Year (2012)	-0.59	0.56 (0.34-0.91)	0.02*
	sinDoT	5.01	1.50e+02(1.83-1.30e+04)	0.02*
	cosDoT	1.84	6.30 (2.17-1.87e+01)	6.81e-04*

Table 7. The percent (and number) of all nighttime Rainbow Smelt movements made to two spawning grounds in the U.S. Gulf of Maine with the tide and against the ebb tide.

	Year	Nighttime movements with the rising tide	Nighttime movements against the ebb tide
Fore River, MA	2012	65.0% (169/260)	35.0 % (91/260)
Mill Creek, ME	2011	68.1% (49/72)	31.9% (23/72)
	2011	79.2% (99/125)	20.8% (26/125)
All movements		70.9% (378/533)	29.1% (155/533)

Figure Captions

Figure 1. Half Duplex (HDX) radio-frequency identification (RFID) systems were placed in Mill Creek, Freeport, Maine. Rainbow Smelt were captured and released upstream of the antenna array. Each set of HDX antennas was made of two side-by-side antennas to span the width of the river channel, fixed to A-frames anchored in the middle of the channel. The channel and stream sides were natural on both sides. Dashed lines represent double-wrapped antenna loops. The antenna loop direction is shown on one side of the cross-section – a mirror image of the looping configuration (not shown) was used on the river left antenna loops.

Figure 2. Half Duplex (HDX) radio-frequency identification (RFID) systems were placed at the head-of-tide of the Fore River, Braintree, Massachusetts. HDX Antennas were placed downstream of the fyke net used to capture Rainbow Smelt. Antennas in the same span were fixed to A-frames anchored in the middle of the river channel. The channel was hardened and

channelized by vertical stone bulkheads on both sides. Dashed lines represent antenna loops that were looped in a pattern to minimize loop size while also cancelling electric noise. The antenna loop direction is shown on one side of the cross-section – a mirror image of the looping configuration (not shown) was used on the river right antenna loops.

Figure 3. Survival probability of control and tagged Rainbow Smelt during a nine-week laboratory study to determine the mortality effect of internally-placed 23mm Passive Integrated Transponders. P-value (log-rank test) indicates that the two mortality curves did not differ throughout the time period.

Figure 4. Mean efficiency values for all Rainbow Smelt tagged with 23mm Passive Integrated Transponders by age. Efficiency values are a measure of the RFID system performance and are calculated for each tagged fish by comparing the number of actual recordings and the number of assumed missed recordings.

Figure 5. The mean ($\pm$ SE) number of Rainbow Smelt repeat spawning returns by the date of tagging. Continuous lines represent modeled number of returns based on date of tagging and site, Mill Creek, ME (abbreviated as ME) and Fore River, MA (MA).

Figure 6. Estimated marginal mean repeat spawning number of returns after accounting for significant effects of other study variables in the best fit generalized linear model for: (a) male and female smelt, after accounting for the effect of site and date of tagging; (b) females by age group; and (c) male smelt by site, after accounting for the effect of year and date of tagging. Error bars indicate $\pm$ 1SE.

Figures

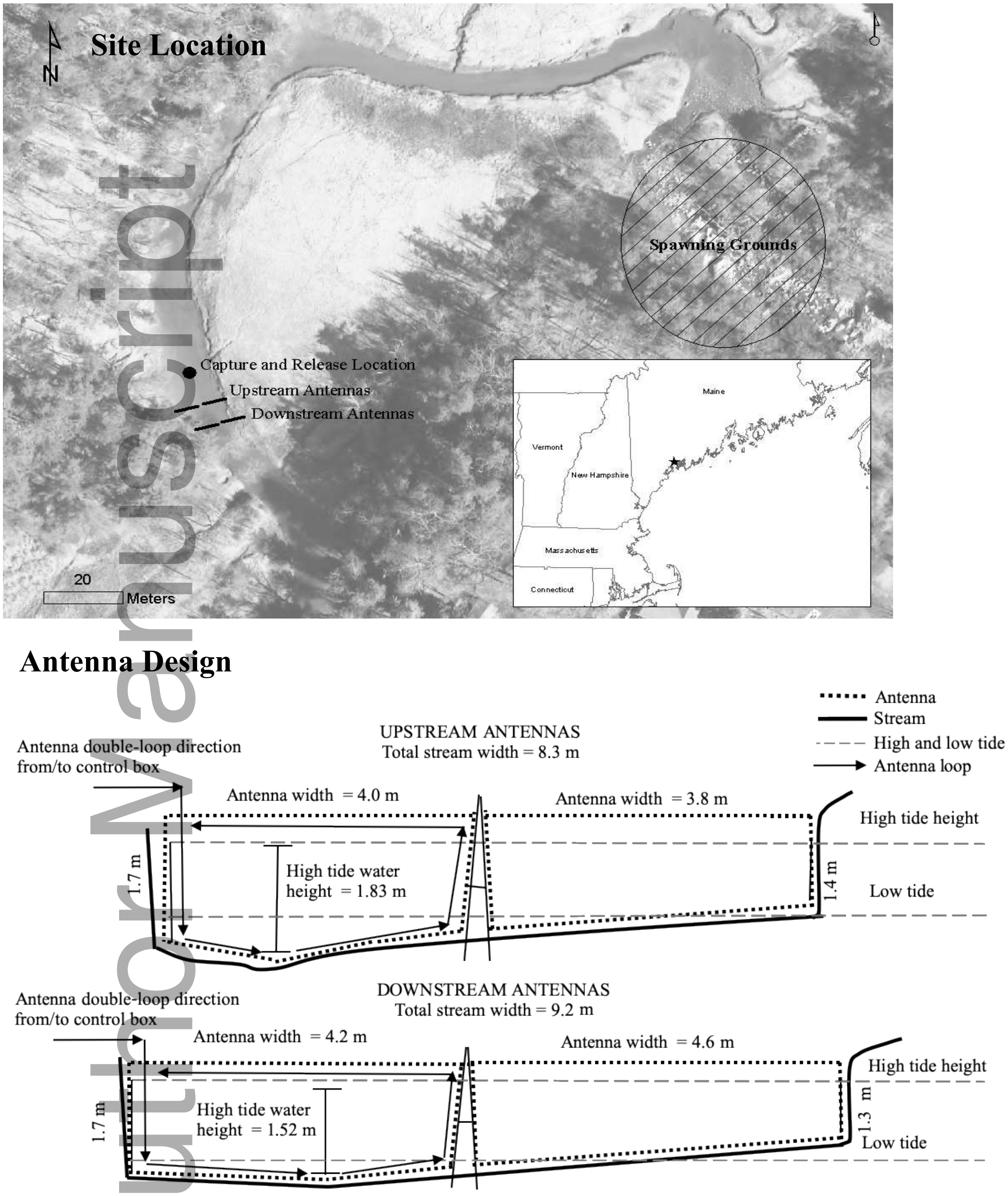
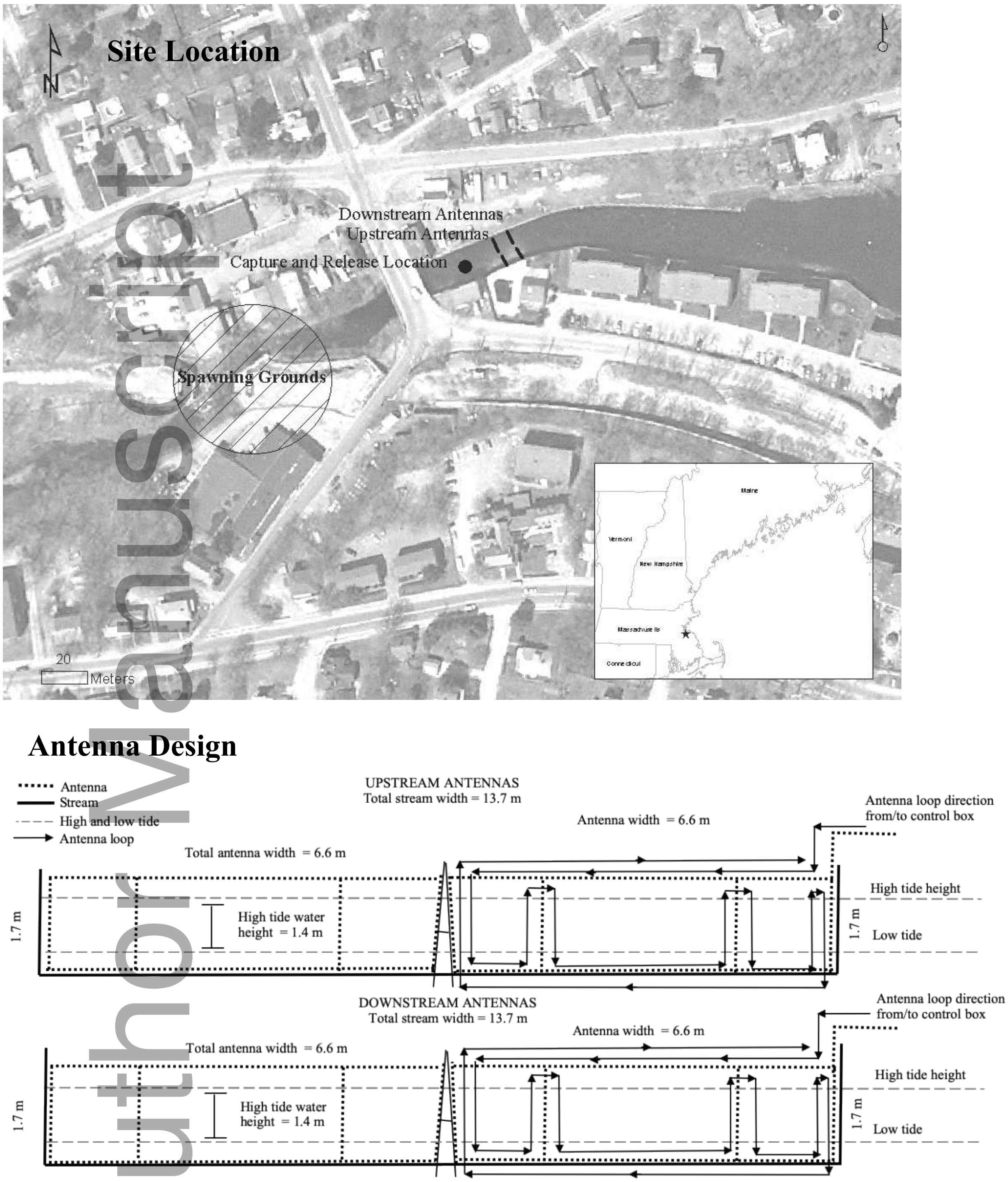


Figure 1. Enterline et al.



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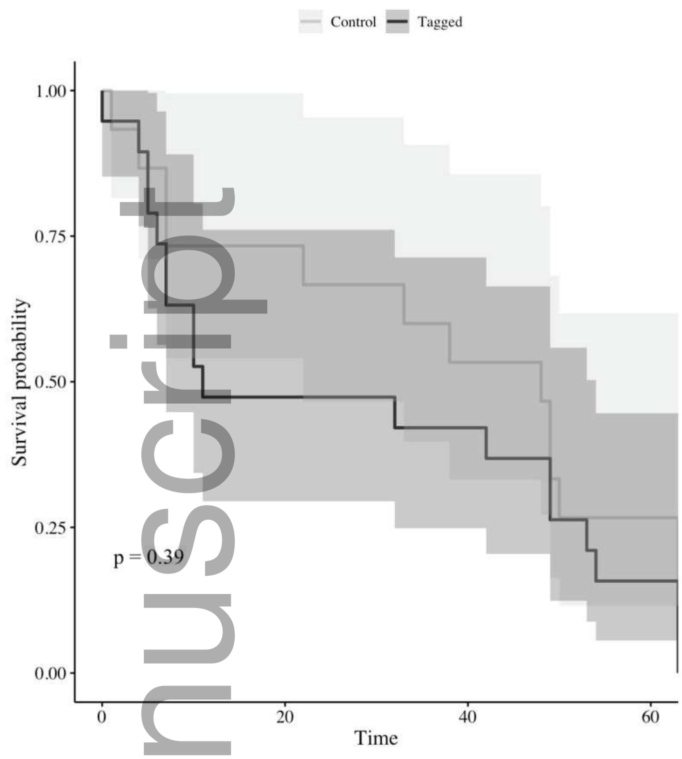

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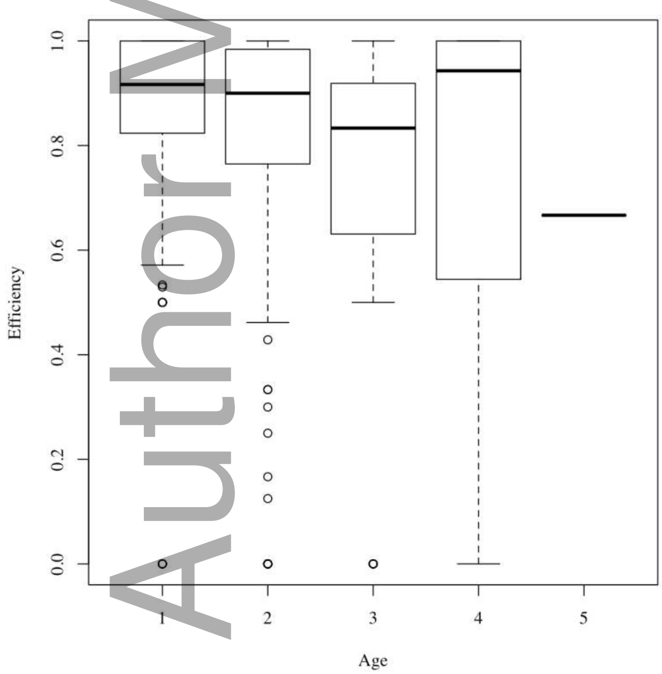

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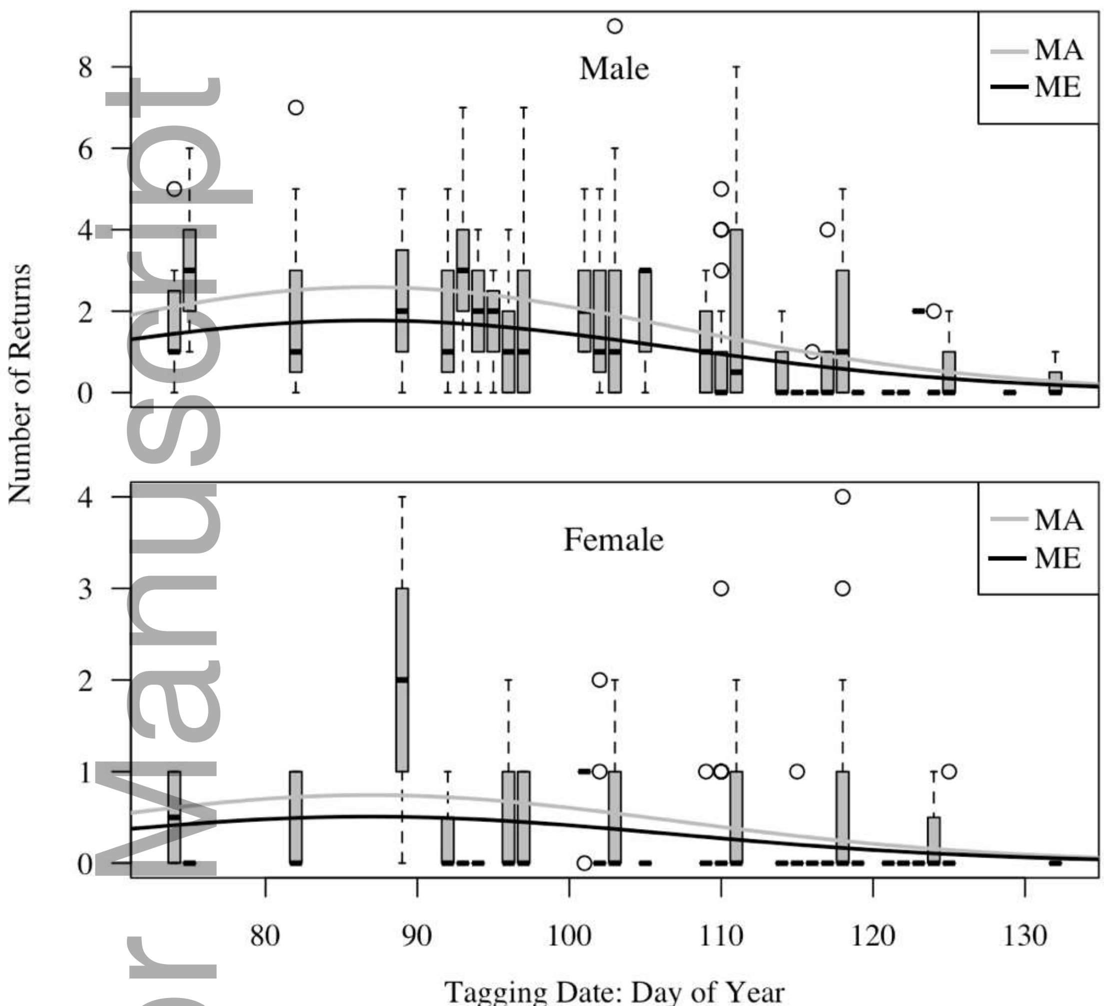

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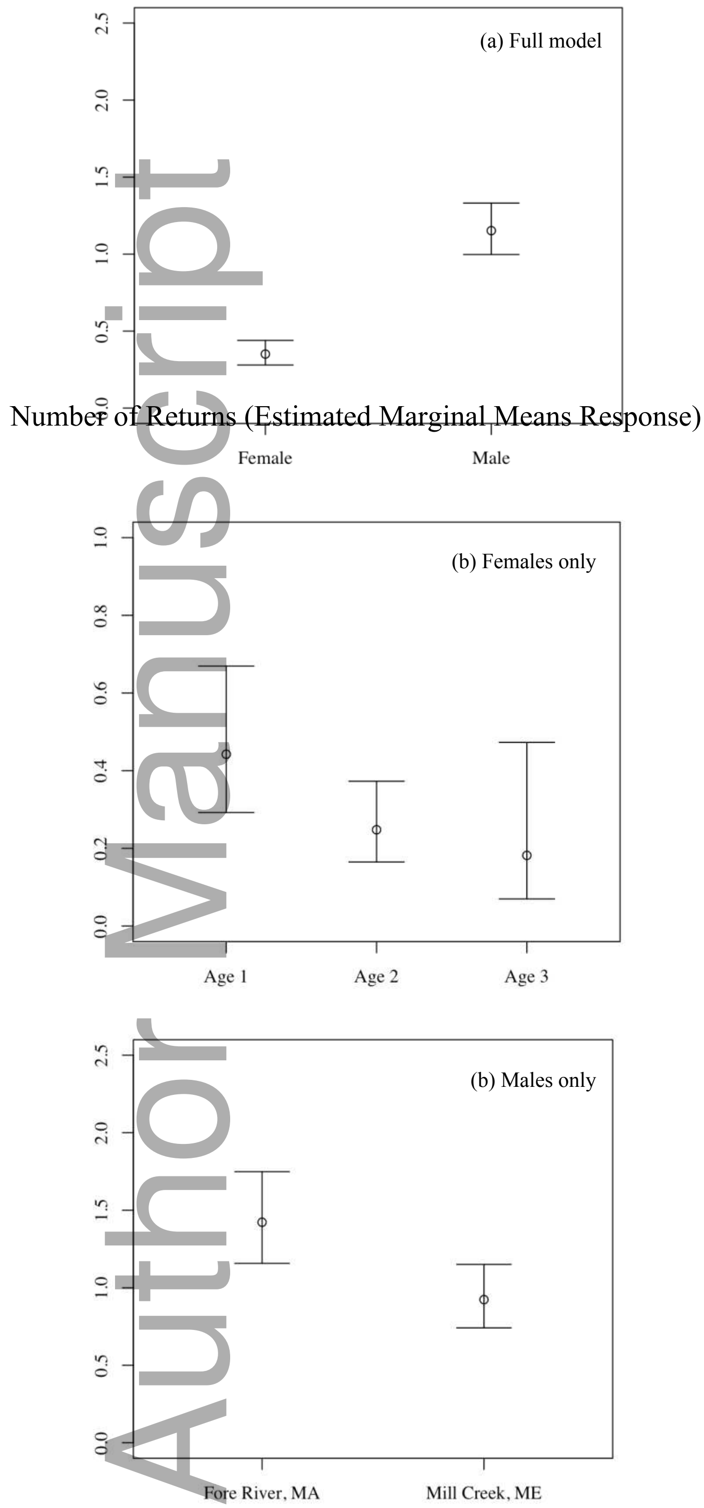

Figure 6. Enterline et al.