Ecological and Phenotypic Factors Influencing the Movement Dynamics of the Blackspotted Topminnow, *Fundulus olivaceus*

Scott Richard Clark

*University of Southern Mississippi*

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ECOLOGICAL AND PHENOTYPIC FACTORS INFLUENCING THE MOVEMENT
DYNAMICS OF THE BLACKSPOTTED TOPMINNOW, Fundulus olivaceus

by

Scott Richard Clark

A Dissertation
Submitted to the Graduate School
and the Department of Biological Sciences
at The University of Southern Mississippi
in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy

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August 2016
ABSTRACT

ECOLOGICAL AND PHENOTYPIC FACTORS INFLUENCING THE MOVEMENT DYNAMICS OF THE BLACKSPOTTED TOPMINNOW, *FUNDULUS OLIVACEUS*

by Scott Richard Clark

August 2016

Movement of individuals throughout a landscape is a complex behavior that has important consequences on numerous ecological and evolutionary processes. Perhaps, most notable is the relationship between movement and reproduction as this has implicit ramifications on individual fitness and consequently, population level processes. Although this relationship has been theoretically and empirically demonstrated across a number of animal taxa, its role in fishes remains understudied and largely unknown. Using a series of outdoor mesocosms, in combination with a field study, I employed passive integrated transponder (PIT) tags to experimentally assess the sex-specific movement dynamics of a small-bodied, sexually-dimorphic stream fish, the blackspotted topminnow (*Fundulus olivaceus*), in response to a suite of local biotic (population density, sex ratio and predators) and abiotic (seasonality and habitat complexity) factors. Movement behaviors were strongly influenced by reproductive timing, with distinct sex-specific movement patterns operating at different temporal scales. More broadly, small-scale (less than 10 meters) movement dynamics of *F. olivaceus* were consistent across mesocosm and field settings, with males displaying increased activity and frequency of movement, patterns that were magnified at lower densities. Concomitantly, I also observed habitat and predator effects at smaller, local scales. Predators were found to restrict movement in the mesocosm study, and movement was reduced in reaches with
increased abundance of local predators in the field study. Simultaneously, however, activity levels were mediated as habitat complexity increased. Consequently, this appeared to promote establishment and defense of territories among males, limiting the longitudinal distance of male movement. In contrast, at larger spatial scales, females tended to engage in longer and more frequent long-distance moves (greater than 30 meters); a pattern that was presumably related to habitat quality and reproductive resource demands. Taken together, these results indicate that local environmental conditions, in combination with phenotypic factors, may function to shape the movement behaviors of stream fishes at various spatiotemporal scales. Moreover, as the magnitude of sex-specific movement responses to local factors appears to be scale dependent, questions that address sex-specific patterns should consider the scale at which movement is quantified in order to capture the nature of sex-biased tendencies.
ACKNOWLEDGMENTS

Although I am acknowledged as the sole author of the cover of this dissertation, so many people contributed and were instrumental to its success and have made my graduate experience at The University of Southern Mississippi one that I will never forget. First and foremost, I will forever be indebted to my advisor, Dr. Jake Schaefer. I certainly could not have asked for a better advisor – his knowledge, patience, unprecedented work ethic, and productivity are truly attributes to marvel. His willingness to not only allow but also encourage me to chase my own ideas and questions, while at the same time offering constructive criticism and guiding me through my mistakes, have been instrumental in not only my growth as a researcher and biologist but also as an individual. Thanks for everything Jake.

I owe a tremendous amount of thanks to the rest of my doctoral committee – the quality of the research and dissertation were greatly improved thanks to all the invaluable support, discussions and constructive insights. Dr. Brian Kreiser was always there to lend an ear and graciously provided thoughtful advice and constant encouragement throughout this process. I am especially grateful for the hundreds of hours we spent in the field together, the good and the bad, that not only contributed to the completion of my dissertation research but the many other projects that would not have ran so smoothly without his help. Dr. Carl Qualls, for enthusiastically jumping aboard and serving on round two of my graduate committees. Carl was always willing to offer advice and sat through many long discussions as I attempted to sort out many of the technical details with my data analysis. Lastly, Drs. Frank Moore and Brett Albanese provided insightful thoughts and critiques that benefitted this project from the beginning stages through the
culmination of this dissertation. Collectively, their broad understanding and experience with animal movement, in both theory and application, prompted stimulating questions and discussions that ultimately aided me to better understand, and consequently communicate, various aspects of my own research.

I need to extend my deepest gratitude to Laura Stewart, as no one person was more influential to the success of my research and this dissertation. Not only for the many hours spent in the field, but for also keeping me sane and always reminding me that I had your ever-present support throughout the last five years. This venture would certainly not have been so enjoyable and rewarding without you by my side. I owe a sincere amount of thanks to Jared Harris for all the help, along with the many distractions, that made many long and grueling days in the field as enjoyable as they could be. I am also especially thankful to all my fellow graduate students and colleagues, past and present, who have helped in the field, provided insights and support, and have contributed to the success of this research. For these reasons, I must unquestionably thank Bjorn Schmidt, Chuck Champagne, Jen Lamb, Tom Sevick, Dr. Nate Franssen, Dr. Paul Mickle, Dr. Wilfredo Matamoros, Jon Barr, Chazz Coleman, and Nkrumah Frazier. I sincerely appreciate the essential contributions all of you have made. I owe a great amount of gratitude to Dr. Mike Davis and the many staff members at The University of Southern Mississippi’s Lake Thoreau Environmental Center. Many hours were spent at this facility completing the research that culminated in this dissertation, and Mike was always amiably responsive to any requests or needs I had along the way. Lastly, funding for portions of this research was provided by the National Science Foundation and the Southwestern Association of Naturalists.
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<td>ANCOVA</td>
<td>Analysis of covariance</td>
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<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
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<tr>
<td>CV</td>
<td>Coefficient of variation</td>
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<td>DOSR</td>
<td>Density and operational sex ratio</td>
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<td>GSI</td>
<td>Gonadosomatic index</td>
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<tr>
<td>HSI</td>
<td>Hepatosomatic index</td>
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<tr>
<td>MANCOVA</td>
<td>Multivariate analysis of covariance</td>
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<tr>
<td>PCA</td>
<td>Principal components analysis</td>
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<td>PIT</td>
<td>Passive integrated transponder</td>
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<tr>
<td>SD</td>
<td>Standard deviation</td>
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<td>SE</td>
<td>Standard error</td>
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<td>SL</td>
<td>Standard length</td>
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<tr>
<td>USGS</td>
<td>United States Geological Survey</td>
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<td>VIE</td>
<td>Visual implanted elastomer</td>
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CHAPTER I - ECOLOGICAL INFLUENCES ON THE LOCAL MOVEMENT

DYNAMICS OF THE BLACKSPOTTED TOPMINNOW, *FUNDULUS OLIVACEUS*


Abstract

Movement of individuals throughout a landscape is fundamental to a wide array of ecological processes; however, the interacting spatiotemporal effects of environmental influences on movement remain poorly understood. Using a series of mesocosm trials, I examined relationships between local abiotic and biotic variables and movement patterns of the blackspotted topminnow, *Fundulus olivaceus*, using passive integrated transponder (PIT) tags. In one series of trials, I assessed the influence of local population factors (density, sex ratio) and phenotypic variables (growth, condition) on movement rates across seasons (spawning, non-spawning). Movement was strongly influenced by different factors seasonally, and movement rates were approximately 7.5 times higher in the spawning season compared to non-spawning periods. Males moved more than females and movement was greater in low density treatments; however, these patterns persisted only during the spawning period. In a second series of trials, I examined abiotic (habitat complexity) and biotic (predator; *Micropterus punctulatus*) influences on the movement dynamics and habitat usage of *F. olivaceus*. Predators were found to suppress movement; however, this response was ameliorated by the presence of habitat structure. Movement rates of *F. olivaceus* were negatively related to predator movement and individuals showed a higher propensity to group in the presence of the predator. The predator induced shifts in habitat usage, as individuals utilized the shallower habitat at a
greater frequency and for longer durations compared to trials without a predator. Taken together, these results suggest local environmental variables may strongly influence spatiotemporal movement behaviors of *F. olivaceus*.

Introduction

Numerous ecological processes including resource acquisition, mate searching, predator avoidance, dispersal and migration, are inextricably linked to movement behaviors (Gurarie & Ovaskainen 2011) that inherently contribute to local and regional ecosystem processes (Nathan et al. 2008). In particular, studies devoted to the interaction between individuals and their environment have highlighted the capacity in which the spatiotemporal variability of local conditions may influence movement strategies (Aparicio & De Sostoa 1999; Albanese et al. 2004; Petty & Grossman 2004; Roberts & Angermeier 2007). These strategies are influenced through trade-offs in the local environment that ultimately impact fitness (Bonte & de la Pena 2009). Pervasive factors such as kin competition (Le Galliard et al. 2003) and inbreeding avoidance (Perrin & Mazalov 1999) may increase mobility; while predation risk and lack of suitable local habitat within fragmented landscapes may function to suppress it (Gandon & Michalakis 2001).

The abiotic and biotic environments of streams vary in both time and space at multiple spatial scales, from individual pools to river drainages (Frissell et al. 1986). Upland tributary reaches tend to be more laterally heterogeneous (i.e. structurally complex) compared to lowland habitats (Schlosser 1995; Alldredge et al. 2011) and increase habitat fidelity of residents (Gorman 1986; Harvey et al. 1999). At the same time, resources (e.g. foraging areas, mates, refugia) may not be localized, requiring
individuals to move in order to obtain these resources or find more suitable patches (Schlosser 1995; Petty & Grossman 2004). However, the relative quality of a particular habitat is species specific and defined by spatiotemporal resource availability, environmental variability, and intensity of biotic interactions (Gowan & Fausch 1996; Ims & Hjermann 2001; Albanese et al. 2004). If local habitat quality correlates with fitness, increased movement is predicted in response to deteriorating environmental conditions or any factor reducing fitness (Croft et al. 2003; Matthysen 2012). Thus, identifying drivers of movement behavior may lend valuable insight into a variety of ecological processes of interest (e.g. population composition, metapopulation dynamics, gene flow).

Movement strategies are thought to respond to local factors such as local population density, sex ratio, resource availability, predation risk, and environmental heterogeneity (Gilliam & Fraser 2001; Croft et al. 2003; Petty & Grossman 2004; Alldredge et al. 2011). However, these factors may exert different spatiotemporal pressures on movement, resulting in variable rates across populations (Gatz & Adams 1994; Kobler et al. 2008). For instance, the relative density and sex ratio within a given habitat will influence inter- and intrasexual encounter rates which may drive differential sex-specific movement patterns (Lawrence 1987). However, the magnitude of these effects may be limited to specific temporal periods (e.g. spawning) as the density and operational sex ratio of conspecifics will contribute to mating dynamics, and in turn, the movement of individuals within a local population. Such patterns may be further influenced by external factors such as resource availability, predation risk, and habitat suitability, that will additionally act as feedback mechanisms influencing the relative
density of conspecifics, and thus interactions, within a given habitat. Thus, movement dynamics of populations are likely influenced by a series of interacting ecological factors operating at different spatial and temporal scales.

While movement of fishes has been well-studied, most studies are observational (e.g. mark-recapture studies), and few studies have experimentally manipulated putative ecological drivers of movement (e.g. Fraser et al. 1995; Gilliam & Fraser 2001; Schaefer 2001). While many ecological factors have been linked to movement, these are largely contingent on the spatiotemporal scale at which movement is quantified (Bowler & Benton 2005). At localized scales, movement has been attributed to resource acquisition and assessing changes in the local environment (Power 1984; Albanese et al. 2004; Gowan 2007; Roberts & Angermeier 2007). The purpose of this study was to assess the influence of local environmental factors on the movement dynamics in an experimental framework using a broadly distributed, tributary specialist, Fundulus olivaceus. Specifically, I ask the following questions: (1) Does the structuring of local populations (i.e. density and sex ratio) influence rates of movement? If so, do different factors influence movement temporally? (2) How do abiotic (habitat complexity) and biotic (predator) variables interact to influence movement? (3) Is there a relationship between movement and fitness surrogates (i.e. growth and body condition)? (4) Are movement patterns similar among sexes? I address these questions using passive integrated transponder (PIT) technology to quantify the movement dynamics of F. olivaceus in a series of mesocosm experiments.
Methods

Fish collection and PIT-tagging

Adult *F. olivaceus* (>50 mm SL; standard length) were collected via dip-netting local tributaries in the Pascagoula River drainage (Mississippi, USA). Fish were subsequently placed into outdoor mesocosm arrays (Matthews et al. 2006) and allowed a two week acclimation period prior to tagging procedures. I implanted 82 fish (mean ± 1 SE; 58.1 ± 0.6 mm SL; 2.37 ± 0.08 g) with 8 mm PIT tags (Biomark, Inc.) and 58 (57.7 ± 0.7 mm SL; 2.35 ± 0.09 g) with 12 mm PIT tags (Oregon RFID). I anesthetized fish using tricane methanesulfonate (MS-222) and injected tags with a syringe-style implanter by puncturing the body wall just off the midventral line, anterior to the vent and implanting the tag in the posterior portion of the peritoneal cavity. All individuals were sexed (*F. olivaceus* is sexually dimorphic; Schaefer et al. 2012), measured to the nearest millimeter (SL), and weighed (grams) prior to tagging. Fish were held in a series of holding tanks (380 L) for a minimum of 10 days post tagging and monitored daily for survival and normal feeding behavior. Fish were fed daily, *ad-lib*, with freeze-dried blood worms. Tag retention (96.5 %) and survivorship (92.3%) was comparable to other studies (e.g. Knaepkens et al. 2007; Dixon & Mesa 2011) and did not differ between tag sizes. Fish from each sex/tag size combination were then housed in separate holding tanks (1500 L; n = 8; 15-20 individuals per holding tank).

Mesocosm setup

Movement trials were conducted in outdoor mesocosms designed to mimic a natural flowing, pool-riffle stream morphology (Matthews et al. 2006). The experimental units consisted of two pools (183 cm in diameter, with mixed sand/gravel substrate)
connected via a riffle (183 cm × 46 cm with gravel/cobble substrate) with a recirculating pump and a continuous inflow of groundwater. Temperature loggers (Onset Computer Corporation) recorded water temperature at 15 min intervals throughout trials. The movement of interest within the experimental unit was across the riffle, as *F. olivaceus* predominantly inhabit the slower-moving margins of stream pool habitats (Braasch & Smith 1965; Blanchard 1996), thus naturally-occurring riffles represent suboptimal habitats and potential barriers to movement between adjacent pools (Schaefer 2001).

I used two antennae configurations to quantify movement patterns. In density and operational sex ratio (hereafter DOSR) trials (8mm PIT tags), a 69 × 33 cm flat plate antenna (Biomark, Inc.) was buried in the substrate so that individuals crossing the riffle passed over the detection field. Water depth in the pools and riffle was set at approximately 45 cm and 4 cm, respectively, with a mean current velocity of 15 cm/s across the riffle unit. For environmental trials (12 mm PIT tags), I used two smaller antennae (46 × 9 cm) at both ends of the riffle to capture movement direction and specific habitat occupancy data. Smaller antennae were connected to a multiplexing reader (Oregon RFID). The large flat plate antenna was connected to a FS2001F-ISO reader (Biomark, Inc.).

**DOSR movement**

I manipulated population density and operational sex ratio across reproductive seasons (spawning vs. non-spawning season) to test for responses in movement patterns. Trials consisted of low (4 individuals) and high (12 individuals) density treatments, with skewed and even (3:1, 1:1, 1:3; male:female) sex ratios. Ten replicates of each cell in a fully-crossed design resulted in a total of 120 trials. To verify spawning periods,
spawning media was placed in both pool units, and deposited eggs were collected following each trial. The observed spawning period (presence of eggs) was consistent with published reports defining the spawning season based on the presence of mature ova in the gonads (Blanchard 1996). For each trial, fish were drawn from holding tanks, identified, and subsequently released in the center of the riffle unit to acclimate for at least 20 hours. Data collection commenced the following morning (0800 h) and recorded movement for a continuous 24 hour period. Trials ran continuously from April to February, allowing for the acclimation period between trials.

Individuals were reused an average (± 1 SD) of 10.6 ± 3.25 times across the duration of DOSR trials, approximately once every eleven trials. Following usage in a trial, all individuals were returned to their respective holding tanks and were not reused for, at minimum, five consecutive trials (minimum of 10 days between successive uses). As the assemblage composition and density of invertebrates colonizing the experimental mesocosms closely resembles that of local, natural streams (Matthews et al. 2006; Wesner 2010), I did not provide a source of food to individuals during the 24 hour trial period. However, because of the higher density in holding tanks, I supplemented the diet with a daily, ad-lib addition of freeze-dried bloodworms. A total of 17 individuals (10 males, 7 females) died throughout the duration of trials. As the maximum life span is 2 to 3 years (Ross 2001) and I tagged adult fish (i.e. > 1 year old), some natural mortality was expected over the course of a 10 month study period.

Movement rate was defined as the mean per capita sex-specific rate (crosses per hour; cph) within each trial. As substantial seasonal differences in movement rates have been observed (e.g. Gatz & Adams 1994; Koed et al. 2006) and following an exploratory
analysis with the data, I analyzed each season independently to reduce the possibility of large seasonal influences masking more subtle trends. For each season (i.e. spawning and non-spawning) I used a mixed model ANCOVA to assess the relative contributions of population density, operational sex ratio and sex on the per capita movement rates of each sex with log-transformed mean daily water temperature as a covariate. Movement rates were log-transformed and each trial served as a block. For this analysis and all subsequent models, all non-significant higher-order interaction terms were removed from the final models. As individuals were reused across trials, I assessed whether individual movement behaviors changed temporally across the number of uses. As movement rates were found to be correlated with temperature, I calculated the log-transformed movement and temperature residuals for each individual across all uses and subsequently correlated this with the number of days elapsed between each use. I used a one sample t-test to test if the mean slope (pooling all individuals) was different from zero.

Fitness-related movement

Measures of growth and body condition are commonly used in telemetry or biologging studies as proxies for fitness (Cooke et al. 2013). All individuals were measured (SL and wet weight) at five intervals throughout the duration of DOSR trials (at the initial tagging [27 March and 5 April 2012], 9 July 2012, 19 September 2012, 5 December 2012 and 15 March 2013) to quantify body condition (Fulton’s condition index; 10,000 × wet weight/SL^3) and growth (mass-specific growth rate). Growth rates were calculated using the equation \[100 \times \left(\frac{\log (w_f) - \log (w_i)}{t}\right)\], where \(w_f\) was the current wet weight, \(w_i\) was the wet weight at the previous measurement, and \(t\) was the number of days between successive measurements. Only individuals \((n = 59)\) for which
measurements were made at all times were used in analyses. Post tagging, tag weight was subtracted from an individual’s wet weight. I assessed changes in log-transformed body condition using an ANOVA with repeated measures (individuals as subjects) with sex and measurement date as main effects.

To evaluate the relationships between movement and measures of growth and condition, I used a multiple linear regression model to test for the effects of mass-specific growth rate (log-transformed) and body condition (averaged across all measurements) on the standardized movement rate (log-transformed) across all trials. As reproductive seasonality explained the largest amount of the variance in movement rates throughout DOSR trials, I standardized (z-scores) the movement rates of individuals by the mean seasonal movement rate (pooling all individuals). This resulted in two z-scores for each individual (i.e. representing spawning and non-spawning movement), and I retained the mean of the two seasonal rates as a measure of overall movement tendency for each individual. Thus, individuals with positive values tended to move, on average, at higher rates relative to the mean; while negative values reflected decreased movement.

**Environmental movement**

Using similar methods as above, I tested the effects of predators and structural habitat complexity on movement of *F. olivaceus* in separate, identical experimental mesocosm units (*n* = 2; two environmental trials ran simultaneously) as used in DOSR trials. Structural complexity (large complexes of woody debris) treatments included (1) absent (absent from entire experimental unit), (2) intermediate (present in one randomly assigned pool) and (3) high (present in both pools). Complexes of woody debris consisted of 10 – 12 pieces of woody debris (approximately 100 × 10 cm; occupying approximately
20% of the pool volume) haphazardly placed along the outer margin of the mesocosm pools. Predators (presence or absence of a single *Micropterus punctulatus*) were free to roam throughout the entire experimental unit but had their mouth surgically immobilized (Knight & Gido 2005) to restrict predation of *F. olivaceus*. I anesthetized predators approximately two hours prior to the trial, securing the jaw with a single loop of 30 lb monofilament line leaving ample gape for respiration. I then allowed the predator to recover from the anesthetic in 380 L holding tanks until they were placed into the downstream pool of the experimental unit fifteen minutes prior to the start of the trial. Following each predator trial, the predator was removed from the experimental unit, and I removed the jaw restraint under anesthesia. Each predator was subsequently housed in a separate holding tank (380 L), offered food (daily addition of live fish) and monitored daily for normal feeding behavior. Each predator (115 – 160 mm SL; *n* = 18) was reused approximately three times throughout the experiment duration (with a minimum of 14 days between successive uses) and were similarly PIT-tagged to collect data on their movement and location, and allow evaluation of *F. olivaceus* responses (i.e. residency time or emigration from pools). No mortality of predators occurred throughout the study. Density (4 individuals) and sex ratio (1:1; male:female) of *F. olivaceus* were held constant across all trials. I replicated each treatment 15 times, resulting in a total of 90 trials. Water depth in the riffle unit was increased to 12 cm (mean velocity = 3 cm/s) to ensure unimpeded passage of the predator between pool units. All environmental trials were completed from May to September and I observed 7% (4 individuals) post-tagging mortality.
I used an ANCOVA with repeated measures (individual *F. olivaceus* as subjects) to assess the effects of predator, structural complexity, sex, and mean daily water temperature on log-transformed rates of movement. To further explore the relationship between predator and prey, I used an ANCOVA on only the trials containing a predator, with predator movement rate (log-transformed) as an additional covariate. As group size has been shown to increase in the presence of a predator (Schaefer 2001), I calculated the mean maximum group size within each trial to assess the effects of habitat complexity and the predator on grouping tendency of *F. olivaceus*. At each 5 minute interval across the 24 hour trial period, I calculated the maximum number of individuals occupying the same mesocosm habitat (riffle, upstream or downstream pool). For trials with four fish, maximum group size was 2, 3, or 4. I retained the mean across all time intervals within each trial and used an ANOVA to test for effects of habitat complexity and predator presence on mean group size.

To assess patterns of habitat use, I calculated the mean residence time (minutes) for each individual in each experimental habitat (pool or riffle, area corrected) across the 24 hour trial period. Residence was defined as the amount of time an individual occupied one habitat of the mesocosm unit before changing location. I used a linear mixed model to assess differences in residence time, with predator, structural complexity, sex and habitat (pool or riffle) as fixed effects. Individuals (nested within sex) and trial (block) were set as random effects. As I was not interested in the actual position of fish throughout the entire experimental unit (i.e. specific pool occupied), pool units were combined for analysis. I controlled for relative habitat size by adjusting residence times.
to account for size differences (surface area) of available mesocosm habitat types. All analyses were performed in the R statistical language (R Core Team 2014).

Verification of PIT tag detection

To ground-truth the detection efficacy of the PIT tag antennae, I recorded approximately four hours of video (Sony Handycam DCR-DVD610; mounted above antennae) during the acclimation period across a randomly selected subset of trials (n = 12; 6 for each trial set). Three 30 min segments were randomly chosen for comparison with detections captured by the PIT tag antennae. Videos were scanned for crosses and were subsequently compared to time-stamped tag detections recorded by the PIT tag readers. Detection efficiency was over 99.3%, with the small number of unsuccessful detections representing individuals crossing the field at uncharacteristically high velocities.

Results

DOSR movement

No eggs were recovered from the spawning media after 11 September. The global spawning season model included no significant higher-order interactions (≥ 3 terms) so the final model included all main effects and two-way interactions. Spawning season movement was influenced by density ($F_{1,50} = 43.01, p < 0.0001$), sex ($F_{1,55} = 65.67, p < 0.0001$), and an interaction between sex and sex ratio ($F_{2,55} = 3.57, p = 0.031$). Movement rates (± SE) were significantly greater in low density treatments (5.17 ± 0.48 cph) compared to high density treatments (2.17 ± 0.19 cph), and males (4.75 ± 0.47 cph) moved more than females (2.59 ± 0.28 cph)(Figure 1). Throughout the spawning season, mean water temperature ranged from 19.9 to 30.9 ºC and movement was inversely related
to daily water temperature ($F_{1,50} = 15.75$, $r^2 = 0.10$, slope = -2.75, $p = 0.0002$). Females moved at a similar rate across all levels of sex ratio (all post hoc Tukey $p > 0.42$); however, males moved significantly more in female biased treatments ($6.15 \pm 1.09$ cph) compared to treatments of equal sex ratio ($3.83 \pm 0.55$ cph; $p = 0.027$). There were no differences in male movement rates between female and male biased treatments ($4.28 \pm 0.55$ cph; $p = 0.27$) or equal and male biased treatments ($p = 0.95$).

No significant interactions existed in the global non-spawning season model, thus I interpreted only the main effects. In contrast to the spawning season, female non-spawning season movement ($0.81 \pm 0.11$ cph) was significantly greater than male movement ($0.69 \pm 0.10$ cph; $F_{1,59} = 6.17$, $p = 0.016$), and overall movement was positively related to mean water temperature ($F_{1,55} = 15.63$, $r^2 = 0.16$, slope = 1.24, $p = 0.0002$). Mean daily water temperatures ranged from 9.8 to 25.2 ºC during the non-spawning season. There was no indication of a temporal change in individual movement behavior across DOSR trials (i.e. mean slope of individual movement rates across uses not different from zero; $t_{81} = -0.30$, $p = 0.77$).

**Fitness-related movement**

Mean body condition increased in both sexes throughout the spawning periods (tagging through July), followed by a reduction post spawning season (September through December) and a subsequent increase the following March (Figure 2). Body condition was similar among sexes throughout spawning season measurements (all post hoc Tukey comparisons $p > 0.12$); however, female condition declined faster after spawning (sex × measurement date interaction: $F_{4,236} = 13.10$, $p <0.001$; Figure 2).
Total specific growth was correlated with standardized movement rates (multiple linear regression; $F_{1.56} = 10.49, p = 0.002$) across all trials. Movement rates were negatively related to specific growth (Figure 3), indicating increased vagility of larger individuals with lower growth capacities. No relationship existed between movement rates and body condition ($F_{1.56} = 1.62, p = 0.21$).

**Environmental movement**

A total of 40 individuals (19 males, 21 females) completed all treatment combinations and were used in analyses. The final interpreted model consisted of the main effects and two way interactions. Males ($5.69 \pm 0.93$ cph) moved significantly more than females ($3.11 \pm 0.89$ cph; $F_{1.33} = 7.67, p = 0.009$) and both sexes responded similarly to the presence of a predator (predator × sex interaction; $F_{1.187} = 0.33, p = 0.57$). The presence of a predator significantly decreased movement rates, but only in the absence of habitat complexity (predator × structure interaction; $F_{1.187} = 3.81, p = 0.024$). In the absence of structure, movement rates were significantly lower in the presence of the predator ($2.22 \pm 0.39$ cph) compared to its absence ($4.85 \pm 0.82$ cph; $p = 0.003$); however no differences in movement rates were found at intermediate (predator absent: $5.87 \pm 0.99$ cph; predator present: $4.15 \pm 0.71$ cph; $p = 0.54$) or high (predator absent: $4.41 \pm 0.75$ cph; predator present: $4.38 \pm 0.74$ cph; $p = 0.99$) complexity levels (Figure 4).

Temperature did not influence the movement of individuals (between subjects $F_{1.33} = 0.24, p = 0.63$) or across trials (within subjects: $F_{1.187} = 3.38, p = 0.067$). Movement rates were inversely related to predator activity (within subjects: $F_{1.72} = 4.87, p = 0.03$; mean slope of individuals = -0.25 \pm 0.08 SE) and average group size (± 1 SE).
was significantly greater in the presence of the predator ($F_{1,84} = 9.62, \ p = 0.003$; mean $= 2.96 \pm 0.04$ individuals) compared to predator absent trials ($2.81 \pm 0.03$ individuals).

The presence of the predator significantly increased mean residence time of individuals in all mesocosm habitats ($F_{1,84} = 25.50, \ p < 0.0001$) and facilitated disproportionately higher occupancy of the riffle habitat compared to the pools (predator $\times$ habitat interaction: $F_{1,621} = 26.52, \ p < 0.0001$; Figure 5). Across all trials, mean ($\pm$ 1 SE) residence times in the riffle habitat significantly increased from $4.8 \pm 0.90$ to $15.4 \pm 2.57$ minutes (post hoc Tukey $p < 0.0001$) and proportional riffle use increased from $13.3 \pm 0.7\%$ to $21.6 \pm 1.0\%$ ($F_{1,356} = 93.26, \ p < 0.0001$) with the addition of predators. Mean residence time in pools increased from $13.1 \pm 2.31$ to $17.7 \pm 2.13$ minutes (post hoc Tukey $p = 0.07$) in the presence of the predator (Figure 5).

**Discussion**

Movement patterns of *F. olivaceus* were influenced by multiple factors across mesocosm trials. Different factors influenced movement patterns seasonally and mean movement rates were approximately 7.5 times greater during the spawning season. These seasonal results are consistent with other studies evaluating seasonal movement patterns (Gatz & Adams 1994; Albanese et al. 2004; Koed et al. 2006) and are presumably correlated with the increased metabolic demand (Chipps et al. 2000; Schaefer & Walters 2010) and the search for mates (Lucas & Batley 1996). As movement has been repeatedly linked to mating system dynamics (Greenwood 1980; Perrin & Mazalov 2000; Shaw & Kokko 2014), the data provides strong evidence highlighting the importance of reproductive processes and timing in relation to movement. While responses to other seasonal cues (e.g. temperature, day length) presumably influence behavior, the disparity
in movement rates immediately following the termination of spawning (65% reduction) suggests movement patterns of *F. olivaceus* are closely tied to spawning behavior.

Opposed to other taxa in which sex-biased movement is pervasive (Greenwood 1980; Sandell et al. 1990), evidence of sex-biased movement in stream fishes is sparse, in part due to the lack of consistent sexual dimorphism. Movement patterns in DOSR (spawning season) and environmental trials were strongly male-biased, consistent with predictions of polygynous mating systems (Greenwood 1980; Perrin & Mazalov 2000; Gros et al. 2008). Asymmetrical reproductive investment in polygynous mating systems would theoretically result in male-dominated movement, due to increased male competition for female access and increased reproductive costs of females associated with egg production and maintenance. In addition, movement was positively correlated to body size. Larger individuals are often thought to be superior competitors, thus juveniles and younger adults may attempt to minimize mobility costs (e.g. energetic expenditure) and risks (e.g. predation) to reach larger sizes which would seemingly promote increased, long-term fitness. For instance, larger male *F. olivaceus* were found to sire a disproportionately higher number of offspring compared to smaller males in a series of mate choice trials (Schaefer et al. 2012), a pattern that likely arose in one of two ways. Females may have preferentially selected larger males due to size-fitness correlates, or male-male dominance resulted in the larger males gaining increased access to females. Regardless of the mechanism, both scenarios could lead to larger males increasing activity to compete for mating opportunities. This strategy would benefit higher quality males as moving between patches would theoretically result in an increased number of mating opportunities. As *F. olivaceus* is a daily spawner (Vigueira et al. 2008) with a
protracted spawning season (upwards of six months; Blanchard 1996), this may play a substantial role in the evolution of movement dynamics.

In contrast to males, females moved little throughout the spawning season; however movement significantly increased during the non-spawning season as water temperatures began to rise. This increased movement, coupled with a concomitant increase in body condition, may reflect attempts to utilize foraging opportunities in preparation for the upcoming spawning season. Female *F. olivaceus* have been shown to allocate up to 16.8% of their daily caloric intake to reproductive tissue (Harris 2013); thus females likely need to feed more than males throughout the spawning season to fuel egg production and maintenance (Thomerson & Wooldridge 1970). Such allotment would seemingly limit the capacity for females to invest in extensive mobility, as this may result in suboptimal fecundity. Furthermore, dispersal costs (e.g. predation, probability of finding suitable habitats) may exceed the benefits of remaining sedentary once established in a suitable patch with an ample resource base.

Collectively, my data indicate that local habitat quality plays a substantial role in moderating movement rates among populations. Variability in population structure (i.e. density and sex ratio), habitat complexity and biotic interactions presumably interact to alter the relative profitability of a given habitat patch at different spatiotemporal scales (Bell et al. 2009). Thus, individuals must be capable of responding to the local conditions as fitness is expected to correlate with patch quality (Matthysen 2012). Across a variety of taxa, population density has been positively correlated with movement rates, as density dependent competition appears to outweigh the benefit of staying in a specific patch (reviewed in Bowler & Benton 2005; Matthysen 2005). However, stream fishes often
exhibit an inverse relationship between density and movement (Gilliam & Fraser 2001; Petty & Grossman 2004; Alldredge et al. 2011). These results are consistent with the latter, as spawning season movement in low densities increased two-fold over high density treatments. Increased mobility may be adaptive at lower population densities, as individuals may need lengthier or more frequent excursions to find potential mates to avoid population growth declines (i.e. Allee effects) or to seek a more suitable home range (Crook 2004).

Similarly, variation in sex ratios has been linked to sex-specific movement (Lawrence 1987; Sandell et al. 1990), and usually results in a positive relationship between the proportion of males and male movement (i.e. increased mate competition). At first, these results seem to be in opposition to documented relationships, as males increased movement in female-biased sex ratios. However, this may be attributed to differing definitions of movement or the scale in which it is quantified compared to this assay of movement. Ultimately, the probability of successful mating is related to the density and operational sex ratio of local conspecifics. Competitive processes (e.g. kin, mate or resource) have been implicated as putative drivers of movement (Hamilton & May 1977; Gandon 1999; Lehmann & Perrin 2003) and the experimental units essentially minimized the ability for individuals to emigrate to less competitive patches. Consequently, male-male competition was relatively invariant within trials at a given density and sex ratio. In natural populations dominated by female-biased sex ratios, males may increase local movement to gain access to females in the absence, or in reduced levels, of mate competition. Alternatively, as sex ratios become more male-biased and mate competition intensifies, males may either emigrate to less competitive patches or
opt to reduce movement in order to establish and defend territories, driving out subordinate competitors (Carranza & Winn 1954).

Predators are thought to influence prey by promoting movement (e.g. predator avoidance tactics, emigration), suppressing movement (e.g. limiting immigration or emigration from hostile reaches), or inducing a conditional response in which a threshold level of external stimuli (e.g. threshold predatory threat; Poethke et al. 2010; Flaxman et al. 2011) may trigger or halt movement (Weisser 2001). Increased levels of habitat complexity mediated the tendency of *F. olivaceus* to move in spite of the presence of the predator. This supports the hypothesis of a conditional response to the predator as movement was facilitated by the presence of structure. Areas of high habitat complexity may be increasingly important for small-bodied fishes, as structure may simultaneously provide effective refuge from local predators and facilitate movement throughout high risk reaches (Gilliam & Fraser 2001). Prey activity level is a common measure of predator avoidance behavior (Lima & Dill 1990), influenced by a suite of visual and chemical (Wisenden & Harter 2001) cues initiating anti-predatory behaviors. While the nature of this design precluded the ability of *F. olivaceus* to completely emigrate to predator free patches, predators are ubiquitous in streams occupied by *F. olivaceus*. Individuals responded to increased predator movement by significantly decreasing activity levels and increasing group size. In addition, the increased riffle use in the presence of the predator is consistent with predators inducing prey into less profitable or sub-optimal habitats (Fraser & Cerri 1982; Power et al. 1985; Schlosser 1988a; Schlosser 1988b; Harvey 1991; Gilliam & Fraser 2001).
While movement patterns of *F. olivaceus* responded to multiple environmental factors in this study, I caution on generalizing these patterns to natural streams for a few reasons. While trial density in the low density DOSR treatments and environmental trials (0.75 fish per meter) was within the range of natural densities (Alldredge et al. 2011), the high density treatments (2.2 fish per meter) likely resided above. Consequently, both intraspecific and predator-prey interactions may have been exacerbated at this scale, as the magnitude of responses may be strongly influenced by fish-habitat size relationships (Schloesser 1988b). Secondly, only adult fish (> 50 mm) were used in this study, thus any ontogenetic changes in movement propensity or behavior is unknown. As movement tendencies (Skalski & Gilliam 2000; Petty & Grossman 2004) and habitat selection (Werner et al. 1983) can change with size or life stage, intraspecific and predator-prey interactions are also likely influenced temporally by shifts in these behaviors. Lastly, although unlikely, I cannot rule out the difference in tag size between trial series affecting rates of movement. While tag to body weight ratios should be minimized as much as possible, it also must be evaluated in the context of the study design, implantation method, focal species and life stages involved (Jepsen et al. 2002). Various studies have documented no effects of internal transmitter implantation on swimming performance or growth employing tags up to 12% of the body weight (e.g. Brown et al. 1999; Ammann et al. 2013). Comparison of movement rates between treatments of similar density and sex ratio differing in tag size (DOSR low density and equal sex ratio versus predator absent) resulted in higher movement rates of the 12 mm PIT tagged fish. However, this was likely due to the increased depth (and decreased velocity) throughout the riffle segment in environmental trials, making riffles more passable. Similar riffle
characteristics (i.e. depth and current velocity) have been shown to influence movement propensity (Schaefer 2001).

The results from this study indicate that multiple interacting factors may contribute to the local movement dynamics of small-bodied stream fishes such as *F. olivaceus*. While this study highlights the influence of various factors on fine scale movement, such knowledge is critical for extrapolation across larger spatial and temporal scales. Integration of factors thought to influence patterns of movement with other aspects of an organism’s life-history and ecology may provide a more holistic view of population-level processes. For instance, as the potential for long distance, exploratory movement exists in *Fundulus* spp.; movement of *F. olivaceus* was observed to be more restricted (< 1 m per day; Alldredge et al. 2011). These data, coupled with data from this study, suggest that *F. olivaceus* may primarily remain within an established home territory and may engage in frequent, small inner-patch bouts of movement influenced by local environmental factors. Future work, investigating these fine-scale movement patterns within natural settings may be an appropriate test to assess the accuracy of controlled artificial studies in application to natural systems.
Figure 1. Sex-specific movement rates across seasons and densities.

Mean per capita movement rates (± 1 SE) of *F. olivaceus* during the spawning (circles) and non-spawning (triangles) seasons for males (closed symbols) and females (open symbols) across densities.
Figure 2. Body condition throughout DOSR trials.

Mean body condition (± 1 SE) of male (closed symbols) and female (open symbols) *F. olivaceus* across the five measurement periods throughout DOSR trials.
Figure 3. Relationship between movement rates and specific growth rate.

Relationship between weight-specific growth rate (% body weight gain per day) and individual standardized movement rates (crosses per hour) throughout DOSR trials.
Figure 4. Movement rate across levels of structural complexity and predators.

Mean movement rates (± 1 SE) of *F. olivaceus* in the absence (open circles) and presence (closed circles) of the predator (*M. punctulatus*) across levels of structural habitat complexity (woody debris complexes).
Figure 5. Habitat specific residence time in the presence and absence of the predator.

Area corrected (m$^2$) mean (± 1 SE) residence time (minutes) of *F. olivaceus* in each mesocosm habitat in the absence (open circles) and presence (closed circles) of the predator.
References


CHAPTER II - SEX-BIASED MOVEMENT AND HABITAT USE IN THE BLACKSPOTTED TOPMINNOW, *FUNDULUS OLIVACEUS*: THE IMPORTANCE OF SPATIAL SCALE

Abstract

The relationship between movement and reproduction has important consequences for individual fitness and population level processes. While sex-biased movement has been repeatedly linked to mating system attributes in birds and mammals, limited data exists on this relationship in fishes. Using passive integrated transponder tags and a series of stationary antennae, I evaluated the movement dynamics and habitat use of a small-bodied, sexually-dimorphic stream fish, *Fundulus olivaceus*, over a 30 day period in a fourth-order tributary to the Pascagoula River in south Mississippi. I observed contrasting sex-biased patterns of movement at two spatial scales that were likely facilitated by differential resource demands and habitat usage. At smaller, local scales, males were more active (number of moves, movement rate) than females (approximately six-fold higher), and their spatial distribution throughout the study reach coincided with measured habitat complexity. Collectively, these data suggest that local mate competition may suppress the movement distance of males and may promote the establishment and monitoring of territories. In contrast, females exhibited an increased propensity to engage in longer distance moves and displayed larger (distance) daily movement spread. Females were more patchily distributed throughout the study reach, aggregating and residing in habitats that contained increased amounts of presumed resource availability (e.g. foraging and spawning) and decreased predator density. Although I was able to document sex-biased movement, biases were inconsistent with the predicted sex-biased movement and
mating system relationship at the contrasting scales in which I quantified movement. These results highlight that importance of spatial scale when evaluating patterns of sex-biased movement tendencies.

Introduction

The link between movement and mating have direct consequences on individual fitness and patterns of gene flow among populations (Clobert et al. 2001; Clobert et al. 2012). Historically, the capacity for movement has often been treated as fixed for a given species, with most efforts focused on inter-specific differences in movement patterns. Recently, there is increased interest in intra-specific movement dynamics as putative drivers of population level processes (e.g. gene flow, metapopulation dynamics) (Clobert et al. 2009; Stevens et al. 2010; Clobert et al. 2012). At the intra-specific level, differences in the causes and consequences of movement may be most pronounced between the sexes, driving the evolution of sex-biased movement. The evolution of sex-biased movement strategies has been the focus of theoretical work (Gandon 1999; Perrin & Mazalov 2000; Lehmann & Perrin 2003; Gros et al. 2008; Gros et al. 2009) and empirically demonstrated in a large number of animal taxa (Greenwood 1980; Sandell et al. 1990; Croft et al. 2003; Bowler & Benton 2009). Generally, kin competition and inbreeding avoidance have been identified as complementary mechanisms driving the evolution of sex-biased dispersal (Hamilton & May 1977; Johnson & Gaines 1990; Gandon 1999; Lehmann & Perrin 2003).

Sex-specific movement patterns have been further linked to mating system attributes and sex-specific costs of reproductive investment (Greenwood 1980). Movement tends to be female-biased in monogamous mating systems, as males typically
invest more heavily in parental care and the benefits of philopatry outweigh those of dispersal. In contrast, males are often the more mobile sex in polygynous systems where females invest more into direct reproductive costs (Perrin & Mazalov 2000). More recent work suggests that mating strategy alone may not necessitate a particular pattern of sex-biased movement, but movement may also be influenced by the relative temporal framework of reproduction and dispersal. Theoretically, males are expected to be more mobile in species that mate throughout the dispersal process; while female-biased movement should evolve in instances where dispersal follows mating (Shaw & Kokko 2014).

Regardless of the role of mating systems dynamics, asymmetries in resource competition between the sexes may also drive the evolution of sex-biased movement (Perrin & Mazalov 2000; Gros et al. 2009). Specifically, the relative intensity of competition for reproductive resources varies drastically between sexes depending on the mating system. Inter-sexual resource competition may be most divergent (i.e. weakest) in polygynous or promiscuous systems, where males largely compete for territories and/or access to females (i.e. mate competition), while female competition is most intense for local resources (e.g. food, spawning habitats). This further presents a dichotomy of relative reproductive investment. Male reproductive success is largely contingent on access to females, while female fitness is linked to the assimilation efficiency of acquired resources into reproductive products. However, resource availability is expected to be spatially and temporally variable (i.e. patchy), resulting in a matrix of available habitats differing in quality (Wiens 1989; Cooper et al. 1997; Thompson et al. 2001). Animals are expected to distribute themselves optimally (e.g. ideal free distribution; Fretwell & Lucas
1970); however local competition intensity, dispersal costs or other biotic interactions (e.g. inter-specific or predator-prey) likely facilitate departures from this optimum (Bell et al. 2009; Matsumura et al. 2010).

As individual fitness is expected to correlate with local population size, the propensity to move is likely to be influenced by the local conspecific density (Matthysen 2005). However, despite a wealth of theoretical models (Travis et al. 1999; Poethke & Hovestadt 2002) and empirical data suggesting movement tendencies are predominantly positively density dependent (Aars & Ims 2000; Andreassen & Ims 2001; French & Travis 2001; reviewed in Matthysen 2005), studies of stream fishes have often documented an inverse density dependent relationship (Gilliam & Fraser 2001; Petty & Grossman 2004; Alldredge et al. 2011; Clark & Schaefer 2016). The restricted movement paradigm (Gerking 1959; Gowan et al. 1994; Gowan & Fausch 1996) has remained a central tenet describing patterns of stream fish movement, positing that most individuals restrict their movements to a limited range of available habitats. As a consequence, many populations of stream fishes display highly leptokurtic distributions of movement distances (Smithson & Johnston 1999; Skalski & Gilliam 2000; Fraser et al. 2001). Various explanations, relating to mate-searching or acquisition (Alldredge et al. 2011), resource competition (Petty & Grossman 2004), resource density (Power 1984), predator avoidance (Gilliam & Fraser 2001), habitat persistence (Aparicio & De Sostoa 1999; Schaefer et al. 2003) and complexity (Gorman 1986; Roberts & Angermeier 2007) and behavioral traits (e.g. boldness; Fraser et al. 2001) have been suggested as possible mechanisms for the observed leptokurtic movement distributions.
In contrast to other taxa (e.g. Greenwood 1980; Clarke et al. 1997; Bowler & Benton 2009), empirical data demonstrating sex-biased movement in stream fishes is limited (Hutchings & Gerber 2002; Croft et al. 2003; Marentette et al. 2011; Clark & Schaefer 2016) or has been elusive (Aparicio & De Sostoa 1999; Alldredge et al. 2011). The purpose of this study was to characterize the sex-specific patterns of movement and habitat use of a tributary-dwelling stream fish, *Fundulus olivaceus* (blackspotted topminnow). It is a broadly distributed, sexually-dimorphic stream fish with a mating system and reproductive behavior that would seemingly predict a male-biased movement strategy (Perrin & Mazalov 2000; Shaw & Kokko 2014). It exhibits a polygynous mating system (Schaefer et al. 2012) with a protracted spawning period (March - September; Blanchard 1996) in which females spawn daily throughout much of the season (mean of 1.6 eggs/day; Vigueira et al. 2008). Territorial male behavior has been observed among *Fundulus* species in both laboratory and natural settings (Carranza & Winn 1954; Baugh 1981). For instance, males of a closely-related species, *F. notatus*, have been observed to exhibit a territorial patrolling behavior, actively monitoring a 6-12 meter stretch of stream parallel to the bank, and driving away conspecific males entering the territory (Carranza & Winn 1954).

In this study, I tested hypotheses relating to sex-specific movement patterns by monitoring movement of passive integrated transponder (PIT) tagged fish in a natural stream. The primary motivation was to assess sex-specific movement in response to local environmental factors and predicted contrasting sex-specific movement dynamics at different spatial scales. If female movement is driven by locating high quality patches to utilize resources, I expected females to exhibit an increased propensity to engage in
lengthier (distance) movement bouts, but to minimize movement once a suitable habitat was encountered. Conversely, I expected males to be more locally active, monitoring an established territory, and to engage in fewer long-distance moves. Consequently, I expected females to display an aggregated distribution, segregating (and residing) in areas of high patch quality (e.g. available foraging, spawning habitats); while the expectation was that males would conform to a more uniform spatial distribution, thereby reducing male-male competition. Lastly, I was interested in the interactive effects of density on sex-specific movement patterns. As density may exert differential effects on the sexes (De Meester & Bonte 2010), I examined density effects by manipulating population size and assessing the response of the focal tagged fish.

Methods

Study location

This study was conducted from July to September 2014 in a 180 m section of Big Creek, a fourth-order tributary of Black Creek, located within the Pascagoula River drainage (Mississippi, USA) (Figure 6). The length of the study reach was determined from movement rates and population estimates obtained from a previous mark-recapture study of *F. olivaceus* (70 males and 74 females) at the same locality (Alldredge et al. 2011). Observed mean daily movement rates were less than 1 m per day and the maximum distance detected was 230 m over the course of 49 days (Alldredge et al. 2011).

Fish collection and tagging

I collected adult fish (>50 mm standard length; SL) by dipnet from areas of Black Creek below the confluence of Big Creek in June 2014. Fish were collected from Black
Creek to avoid any potential conditioning effects (e.g. knowledge of present habitats) associated with resident fish in Big Creek. Fish were temporarily housed in a series of outdoor mesocosms (Matthews et al. 2006) and acclimated two weeks prior to tagging. All individuals were sexed, measured to the nearest millimeter (SL) and weighed (grams) prior to tagging. I implanted 27 males (55.5 ± 3.8 mm SL; 2.11 ± 0.46 g) and 27 females (54.1 ± 3.7 mm SL; 2.01 ± 0.51 g) with 12 mm HDX PIT tags (Oregon RFID). Fish were anesthetized using tricane methanesulfonate (MS-222) and tags were injected using a syringe-style implanter by puncturing the body wall just off the midventral line, anterior to the vent and implanting the tag in the posterior portion of the peritoneal cavity. Tagged fish were held in the mesocosms for a period of four weeks and monitored daily for survival, external signs of infection and normal feeding behavior.

**Stream setup**

Four pairs of pass-through antennae were placed throughout the study reach that divided it into five sections within the 180 m study reach. Stream widths precluded construction of antennae spanning the entire stream width, thus I utilized in-stream habitat features (e.g. stream constrictions or impassable barriers [e.g. large woody structure]) to funnel individuals through the antennae fields. I paired antennae (approximately 2 m apart) to assess the directionality of movement between sections and to capture fine scale movement. Antennae consisted of two loops of 12 gauge wire, suspended above the stream using 5 mm static rope and secured into the substrate with plastic stakes. Antennae were connected to two multiplexing HDX PIT readers (Oregon RFID, Portland Oregon) that continuously logged data throughout the study period (30 days of data collection). Each antenna scanned at an average rate of 2.3 (± 0.2 SD) scans
per second with a detection range of approximately 40 cm across each antenna (i.e. 20 cm in both the up- and downstream directions). Data readers and antennae arrays were powered by deep-cycle marine batteries (12 V, 114 amp hours), replaced every three to four days.

I deployed permanent block nets at the up- and downstream ends of the reach to prevent emigration of focal fish or immigration of conspecifics into the study reach. Block nets (steel hardware cloth; 1.2 m tall; 0.635 cm mesh) were constructed to span the entire stream width and anchored into the substrate at the up and downstream termini of the study reach. After block-nets were set, I removed 88 adult *F. olivaceus* (>40 mm SL; 46 males, 42 females) by dipnet that were subsequently released downstream of the study reach (>200 m). As predators presumably alter relative habitat quality (e.g. Power & Matthews 1983; Power et al. 1985), I surveyed predator density in each of the stream sections using two-passes with a backpack electro-fisher to obtain an estimate of the resident predator assemblage. I classified predators as those that are principally piscivorous (*Esox americanus, Lepisosteus oculatus, Micropterus punctulatus*) and also included large-bodied centrarchids (*Ambloplites ariommus, Lepomis megalotis* and *L. gulosus*) and ictalurids (*Ameiurus natalis*) over 100 mm SL. All predators were enumerated, measured and released in the same section of capture.

**Habitat data**

I collected habitat data along transects established perpendicular to stream flow at 2 m intervals (N = 90) throughout the study reach. At each transect, I recorded stream width (m), visually estimated canopy cover (0, 25, 50, 75, 100%) and characterized bank features (stability, slope, littoral vegetation type [herbaceous, shrub, sapling, tree].
presence of undercut banks and root wads). At 1 m intervals along each transect, I recorded depth (cm; The Kreiser™), current velocity (m/s; Marsh-McBirney Flowmate), dominant substrate (modified Wentworth scale; Cummins 1962) and the presence or absence of detritus, small (<10 cm diameter or <1.5 m in length) and large woody structure and aquatic vegetation. Each day throughout the study period, I recorded depth and current velocity at a fixed point to evaluate relative changes in hydrology. For analyses, I calculated means for all quantitative measures and expressed qualitative data as a percentage for each transect. As a measure of variability, I calculated the coefficient of variation (CV) of depth, current velocity, and substrate within each transect. I first used principal components analysis (PCA) to distill the bank and littoral zone characteristics and subsequently included the first two axes in a full PCA to summarize the habitat data along transects.

*Fish introduction and manipulation*

On 30 July I introduced 90 *F. olivaceus* (54 PIT tagged, 36 untagged; equal sex ratio) into the study reach. The untagged individuals (collected from Black Creek) were added simultaneously in order to allow manipulation of population density during the study period (below). I evenly distributed individuals (sex and tagging treatment) between the three interior sections (2, 3 and 4) of the study reach. Fish were allowed to distribute and acclimate throughout the study reach for three days prior to data collection (data loggers were operational to track activity). I chose to add untagged individuals at the beginning of the study to avoid a temporal lag in acclimation that might result from non-focal fish being added to increase the population density. Following the fifteenth day of data collection, four passes (two upstream, two downstream) were made with a two
person crew, dip-netting as many *F. olivaceus* as possible. All fish were temporarily housed in stream-side buckets according to the section captured and subsequently scanned for PIT identification. Captured fish within each section were enumerated and PIT tagged individuals were released back into their section of capture. Non focal (untagged) individuals were released downstream of the study reach, allowing evaluation of focal fish movement responses at contrasting levels of population density. Following collection and handling, I allowed one day of acclimation prior to resuming data collection.

To estimate rates of focal fish potentially escaping the study reach, I sampled *F. olivaceus* above and below the study reach every five days and scanned each for PIT identification. Multiple passes through approximate 75-100 m stretches (i.e. above and below the study reach) were sampled by one or two individuals. Following the completion of the study, the reach was sampled periodically over two days in an attempt to recover remaining focal fish. I continued to run the PIT detection loggers throughout these days to account for any remaining individuals not recaptured.

*Movement metrics*

Using the individual detection data, I generated a suite of movement metrics to assess sex-specific movement patterns at multiple spatial scales across the 30 days of data collection (Figure 7). To first assess a general daily activity level, I calculated the daily sex-specific proportion of individuals that were detected at least once by any antennae. Secondly, I obtained hourly movement rates for each individual by summing the distance (absolute value in meters) between successive detections (minimum total distance moved) within each day (defined as 0700-0700) and divided each by 24 h. Movement
rates were averaged across sex to obtain a daily average (meters per hour) for each sex. To evaluate displacement patterns (i.e. degree of kurtosis), I used the signed distance in meters (+ for upstream; – for downstream) between successive detections to calculate a daily net displacement for both sexes. I used one-sample Wilcoxon Rank Sums tests to examine deviations from zero for both males and females. A Chi-square test was used to test for an overall directional bias (up- or downstream) for males and females pooling all movement events. To estimate the degree of population spread, I calculated the daily standard deviations of the signed distance and used an analysis of covariance (ANCOVA) to test for sex and day effects.

I further classified movement into two qualitative categories to evaluate the influence of predictor variables on movement patterns at different spatial scales (Figure 7). The longitudinal distance between detections was used to assign an individual movement event into two broad categories of movement. Moves which resulted in distances of 0 or 2 m (absolute distance), corresponding to successive detections at the same antenna or pairs of antennae, respectively, were defined as local moves. Larger moves that represented individuals traversing, at minimum, an entire antennae section (i.e. minimum distance >28 m) were characterized as exploratory moves.

To derive each population movement metric, I first constructed sex-specific individual × day rectangular matrices for each of the response variables described above. Daily averages (i.e. movement rates, mean number of local and exploratory moves) and proportions (i.e. the proportion of individuals displaying each metric) were computed and retained for analysis. Because I had minimal knowledge of mortality or potential emigration from the study reach that may have occurred throughout the study, I opted for
a conservative approach and adjusted the values in the individual × day matrices by the last known occurrence of each individual (either by detection history or post-study recapture). A second individual × day matrix constructed of presence/absence data was used to filter values used in calculating daily average and proportion metrics. For example, if an individual last appeared in the detection log on day 16 and was not captured following completion of the study, that individual (and its presence) was excluded from the calculation of daily values throughout days 17-30. I used a series of mixed models to assess the influence of sex and density on the various movement metrics. Using the daily measurements of depth and current velocity, I estimated the daily change in discharge (m³/second) and included this as a covariate in each model. Day (i.e. 1-30) was nested within density and treated as a random effect to account for multiple measurements (i.e. sex) within each day. All response variables were transformed, if needed, prior to analysis.

Section occupancy and residence

The spatial distribution of individuals throughout the study reach was used to test patterns of differential occupancy of the sexes among the sections. At every hour (across all 30 days), I used the detection data to assign an individual to a specific stream section based on the last antenna detection. The number of individuals of each sex/section combination was quantified every hour and averaged across each day to obtain a mean daily sex-specific occupancy in each section. The observed daily values were corrected for section area (i.e. individuals per 100 m²) in an effort to standardize individual density across sections. To calculate section area, I used the transect data to estimate the mean stream width within each section and multiplied this by the known distance between
antennae pairs. I used a mixed model to test for effects of sex and section (day served as a blocking variable) on daily occupancy across the 30 days.

To assess the duration of section-specific use, I calculated the residence time (hour) each individual spent in a specific section across the entire study period. I defined residence time as the amount of time an individual occupied one section before crossing a pair of antennae fields into an adjacent section. Detection by only one antenna (i.e. the interior-most antenna of the antennae pair) did not result in a new residence event.

Similar to the movement metrics, both occupancy and residency times were corrected for individuals known to be present in the study reach; thus the sum of residence times for an individual corresponded to the total time in the study reach. I used a mixed model (individuals as a random effect) to test the effects of sex and section on residence times across the trial period. To assess residence time relative to derived habitat gradients (i.e. PCA axes), I used sex-specific multiple linear regression models to assess the relationship between mean section residence times (pooling all individuals) and section centroid scores (Axes I and II). As residence has been shown to increase with habitat complexity (Gorman 1986; Harvey et al. 1999; Van Moorter et al. 2016) I accounted for the amount of available habitat (i.e. heterogeneity) in each section by correcting mean residence times by the area each stream section occupied in PCA space. To quantify the heterogeneity within each section, I calculated the convex hull (area in PCA space) occupied by all transects within each section. This provided a general measure of habitat availability determined by the area of the enclosed polygon. All analyses were performed in the R statistical language (R Core Team 2014).
Results

Detection and recapture

A total of 19,115 detections were logged from 47 individuals (24 females; 23 males) throughout the 30 day period (Table 1). I lowered the population density following the fifteenth day and collected 39 adults (>50 mm SL) from the study reach and 12 (6 females, 6 males) were positively identified with PIT detection. Following the completion of the study, I recovered a total of 50 adult fish (17 PIT fish; 7 females, 10 males). One male not captured remained in the detection logs two days post-study and was thus considered to have been present for the entire 30 days. Collectively, the section where individuals were physically captured corresponded to the assumed location based on detection records for 28 of the 29 PIT captured fish. The lone individual that did not match was captured in an adjacent section based on the detection logs. Thus, I was confident that the majority of individuals were detected while crossing antennae fields while moving between sections. Of the remaining 29 detected individuals not recaptured post-study and the seven that were released and never detected or recaptured, 15 (42%) were last assumed to occupy sections 1 or 5 based on detection records, indicating it unlikely that a large portion of these individuals escaped the study reach. Furthermore, I did not capture any focal PIT fish outside the reach during periodic sampling above and below the study reach (76 individuals captured across all sampling).

Habitat data

The principal components analysis (PCA) of the bank characteristics described gradients related to bank morphology (PC I; 23.0%) and the composition of littoral zone
vegetation (PC II; 12.5%). The first axis separated transects based on bank slope and the presence of undercut banks and root wads. The second axis corresponded to the distribution and size of littoral zone vegetation types. The relative size of the dominant vegetation type increased along axis II, lower scores featured herbaceous and shrub types, while larger scores represented transects with sapling and trees dominating the littoral zone.

I retained the first two axes of the full PCA (all habitat variables) that accounted for 42.6% of the variance in the habitat data (Figure 8, Table 2). The first axis (25.5%) described a gradient related to stream slope. Transects with negative values along the first axis tended to be faster moving with larger, less variable substrates and increased amounts of small woody structure. The second axis (17.1%) reflected a stream size gradient. Transects with positive values were on average wider, shallower and had an increased occurrence of submerged aquatic vegetation. The three interior sections (2, 3 and 4) tended to be more structurally complex (woody structure, undercut banks), and the outer sections (1 and 5) were wider and contained higher proportions of submerged aquatic and larger littoral zone vegetation (Figures 8 & 9). Sampled predators were patchily distributed among the sections, and predator density followed a pattern relative to the second PCA axis. Predator density (individuals per 100 m²) was highest in sections 2, 3 and 4 (9.7, 7.8 and 5.5 individuals per 100 m², respectively); while sections 1 and 5 contained fewer predators (2.9 and 1.6 individuals per 100 m², respectively). Most predators were centrarchids (Lepomis spp., A. ariommus and M. punctulatus), representing 86.4% of all predators collected.
Movement patterns

Overall, the movement distribution (signed minimum distance) was highly leptokurtic and neither sex indicated an up- or downstream bias in distance (i.e. net signed movement not different from zero; females: $V = 191, p = 0.97$; males: $V = 155, p = 0.90$) or movement direction (Chi-square; both $p > 0.96$). Daily population spread was greater among females ($15.1 \pm 1.8$ m) compared to males ($4.0 \pm 0.5$ m; $F_{1,56} = 28.1, p < 0.0001$) and decreased throughout the study period ($F_{1,56} = 14.2, r^2 = 0.14, p = 0.0004$).

Daily activity level (proportion of individuals moving) differed among sexes ($F_{1,26} = 25.91, p < 0.0001$) and density ($F_{1,26} = 12.24, p = 0.002$). Males ($0.49 \pm 0.02$) were more active than females ($0.35 \pm 0.03$) and overall, activity levels declined from high ($0.48 \pm 0.03$) to low density ($0.35 \pm 0.03$). Mean daily movement rate was influenced by sex ($F_{1,26} = 28.61, p < 0.0001$) and sex × density interaction ($F_{1,26} = 6.69, p = 0.016$). Mean ($\pm 1$ SE) movement rates were greater for males and increased from high to low density ($1.47 \pm 0.15$ to $2.12 \pm 0.18$ meters per hour, respectively); while female movement rates fell from $1.03 \pm 0.15$ to $0.87 \pm 0.22$ meters per hour from high to low density (Figure 10).

The proportion of individuals displaying local moves was influenced by sex ($F_{1,26} = 62.49, p < 0.0001$), density ($F_{1,26} = 5.03, p = 0.034$) and a sex × density interaction ($F_{1,26} = 16.30, p = 0.0004$). The proportion of males ($\pm 1$ SE) making local moves was similar across density treatments (high density: $0.60 \pm 0.03$; low density: $0.62 \pm 0.03$); while the proportion of females declined from $0.51 \pm 0.03$ in high density to $0.34 \pm 0.03$ in low density.
density. The mean number of daily local moves (log-transformed) was influenced by sex ($F_{1,26} = 102.52, p<0.0001$) and density ($F_{1,26} = 34.06, p<0.0001$). The number of local male moves ($46.2 \pm 3.6$) was greater than females ($14.1 \pm 2.1$) and local movement increased from high ($20.44 \pm 2.6$) to low density ($39.9 \pm 4.7$) (Figure 11).

The proportion of individuals displaying exploratory moves (arcsin square root-transformed) was influenced by sex ($F_{1,26} = 7.50, p = 0.011$) and density ($F_{1,26} = 16.84, p = 0.0004$). Proportionally, females ($0.16 \pm 0.02$) tended to engage in longer distance moves more frequently than males ($0.10 \pm 0.02$) and the propensity of all individuals to engage in exploratory moves decreased from high ($0.19 \pm 0.02$) to low density ($0.08 \pm 0.01$). The mean number of daily exploratory moves was not significantly related to any of the predictor variables (all $p>0.24$ with the exception of sex [$p = 0.064$]).

Section occupancy and residence

The spatial distribution (occupancy; density of individuals in each section) differed among sections and between sexes (section × sex interaction, $F_{4,261} = 81.85, p<0.0001$; Figure 12). Among females, mean occupancy was greatest in section 4 (all pairwise post-hoc Tukey comparisons $p<0.0001$) and lowest in section 2 (all $p<0.0001$). Female occupancy was similar in sections 1, 3 and 5 (all $p>0.95$). Male occupancy was lowest in sections 1 and 5 (all pairwise $p<0.0001$) but were not different from one another ($p = 0.99$). Males utilized sections 2 and 4 similarly ($p = 0.99$), and occurred most readily in these sections compared to the other sections (all $p<0.0001$). Male occupancy in section 3 (intermediate to all sections) was significantly different from all other sections (all $p<0.007$). Between sexes and within sections, mean male occupancy was greater in sections 2 ($p<0.0001$) and 3 ($p = 0.002$) compared to females, while female occupancy
was greater in section 4 compared to males \((p<0.0001)\). Mean occupancy was similar between the sexes in sections 1 and 5 \((p = 0.23\) and 0.65, respectively).

Mean residence time (log-transformed hours) differed among sections \((F_{4,11629} = 34.95, p<0.0001)\) and between males and females across sections (section \(\times\) sex interaction, \(F_{4,11629} = 4.17, p = 0.002\); Figure 13). Post-hoc Tukey comparisons among females indicated residence time was significantly lower in section 3 compared to all other sections \((all\ p<0.0001)\). Female residence time was not different between the other sections \((all\ p>0.12)\) with the exception of lower mean residence time in section 4 compared to section 5 \((p < 0.0001)\). Among males, all pairwise section combinations were significantly different \((all\ p<0.0001)\), with the exception of sections 4 and 5 \((p = 0.15)\). Male residence time was highest in section 3, followed by section 2, section 4 and 5, and mean residence time was lowest in section 1. Within sections, female residence time was higher than males in all sections \((all\ p<0.0001)\) with the exception of section 3 \((p = 0.96)\).

Section 3 was the most heterogeneous section \((0.0354; area\ occupied\ in\ PCA\ space)\), followed by section 4 \((0.0348)\), section 2 \((0.0329)\), section 5 \((0.0249)\) and section 1 \((0.0127)\). Mean residence time (area-corrected) of females was not related to section centroid scores along axis I \((F_{1,2} = 0.95, p = 0.43)\); however was positively related to axis II centroids \((F_{1,2} = 54.83, p = 0.018)\), indicating longer persistence in wider stream reaches with increased amounts of submerged aquatic vegetation (Figure 14; upper panel). Mean male residence time was not related to section scores of either axis \((both\ p>0.57)\); Figure 14; lower panel).
Discussion

Most studies evaluating movement in stream fish populations have generally concluded a sedentary behavior (Gerking 1953; Gerking 1959; Hill & Grossman 1987; Smithson & Johnston 1999; Gilliam & Fraser 2001); however the capacity for longer distance moves exists (Alldredge et al. 2011; Able et al. 2012; Walker & Adams 2014). My data are consistent with these patterns, as the majority of moves (98.7%) were spatially limited (i.e. movement distribution was highly leptokurtic); however longer distance excursions were observed. Of the 47 focal PIT fish detected throughout the study, 16 (12 of which were female) were inferred to have occupied all stream sections (minimum of 100 m displacement) based on detection histories, with three individuals (two females and one male) traversing this stretch within 24 h. Similar proportions of mobile *F. olivaceus* have been observed (Smithson & Johnston 1999; Alldredge et al. 2011); however, no sex-bias in propensity or movement rates were found by Alldredge et al. (2011).

Following my predictions, I observed distinct sex-specific patterns of movement operating at contrasting spatial scales. However, the predicted pattern of sex-biased movement (Perrin & Mazalov 2000; Shaw & Kokko 2014) breaks down when viewed at these scales, as contrasting movement biases occurred between the sexes. At smaller scales, the predicted movement pattern emerged as males were locally more active compared to females (approximately six-fold) and displayed an increased propensity to move. Furthermore, males showed a high degree of fidelity exemplified by disproportionally strong associations with a single antennae pair. Across all males,
85.2% (± 4.9 SE) of all detections for an individual were logged at one antennae pair, contrasted to only 62.3% (± 4.8 SE) for females.

Conversely, when movement was viewed at a larger scale, the data failed to support the predicted pattern, as females consistently engaged in more frequent and longer exploratory moves. Both sexes initially displayed a higher tendency to make large-distance moves that may have been facilitated through a “novel environment” effect, as individuals were presumably searching for suitable patches (Crook 2004) or attempting to escape overcrowded habitats (Clobert et al. 2009). While males occasionally made longer distance moves, both the frequency and magnitude of these moves were limited in comparison to females and declined throughout the study. Females, however, continued to display a higher tendency to engage in these longer exploratory movement bouts, which I assume reflects a strategy to optimize reproductive resource demands. However, it is unclear as to why this behavior remained relatively consistent through time, especially as densities, and presumably resource competition, became reduced through time. As this study was conducted during the latter portion of the spawning season (Blanchard 1996), it is possible that females varied in their reproductive status, and consequently in physiological and habitat needs, which may have influenced individual movement propensity (Shaw et al. 2013).

While the spatially-explicit movement patterns may reflect intrinsic differences between the sexes or individuals (Table 1), local competitive pressures likely contributed to male fidelity and consequently, the inconsistency in sex-biased movement. In polygynous mating systems, the relative intensity of mate competition among males is generally assumed to exceed resource competition among females (Perrin & Mazalov...
Dependent on local densities and sex ratios, the strength of local mate competition may present a movement dichotomy for males in order to maximize reproductive success. As male aggression has been shown to be related to adult densities and sex ratios (Jirotkul 1999a; Jirotkul 1999b; Spence & Smith 2005), the probability of aggressive encounters likely increases as a function of the local intra-sexual density.

Considering the territorial nature of fundulids (Carranza & Winn 1954; Baugh 1981) and the greater tendency of females to more frequently invest in longer moves, males may benefit more by maintaining position to monitor a territory (Carranza & Winn 1954), attempting to court females during transit, thereby limiting the extent of aggressive male encounters. However at reduced densities, mate competition may be lessened, thereby releasing males to become more expansive in mate-searching behaviors. While it is important to note that the levels of density (i.e. high and low) were not necessarily fixed through time as mortality undoubtedly occurred throughout the study period, this would have served to magnify any observed density effects. Regardless of this magnitude however, the data suggest that changes in population density influenced male movement more so than females (De Meester & Bonte 2010). As the daily proportion of males moving remained relatively constant across the study, movement rates and the number of daily local moves both increased by approximately 50% at the reduced density. Such condition-dependent plastic responses may be spatiotemporally important in mitigating population declines associated with reduced densities (e.g. Allee effects; Gascoigne et al. 2009), while at the same time, may serve to increase the probability of mating success of mobile males.
Anecdotally, increased male movement throughout the latter portion of the study may have been further influenced by the operational sex ratio within the study reach. I observed a fairly even sex ratio when I lowered densities as 54% (12/22) and 48% (13/27) of PIT (captured or detected) and unmarked fish, respectively, were males; however following the completion of the study, males comprised 61% (11/18) of the PIT fish accounted for and 63% (20/32) of the unmarked fish captured. Assuming these ratios were representative of the local population through time suggests a higher rate of female mortality and males may have needed to increase activity (either locally or longitudinally) in an attempt to locate potential mates (Crook 2004; Alldredge et al. 2011). Coupled with lower densities, a more male-biased sex ratio could lead to increased movement that would benefit males that moved into areas with higher female densities (Croft et al. 2003). Furthermore, spatial variation in sex ratios (Figure 12) may have facilitated differential movement patterns within and among the stream sections that may be responsible for some of the individual variability in movement propensity.

Habitat use among the sections support predictions based on putative resource availability and usage. Both sexes differentially aggregated amongst the sections; however, patterns of section occupancy (Figure 12) did not strongly reflect section residency (Figure 13) and this was more pronounced in females compared to males. While individual variability in movement propensity (Table 1) may be to some degree responsible, variability in habitat heterogeneity may have facilitated movement into more heterogeneous stream sections to utilize foraging or refuge habitats (Matheney & Rabeni 1995; Gilliam & Fraser 2001; Van Moorter et al. 2016). Although males did not conform to a strict uniform distribution based on stream area, their occupancy patterns appeared to
be tightly linked to the relative amount of available habitat (i.e. heterogeneity) among the sections. This would seemingly align with the idea of male territoriality, as increased heterogeneity likely allowed for increased densities of conspecifics (Gorman & Karr 1978; Taylor et al. 1993; Belica & Rahel 2008). However, residence in these heterogeneous sections also entailed increased exposure to resident predators. I suspect that increased amounts of structure likely mitigated predation risk to some extent and allowed activity (Gilliam & Fraser 2001) and persistence (Fraser & Cerri 1982; Matthews et al. 1994) in these high risk patches. However, at the same time, increased predation risk may have simultaneously functioned to limit the longitudinal distance males moved (Fraser et al. 2006).

Females on the other hand displayed a more patchy spatial distribution (Figure 12) and increased residence time compared to males (Figure 13). More specifically, multiple lines of evidence suggest that the movement dynamics and habitat use of females were linked to resource availability. First, female residence was highest in sections with increased aquatic vegetation (Figure 14), a substrate readily used for egg deposition (Carranza & Winn 1954; Ross 2001) and habitats in which high densities of *F. olivaceus* have been observed (Blanchard 1996). Secondly, females predominantly resided in sections with increased amounts of overhead canopy and larger littoral zone vegetation types (i.e. saplings and trees). Because of the protracted spawning period, Thomerson and Wooldridge (1970) suggested that females likely feed more than males throughout the spawning season to fuel reproductive costs. As with many inland fundulids, *F. olivaceus* predominantly feeds on terrestrial arthropods that fall onto the water surface (Thomerson & Wooldridge 1970; Champagne 2011; Ross 2013).
Consequently, areas of increased canopy with larger littoral zone vegetation types would presumably result in increased foraging opportunities via allochthonous input (Vannote et al. 1980). Power (1984) observed a similar pattern in an algae-eating loricariid catfish (*Ancistrus spinosus*), as the distribution and local densities were strongly correlated with areas of decreased canopy cover (increased autochthonous algal production). Lastly, and in contrast to males, female residence was inversely related to predator density. This may simply be coincidental and reflect preference for higher quality foraging and/or spawning habitats; however it may indicate a stronger response of females to predators compared to males. Presumably, the largest costs to movement throughout the study reach were predation risk and the probability of locating suitable patches or mates (Bonte et al. 2011). As mentioned above, 12 females were documented to have occurred within all five sections, meaning they had to traverse through the more hostile, predator-dense, interior sections. Increased activity likely makes individuals more conspicuous to predators (Lima 1998) and mortality rates have been shown to greater in mobile individuals (Johnson & Gaines 1990). Furthermore, female residency was highest in the most homogeneous sections, seemingly contradicting residency-habitat heterogeneity relationships (Fraser & Cerri 1982; Belica & Rahel 2008; Van Moorter et al. 2016). However, similar predator-induced habitat shifts have been observed in other systems and at various spatial scales (Power et al. 1985; Gilliam & Fraser 2001). Collectively, these could not only lead to increased longitudinal movement to search for high quality patches, but may subsequently result in increased residence once locating a suitable habitat.
As this study evaluated movement in a relatively short longitudinal reach at one locality, I cannot discount the possibility that the movement behaviors captured were stream or individual-specific. At larger spatial scales, populations and individuals undoubtedly face various environmental pressures that may drive population-specific movement tendencies (Woolnough et al. 2009). However, population density was implicated as a putative mechanism influencing inter-year movement differences in *F. notatus* (Alldredge et al. 2011) and similar sex and density effects influenced the movement of *F. olivaceus* in a mesocosm setting (Clark & Schaefer 2016). Accordingly, I feel this data accurately represented movement behaviors as the current patterns are in broad agreement with the observed patterns captured in both natural and experimental settings. Estimates of movement dynamics were conservative to avoid overestimating patterns due to the assumed unaccountability (detection or capture) of individuals throughout the study period. While no marked fish were captured outside the block nets, it is possible that highly mobile fish (or group of fish) could have potentially left the study reach and emigrated past the surveyed areas above and below the routinely sampled areas. Alternatively, albeit rare in studies employing PIT tags in small-bodied fishes (e.g. Ficke et al. 2012; Bangs et al. 2013), a moderate degree of tag expulsion could have occurred post-release. Although I cannot rule this out, I believe it unlikely as I have extensive tagging experience with this species and any tag loss generally occurs within the first 7-10 days post-implantation when the injection site is healing. More likely though was mortality due to senescence or predation. As with many small-bodied stream fishes, *F. olivaceus* is a generally short-lived species (~2 years; Ross 2001), and mortality of senescent adults has been observed following peak spawning (Alldredge et al. 2011).
Alternatively, it is conceivable that a small proportion of fish displayed a high degree of section fidelity, and patterns displayed by a few individuals support this possibility. Of the 18 individuals that were positively accounted for post-study (recapture or detection), four individuals exhibited extremely sedentary behaviors through time (individuals 22912, 22950, 22958, 22986; Table 1). Most notably was a single female (individual 22958) that was not detected by any antenna throughout the entire study, but was physically recaptured (and positively identified with a handheld PIT scanner) in the same section when I lowered densities and following the study. Similar patterns of highly variable individual movement tendencies have been observed in other stream fish populations (e.g. Smithson & Johnston 1999; Gilliam & Fraser 2001; Hutchings & Gerber 2002; Petty & Grossman 2004; Walker & Adams 2014). Whether such patterns represent populations containing distinct groups of mobile and sedentary individuals (e.g. population heterogeneity hypothesis; Skalski & Gilliam 2000) or result from individual plastic responses to environmental conditions (Alldredge et al. 2011; Booth et al. 2014) remains largely unknown.

Utilizing various movement metrics, I documented contrasting sex-specific movement patterns in a polygynous, small-bodied stream fish. As predicted, my results indicate that intersexual differences in movement behaviors were largely scale-dependent; however this resulted in movement biases that were inconsistent with predictions (Perrin & Mazalov 2000; Shaw & Kokko 2014) across the movement assays. While mating system attributes have been implicated as drivers of sex-biased movement across a variety of taxa (e.g. birds: Greenwood 1980; mammals: Dobson 1982; Clarke et al. 1997; fishes: Hutchings & Gerber 2002; Croft et al. 2003; insects: Beirinckx et al. 1997; mollusks: Hoffman 1999; herps: Hubbs et al. 1999).
2006; Rabasa et al. 2007), it is certainly not a universal phenomenon (Lawson Handley & Perrin 2007). As with many other putative drivers of movement (reviewed in Bowler & Benton 2005), the prevalence and magnitude of movement responses may be largely dependent on a combination of local extrinsic (i.e. biotic and abiotic conditions) and intrinsic (e.g. sex, physiological condition) factors (Nathan et al. 2008; Clobert et al. 2009). Notably, my data provides evidence that the scale at which movement is quantified may be important when addressing the nature of sex-biased tendencies. Understanding the evolutionary and ecological consequences of sex-specific movement may be, in large part, contingent on the ability to document the pervasiveness of such patterns across various spatiotemporal scales.
Table 1

Summary of movement behaviors for each individual throughout the study period

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<tr>
<th>Individual</th>
<th>Days Present in Data</th>
<th>Dominant Section</th>
<th>Mean Residence Time (hrs)</th>
<th>Proportion of Days Active</th>
<th>Mean Daily Movement Rate (m/hr)</th>
<th>Mean Daily Local Moves</th>
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**Males**

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Note: Days present in data indicates the day of last detection for each individual. Dominant section indicates section with cumulative highest residence and occupancy (parentheses). All individuals present for 30 days were captured post-study with the exception of 22910 that remained in the detection logs for two days following completion of the study.
Table 2

*Loadings of the principal components analysis (PCA) summarizing the habitat variables across all transects*

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Note: *N* = 90 transects.
Figure 6. Location of field movement study in south Mississippi.

Map of study location in south Mississippi. Shaded rectangle in the lower inset depicts the study reach in Big Creek.
**Figure 7.** Schematic illustration of potential movement behaviors, detection pattern and interpretation.

Schematic illustration depicting potential observed movement behaviors and associated interpretation. Displacement represents the summed minimum absolute distance an individual moved.

For residency, $t$ represents the amount of time (minutes) an individual resided in a given section before crossing a pair of antennae.
Figure 8. Principal components analysis of transects within the study reach.

Principal components analysis of transects within the antennae sections (shaded symbols) of the study reach. Larger symbols represent centroids (±2 SE) for stream sections. Vectors indicate the magnitude for the habitat variables with the highest loadings (> 0.5; see Table 2 for complete list of variable loadings).
Figure 9. Detail maps of transect measurements in the study reach.

Detail maps of point measurements taken along each transect (N = 90) throughout the study reach. Vertical dashed lines indicate placement of PIT antennae pairs and stream sections (1-5) are visualized from left to right. Circles in the top two panels (stream depth and stream flow) are scaled proportional to observed measurements (larger circles indicate increasing values). The lower panel represents structural complexity present at each point measurement (closed circles: woody structure; open circles: aquatic vegetation). Circle sizes indicate the type of woody structure present (see Methods for description). Small circles indicate small woody structure, medium circles indicate large woody structure and largest circles represent the presence of both small and large woody structure at a point measurement.
Figure 10. Sex-specific movement rates across densities.

Mean (± 1 SE) daily movement rates (meters per hour) of females (open circles) and males (closed circles) across levels of density.
Figure 11. Sex-specific proportion and number of local moves across densities.

Mean (± 1 SE) proportion (circles) and number of daily local moves (triangles) of females (open symbols) and males (closed symbols) across levels of density.
Figure 12. Sex-specific occupancy across sections within the study reach.

Mean (± 1 SD) daily occupancy (area corrected; individuals per 100 m$^2$) across the sections within the study reach.
Figure 13. Sex-specific residency across sections within the study reach.

Mean (± 1 SE) residence time (hours) for females (black) and males (grey) within each section. Values represent back-transformed means following analysis with log-transformed residence time.
Figure 14. Sex-specific section residency in relation to habitat gradients.

Mean area-corrected (PCA space) residence time for females (upper panel) and males (lower panel) in relation to section centroid scores. Symbol sizes are scaled based on residence times (female residence × 0.10; male residence × 0.50). Female residence time was positively related to sections along axis II. Centroids are labeled according to the section.
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CHAPTER III - THE EFFECTS OF PIT TAGS ON THE PHYSIOLOGY AND SWIMMING PERFORMANCE OF THE BLACKSPOTTED TOPMINNOW, *FUNDULUS OLIVACEUS*

Abstract

Within the last few decades, telemetry has proven a valuable tool for tracking or monitoring fishes. While the advent of smaller transmitters has made these methods increasingly applicable to small-bodied fishes, limited information currently exists regarding the efficacy of these transmitters or the putative consequences on small-bodied fishes. Consequently, I evaluated the physiological and performance consequences of passive integrated transponder (PIT) implantation in a small-bodied, sexually-dimorphic stream fish, *Fundulus olivaceus*. First, I used a common garden experiment, in an outdoor mesocosm setting, to assess the long-term effects of PIT tag implantation on a suite of physiological and phenotypic metrics. I randomly assigned 216 individuals (50-74 mm SL; equal sex ratio) to one of three tagging treatments: control (no tag), small (8 mm) or large (12 mm) PIT tag. Survivorship did not vary by treatment, and the presence of the tags was not found to have any effects on the condition or development of the intraperitoneal organs relative to control fish. Fish implanted with large PIT tags showed reduced growth rates relative to small PIT fish; however, this appeared to result in a trade-off that prioritized reproductive output. Secondly, I evaluated swimming performance (kinematics and endurance) to assess any hydrodynamic consequences of PIT tag implantation. Kinematics (tail-beat frequency, amplitude and total displacement) and endurance swimming were similar across all treatments and sexes, with only slight, idiosyncratic effects observed. These results indicate that 8 or 12 mm PIT tags are
appropriate for *F. olivaceus* greater than 50 mm SL, as no detrimental long-term effects were observed.

**Introduction**

The ability to uniquely identify individuals can provide valuable information on a wide range of ecological and management data such as habitat usage, growth and life-history data, movement patterns, population dynamics and mortality. Collectively, these patterns function to shape many ecological and evolutionary processes (e.g. species distributions, community composition), and basic life-history data is fundamental to a broader understanding of how animals interact with their environment. In the last few decades, advances in animal telemetry and logging devices have revolutionized the manner and scale at which we can monitor animals (e.g. Klimley et al. 1988, Hindell et al. 2002, Weng et al. 2005, Gilly et al. 2006; Naito et al. 2013, Schick et al. 2013). New types and sizes of animal tags and transmitters, coupled with technological advances, are transforming the questions that may be asked, as well as the way animal ecology is quantified and viewed (Wilmers et al. 2015; Hussey et al. 2015). These new approaches may provide researchers with an innovative set of tools to simultaneously capture and evaluate a wide array of biological and ecological patterns that were previously unattainable.

Historically, studies employing the use of internal or external transmitters with fishes have generally adhered to an arbitrary 2% rule (tag to body mass ratio; Winter 1983), which, in turn, precluded many small-bodied fish from such studies. However, tag to body mass ratios ranging from 2 – 12% have successfully been applied with no evidence of decline in physiological performance (e.g. Brown et al. 1999; Anglea et al.)
2004; Ammann et al. 2013), which has prompted criticism of the generality of this rule (e.g. Brown et al. 1999; Jepsen et al. 2002; Lacroix et al. 2004). While the tag to body mass ratio should certainly be scrutinized, other factors such as the nature of study design, implantation method and the species involved should be considered when choosing an appropriate type and size of transmitter. As most fishes have the ability to maintain neutral buoyancy in their environment, they possess physiological mechanisms to adjust buoyancy in response to the excess mass of affixed or implanted transmitters (Perry et al. 2001). Furthermore, as body morphology varies greatly among fishes, it is necessary to simultaneously consider other factors such as the dimensions, volume and position of the transmitter along with tag to body mass ratios (Brown et al. 1999).

In recent decades, passive integrated transponder (PIT) tags have become a beneficial tool in both research and management settings to monitor and track fishes. PIT tag technology has been successfully implemented in a wide array of experimental and natural applications including monitoring local habitat usage (Roussel et al. 2004; Teixeira & Cortes 2007), short and long term movement patterns (Bond et al. 2007; Booth et al. 2014; Clark & Schaefer 2016), the efficacy of fish passage systems (Castro-Santos et al. 1996; Archdeacon & Remshardt 2012) and individual growth and survival rates (Ruetz III et al. 2006; Wagner et al. 2007; Knaepkens et al. 2007; Kaemingk et al. 2011; Dixon & Mesa 2011). Furthermore, it has many advantages over the traditional monitoring methods used to collect these types of data. PIT tags are small (8-32 mm), unambiguously coded, relatively inexpensive, and have the potential to stay with an individual over the course of its entire lifespan. Alternative methods of marking individuals (e.g. injected elastomer tags, external tags, heat or freeze banding, fin
clipping) are temporally variable in their ability to identify individuals over time or monitor multiple sites simultaneously (Skalski et al. 2009). This has likely limited the value of datasets generated by these techniques. The size of PIT tags has historically limited studies to relatively large-bodied species (e.g. salmonids); however, recent development of smaller tags (8 and 9 mm) has made them increasingly applicable to small-bodied or juvenile fishes (e.g. Dixon & Mesa 2011; Bangs et al. 2013; Ward et al. 2015).

Independent of tag size, a number of assumptions are often made when employing PIT tags or any method of marking organisms. First, tags should not reduce survival and must remain with individuals for at least the duration of the study period. Numerous studies have evaluated the effects of PIT tags on growth, tag retention and mortality rates of fishes across a wide range of body sizes and morphologies (Table 1). Most have documented high tag retention and survival rates with minimal long term effects, but with some exceptions (Table 3). Secondly, it is assumed that the individual and its behavior are not affected by the presence of the tag. Despite the body of work aimed at assessing mortality and retention rates, evaluation of potential ecological or fitness consequences (e.g. behavioral, performance and physiological effects) has received less attention, especially for small-bodied fishes (Table 4). Lastly, it is also assumed that the behaviors captured during the marking and monitoring or recapture does not alter the expression of the normal behavior. Conventional marking that requires active recapture and handling may induce stress responses (Sharpe et al. 1998); however, PIT tags can provide a generally non-destructive method of resampling that results in minimal handling and disturbance. A variety of resampling methods have been developed for PIT tag studies,
ranging from trapping (Albanese et al. 2004), to semi-intensive resampling with portable underwater detection units (Cucherousset et al. 2005; Cucherousset et al. 2010; Booth et al. 2014) to passively monitored stations via stationary antennae (Bond et al. 2007; Johnston et al. 2009) which is more cost effective compared to more labor-intensive recapture techniques (Barbour et al. 2012; Cooke et al. 2013).

The purpose of this study was to evaluate the potential effects of PIT tag implantation on a suite of physiological and performance metrics in a small-bodied stream fish. Small-bodied fishes numerically dominate many temperate freshwater fish assemblages (Lee et al. 1980) and potentially exert a strong influence on ecosystem and community dynamics (Power et al. 1985); however less is known of their general biology and ecology. Effective individual marking of small-bodied fishes has often been problematic, with long-term studies limited by the relative size and retention rates of potential marking options (Skalski et al. 2009; Bangs et al. 2013). If a particular marking technique negatively affects life-history characteristics, performance or behavior, the data obtained may not be reflective of the population. First, I used a common garden approach to evaluate potential physiological and fitness (gonad development and state) effects due to PIT tag implantation. As intraperitoneal tags can occupy a relatively large volume of the body cavity, I was interested in whether this would have any negative effects on the development or condition of internal organs (i.e. digestive tract, gonads and liver) and if these effects were similar across sexes. Cooke et al. (2011) noted the paucity of data that exists in assessing the effects of intraperitoneal tags between sexes, especially in relation to fitness metrics. Secondly, I quantified swimming and kinematic performance in the
laboratory to assess any detrimental effects of PIT tags on the swimming ability and hydrodynamics.

Methods

Study species, collection and marking

The blackspotted topminnow, *Fundulus olivaceus*, is a small-bodied fish (maximum reported size 97 mm total length; Braasch & Smith 1965), that reaches sexual maturity at approximately 35 – 40 mm (Blanchard 1996; Vigueira et al. 2008). Adults are sexually-dimorphic, with males possessing elongated and more pointed dorsal and anal fins. Spawning occurs daily (mean of 1.6 eggs/day; Vigueira et al 2008) throughout a spawning season that lasts from March through September, with peak activity in May (Blanchard 1996). Throughout their geographic distribution they occur in a wide range of stream sizes; however, they are most abundant in the slow-moving waters of pools and stream margins (Howell & Black 1981). They are predominantly surface-feeders, with short digestive tracts that are somewhat unique in lacking a distinct stomach (Ghedotti & Davis 2013).

I collected adult *F. olivaceus* (> 50 mm SL; standard length) by dipnet during September and October 2013 (post spawning) from tributaries within the Pascagoula River drainage (Mississippi, USA). Fish were placed into outdoor mesocosm arrays (Matthews et al. 2006) and acclimated two weeks prior to marking procedures. Each mesocosm array (*n* = 3) consisted of six pools (183 cm in diameter) connected in series by riffle units (183 × 46 cm) with a recirculating pump and constant inflow of ground water. Substrate contained a mix of sand and gravel mined from local streams and water levels were maintained at approximately 55 cm above the substrate in the pools and 15
cm in the riffles. Spawning media were placed into the pools in early March and checked weekly to document normal spawning behavior. Following acclimation, 252 fish were randomly assigned to one of three treatments: control (no PIT tag), small PIT tag (8.4 mm length × 1.4 mm diameter; 0.032 g in air; Biomark, Inc.) or large PIT tag (12.0 mm length × 2.1 mm diameter; 0.101 g in air; Oregon RFID). Fish were anesthetized using MS-222 (tricaine methanesulphonate) before recording standard length (mm), wet mass (grams) and sex. Fish were marked with color coded visible implant elastomer (VIE; Northwest Marine Technologies, Shaw Island, WA) along the dorsum to indicate treatment and the appropriate PIT tag was surgically implanted (Clark & Schaefer 2016) into individuals receiving a PIT treatment (16 and 12 gauge needle for small and large PIT tags, respectively). No suture was used to close the puncture wound (Skov et al. 2005). I chose not to include a sham treatment (i.e. surgery but no PIT tag implantation) for logistic and housing space purposes, as priority was given to PIT treatments. PIT tags and injection needles were sterilized in ethanol prior to and following each tagging. Treatment specific VIE marking was necessary to identify treatment for any fish expelling tags during the study period. Fish were collected after a 10 day recovery to assess mortality, tag loss, and any external signs of infection. I observed relatively low levels of mortality during the 10 day period for PIT tagged (5.4% [tag sizes pooled]; 5 males, 4 females) and control fish (1 female), and no tags had been expelled. Successfully tagged fish were randomly assigned to one of three mesocosm arrays (12 of each sex/treatment combination into each array; 72 fish total per array; 216 total fish) and released on 5 November 2013. Fish remained in mesocosms until the following spring. I did not supplement the diet throughout the study period as macroinvertebrate
colonization is rapid and continuous, resulting in a similar assemblage composition as found in local natural streams (Matthews et al. 2006). Fish were then removed from mesocosms during the putative peak reproductive period (Blanchard 1996).

I collected additional adult *F. olivaceus* (> 50 mm SL) in the fall of 2014 to assess the potential effects of PIT tags on swimming kinematics and sustained swimming performance. I chose to run trials during the non-spawning portion of the year to eliminate any confounding effects due to reproductive production and maintenance. Fish were laboratory-held in a series of 110 L aquaria (*n* = 30) under a 12:12 h photoperiod at 19ºC for a period of 10 days prior to marking. I randomly assigned each fish to a treatment (same as above) and fitted each with the appropriate VIE and PIT tags. I marked 15 males and 13 females in each treatment (84 total fish) and allowed approximately 30 days to recover from tagging prior to use in performance trials. While in captivity, fish were fed once daily, *ad libitum*, with freeze-dried bloodworms and frozen brine shrimp (*Artemia* sp.). Prior to data collection in performance trials, test fish were fasted for 24 hours to ensure guts were empty and specific dynamic action minimized.

**Gut capacity and fitness assays**

All fish recovered from the mesocosms were transferred to 2,000 L rectangular holding tanks (*n* = 3) to obtain gut capacity data. Fish were acclimated to a diet of freeze-dried bloodworms for five days, fed once daily *ad libitum*. Guts were evacuated by starving fish for 48 hours, covering tanks to prevent external food from entering. Following the fasting period, fish were fed *ad lib* in a series of 15 minute exposures until uneaten food remained to ensure all were satiated. Fish were then overdosed with MS-
222 and stored in a 10% formalin solution until processing. Digestive tracts were extracted from all fish and contents were removed and dried for a period of 48 hours at 60°C and subsequently weighed (nearest 0.0001 g) to obtain a measure of gut capacity.

For each individual, I measured length (SL), gonad mass, liver mass, and body mass without gonads or digestive tract (eviscerated mass) to derive a suite of phenotypic metrics. Body condition was assessed using Fulton’s Condition Index (10,000 × eviscerated mass/SL$^3$), reproductive condition assessed with the gonadosomatic index (GSI; ratio of gonad mass to eviscerated mass), and relative liver size as the hepatosomatic index (HSI; ratio of hepatic mass to eviscerated mass) (Kaufman et al. 2007). As both GSI and HSI were greater in females (order of magnitude and double, respectively), I standardized all response metrics by sex (z-scores) prior to analysis. I used multivariate analysis of covariance (MANCOVA) to test for differences in the standardized metrics among tag treatments and sex using body size (SL) as a covariate and mesocosm array as a blocking factor. I quantified growth as the proportional change in mass of each fish relative to its initial mass when marked (i.e. change in mass/initial mass). As I did not uniquely mark control fish, all individual-based quantitative comparisons of growth was limited to the PIT treatments. I used analysis of covariance (ANCOVA) to assess the influence of PIT treatment (i.e. small vs large) and sex on the changes in growth with initial mass as a covariate.

As the relative proportion of the body cavity occupied by the gonads is greater for females, thus more space-limiting, I evaluated the influence of PIT tags on gonadal development and condition. To assess these putative effects, I counted and weighed (nearest 0.0001 g) the number of mature, hydrated eggs (Blanchard 1996) present for
each female. For each female, I obtained an average egg mass and calculated the proportion of the gonad represented by mature eggs (mass of mature eggs/mass of the gonad). I used individual ANCOVA models (eviscerated mass as covariate, mesocosm array as blocking factor) to test for differences in clutch parameters between treatments.

Swimming performance metrics

I measured swimming metrics within an acrylic swimming flume which measured 2.4 × 1.2 × 0.5 m. A piece of acrylic (1.6 × 0.5 m) divided the flume along the long axis but was open at both ends to allow water movement (Figure 15). The corners of the flume were fit with sheets of plexiglass, producing an oval shape to allow a smooth, unidirectional flow of water. Water was recirculated throughout the flume using a series of submersible magnetic drive pumps (Model 12 and Model 18, Danner Manufacturing, Inc.) that were engaged in series to increase flow to the desired velocity. A flow tunnel (110 cm length; interior diameter 15.2 cm) was located on the opposite side of the divider from the pumps and water within the tunnel was maintained at a depth of approximately 4.5 cm at base flow (5 cm/s). Fish were tested in a smaller section (70 cm) of the tunnel, enclosed in the upstream direction by a honeycomb baffle constructed of 0.95 cm diameter cPVC that served to maintain laminar flow throughout the test area. Wire screen (0.5 cm) restricted movement of fish in the downstream direction. Water temperature within the flume was maintained at 19ºC using a simultaneous combination of a heater and chiller unit.

Each individual swam at four test velocities until reaching exhaustion. Fish were first acclimated to the swimming tunnel for at least 20 minutes at 5 cm/s to recover from handling and to orient to the flow. I subsequently increased the velocity to 10, 20 and 30
cm/s at 5 minutes intervals and maintained 30 cm/s until the fish could no longer hold its position in the water column, remained at the downstream screen and would no longer respond to prodding. All test fish were measured (nearest mm) and weighed (nearest 0.01 g) following each trial. Endurance time (seconds) was quantified as the time spent at 30 cm/s before reaching exhaustion. I used an ANCOVA to evaluate the influence of tagging treatment, sex and body size on endurance time.

I video recorded trials with two GoPro Hero3 cameras (60 frames per second; mounted above swim tunnel) to quantify swimming kinematics at each velocity. For each fish, I extracted several seconds of video at three independent points at each velocity (n = 12 observations per fish) while minimal movement was observed. Videos were later digitized using Tracker video software (Brown 2014) to obtain X, Y coordinates (reference scale affixed to floor of swimming tunnel) to calculate tail-beat frequency and amplitude, averaged over five consecutive complete caudal oscillations. Tail-beat frequency was calculated by dividing 5 by the time required to complete the five consecutive tail oscillations to obtain an average number of beats per second. Tail-beat amplitude was defined as the distance between the dorsal edge of the caudal fin at the maximum lateral positions and averaged over five complete caudal oscillations. I also calculated total tail displacement (frequency × amplitude; Oufiero et al. 2014a) as an aggregate measure that describes the speed and distance the caudal fin travels during a complete oscillation. For all three kinematics traits, I used repeated measures ANCOVA models (individuals as subjects) to test the effects of tagging treatment, flow speed, sex and body size on swimming performance. For all models, I removed any non-significant
higher order interactions and interpreted the reduced models. All analyses were performed in the R statistical language (R Core Team 2014).

Results

**Mesocosm tagging and recovery**

At the time of tagging, males were larger (SL) than females ($F_{1,210} = 9.16, p = 0.003$), but sizes within sex did not differ among treatments (Treatment: $F_{2,210} = 0.42, p = 0.66$; Sex × Treatment: $F_{2,210} = 0.82, p = 0.44$). I first observed eggs on the spawning media on 7 April 2014 followed by free-swimming larvae on 24 April 2014. I recovered 197 fish from the mesocosms on 8 May and survivorship did not differ between treatments or sexes ($\chi^2 = 0.55$, d.f. = 2, $p = 0.76$). Confirmed mortalities and unrecovered PIT fish were of similar initial length and mass to the surviving fish. Three fish lost VIE markings; however all were positively identified by PIT detection. PIT retention was 100% for all surviving individuals. Ten individuals (1 male, 9 females) were removed from analyses upon processing due to the presence of nematodes in the digestive tract or gut cavity.

**Gut capacity and fitness assays**

There were no differences between tag treatments on the standardized physiological response metrics (MANCOVA; Tables 5 & 6). There was a significant effect of SL and a sex × SL interaction on the response matrix, suggesting different allometric trajectories between the sexes in the metrics quantified. Proportional growth of PIT fish was influenced by PIT treatment ($F_{1,115} = 20.16, p < 0.0001$), sex ($F_{1,115} = 22.33, p < 0.0001$), initial mass ($F_{1,115} = 123.73, p < 0.0001$) and a sex × initial mass interaction ($F_{1,115} = 8.68, p = 0.004$). Growth was greater in the small PIT treatment (Figure 16) and
proportional growth was negatively related to initial mass; however males indicated a higher capacity of growth.

Mature eggs were absent in seven females (three control and two in each PIT treatment); however no females were completely devoid of eggs. The average number of mature eggs did not differ between treatments ($F_{2,80} = 0.31, p = 0.74$; Table 4) or with body size ($F_{1,80} = 0.46, p = 0.50$). Similarly, the proportion of the total gonad mass represented by mature eggs did not differ between treatments ($F_{2,80} = 0.14, p = 0.87$; Table 4) or with body size ($F_{1,80} = 0.27, p = 0.61$). Excluding the females with no mature eggs, average mature egg mass differed between treatments ($F_{2,73} = 3.74, p = 0.03$) and across body mass ($F_{1,73} = 11.42, r^2 = 0.10, p = 0.001$). Mature egg mass was positively related to body mass and post hoc comparison among treatments revealed that control females produced significantly smaller eggs compared to the large PIT females ($p = 0.02$); however, mean egg mass did not differ between PIT treatments ($p = 0.81$) or between controls and small PIT tags ($p = 0.11$; Table 6).

Swimming performance

I tested 76 of the 84 individuals initially marked for performance trials. Five mortalities occurred within the first week post-marking (two control, two small PIT and one large PIT) and three died in the following weeks (one control and two large PIT). I further excluded two individuals that did not swim at 30 cm/s and another five individuals for which I did not obtain adequate video for analysis, leaving a final sample size of 69 for kinematic analyses (Table 7). Within each sex, SL did not differ between treatment groups (treatment × sex; $F_{2,63} = 1.18, p = 0.31$), but on average males were larger ($57.6 ± 0.74$ mm) than females ($55.5 ± 0.48$ mm).
There was a significant effect of flow speed \((F_{3,192} = 949.94, p < 0.001)\), SL \((F_{1,59} = 24.79, p < 0.001)\) and a flow speed × treatment interaction \((F_{6,192} = 2.90, p = 0.010)\) on tail-beat frequency (Figure 17A). To further resolve the effects of flow speed and treatment, I subsequently ran flow speed-specific ANCOVAs and post hoc Tukey comparisons on tail-beat frequency. SL was inversely related to tail-beat frequency at all flow speeds (all \(p <0.01\)). There was a significant effect of sex at 10 cm/s, where mean (± 1 SE) female tail-beat frequency \((4.80 ± 0.06 \text{ bps})\) was significantly greater than male \((4.61 ± 0.08 \text{ bps})\) tail-beat frequency \((F_{1,57} = 4.70, p = 0.034)\).

The final tail-beat amplitude model (all main effects and two-way interactions) indicated tail amplitude was influenced by flow speed \((F_{3,192} = 259.70, p < 0.001)\) and a flow speed × treatment interaction \((F_{6,192} = 3.42, p =0.003)\). The ensuing flow speed-specific ANCOVAs indicated no differences in tail-beat amplitude at 5, 10 or 20 cm/s; however tail-beat amplitude was influenced by treatment \((F_{2,57} = 3.53, p = 0.036)\), SL \((F_{1,57} = 7.56, p = 0.008)\) and a sex × SL interaction \((F_{1,57} = 7.23, p = 0.023)\) at 30 cm/s (Figure 17B). Mean tail-beat amplitude (± 1 SE) was greater in large PIT fish \((13.8 ± 1.3 \text{ mm})\) compared to control fish \((12.9 ± 1.2 \text{ mm}; \text{ post hoc Tukey: } p = 0.04)\) at 30 cm/s; however no differences existed among any other pairwise combinations. Mean tail-beat amplitude of females was unrelated to SL at 30 cm/s; however it increased positively with SL among males.

Total tail displacement (Figure 17C) was influenced by flow speed \((F_{3,171} = 3628.68, p <0.001)\) and a flow speed × treatment × SL interaction \((F_{6,171} = 3.69, p =0.002)\). Flow speed specific ANCOVAs indicated a significant treatment × SL interaction \((F_{2,57} = 4.47, p =0.016)\) at 5 cm/s. Total tail displacement was inversely
related to SL in the large PIT fish; but no relationship existed between tail displacement and standard length in the other treatments. At 10 cm/s, tail displacement was negatively correlated with SL ($F_{1,57} = 10.54, p = 0.002$). No significant effects on tail displacement existed at either 20 or 30 cm/s.

The final interpreted model for endurance time featured only the main effects and covariate. Body mass ($r^2 = 0.14, p<0.001$) was used as a covariate as it was a better predictor of endurance time compared to SL ($r^2 = 0.09, p<0.01$). Endurance time was similar among treatments ($F_{2,69} = 0.11, p = 0.90$) and sexes ($F_{1,69} = 1.39, p = 0.24$) and was positively related to mass ($F_{1,69} = 12.33, p = 0.001$; Figure 18). All individuals that swam at 30 cm/s ($n = 74$) were included in analysis of endurance time.

**Discussion**

Fundamental to any tagging study is that the chosen tagging method is not detrimental to basic life-history processes, fitness or behavior of the study organism. The combination of these experiments provides evidence that 8 or 12 mm PIT tags do not strongly influence the long-term physiology, fitness or performance, and are thus a valid tagging method, of adult *F. olivaceus* greater than 50 mm SL. In contrast to a number of studies reporting a positive relationship between observed or predicted survivorship and size (e.g. Baras et al. 2000; Acolas et al. 2007; Richard et al. 2013), survivorship of *F. olivaceus* was unrelated to tagging treatment and body size. Although I have no explanation for this discrepancy, I suspect the adult sizes used in this study likely minimized any detrimental effects related to size. Conversely, tagging success has been shown to be related to tagger experience (Ombredane et al. 1998; Bateman et al. 2006; Richard et al. 2013) and variable across implantation methods (e.g. injection, incision,
While low levels of mortality were observed immediately post tagging in the mesocosm tagged fish (5.4%), the extended period of time before the first confirmed mortality (121 days post tagging) suggests that these were unrelated to the tagging procedure. Although I did not incorporate a sham treatment as a secondary control and therefore cannot rule out potential surgery effects, mortality did not differ among treatments, thus I was confident that mortality throughout the study was related to senescence or other extrinsic factors. As tagging success has been shown to be dependent on a variety of factors (e.g. focal species, tag size, implantation method and tagger experience), I suggest that these factors should be considered and tested in the species of interest prior to implementing any tagging study.

I found no long-term effects of PIT tag implantation on the suite of intraperitoneal traits assessed in this study, indicating the presence of the tags did not negatively affect the development or condition of the primary visceral organs. Only a few studies that I am aware of evaluating the efficacy of PIT tags have used reproductive metrics or condition as evaluation endpoints (Baras et al. 2000; McCormick & Smith 2004). My results are in concordance with these studies as intrasexual GSI values were similar across all tagging treatments (Table 4). Aside from the females implanted with large tags having slightly larger eggs compared to control females, clutch parameters were similar across tagging treatments, suggesting the relative volume occupied by the tags (approximately 13.1 and 42.4 mm$^3$ for small and large tags, respectively) did not adversely affect the development of the ovaries or ova. However, this study provided only a snapshot of gonadal development in *F. olivaceus*. It may be of more utility to explore the effects of internal tags on clutch parameters (i.e., egg size, frequency, number) across an entire spawning
season to assess potential fecundity effects. For instance, species with protracted spawning periods, such as *F. olivaceus*, have ova that persist in a gradient of developmental stages within the ovaries (Heins & Rabito 1986; Blanchard 1996). Regardless, it appears that egg development and the ability to successfully deposit eggs were unaffected by PIT tag implantation.

Growth was reduced in large PIT fish compared to small PIT fish and more so for females than males. Different growth trajectories between the sexes was not surprising in itself, as male *F. olivaceus* are generally larger than females (Schaefer et al. 2012), and females must allocate a greater proportion of energy to reproduction (GSI; Table 4). Lacking appropriate comparisons with the control fish (not uniquely marked) limited the ability to assess the relative magnitude of these responses; however, I surmise any putative effects of tags on growth would most likely occur in females implanted with the large tags. However, the data collectively suggests that this may have resulted from females allocating energy to prioritize reproductive capacity as opposed to investing heavily into somatic growth (Wootton 1977). Across all treatments, females displayed similar reproductive investment (e.g. GSI, number and proportion of mature eggs) indicating that the presence of the tags had minimal effects on female fitness. Additionally, I also observed a slight trend of increased HSI for the fish implanted with large tags. As the liver functions as an important energy store and relates positively with fecundity (Marshall et al. 1999), the increased HSI may further provide support for a trade-off between energy allocation and somatic growth as females may have been storing energy to invest in egg maintenance. Thus, while females implanted with large
PIT tags may have incurred a tag-induced reduction in somatic growth, this was compensated for by maintaining reproductive output.

In contrast to my results, many studies evaluating growth effects of PIT tags have generally concluded that the tags typically do not impair growth or that the effects are limited to a short period immediately post-tagging (Table 1). However, due to the highly controlled nature (i.e. laboratory) and short duration of many of these studies, extrapolation to natural field settings should be exercised with caution. The fish assessed in this study were housed in outdoor mesocosms that closely reflected patterns experienced in natural streams. For instance, photoperiod and temperature are two factors that are known to temporally trigger certain physiological and life-history processes (e.g. spawning; Pankhurst & Porter 2003). Water temperatures in the mesocosms throughout the study period (mean = 12.1°C; range = 0.2-26.5°C) were comparable to those of a local third-order tributary (Cypress Creek, mean = 11.7°C; range = 1.5-22.6°C; USGS gauging station 02479155). Laboratory-held fish may lose synchronicity associated with these cues, consequently disrupting normal life-history patterns. The short-term duration of many studies further precludes assessment of transmitter effects across various physiological or life-history stages. Additionally, in opposition to the daily ad libitum feeding regimens implemented in many lab-based studies, I did not supplement the diet as the density and assemblage of invertebrate colonizers in the mesocosms are comparable to natural streams (Matthews et al. 2006; Bertrand & Gido 2007). Taken together, 8 or 12 mm PIT tags appear to be a viable monitoring tool for *F. olivaceus* in natural systems as daily (e.g. foraging) and seasonal (e.g. spawning) processes did not appear to be negatively impacted by the tags or by the ambient environment conditions.
Results of intraperitoneal transmitter implantation (i.e. not exclusively PIT tags) on swimming performance have been mixed across species examined. On one hand, detrimental effects have been seen at relatively low tag to body mass ratios (e.g. Zale et al. 2005); however, transmitters up to 12% of the body mass have successfully been implanted with no observable performance effects (Brown et al. 1999; Anglea et al. 2004; Ammann et al. 2013). In this study, tag to body mass ratios ranged from 0.9-2.1% and 2.6-5.8% for small and large PIT tagged fish, respectively, and did not consistently impair swimming kinematics or endurance swimming. Most of the observed differences in swimming kinematics were related to SL with a few idiosyncratic treatment and sex effects within certain velocities. For instance, within each speed, there was a noticeable inverse relationship between tail-beat frequency (Figure 17A) and amplitude (Figure 17B). Despite these patterns however, the cumulative effect (total tail displacement) was apparently negligible across treatments, especially at high velocities when individuals would seemingly need to optimize swimming biomechanics and thrust (Figure 17C). Behavioral differences may have allowed for more variable swimming strategies (e.g. increased frequency and decreased amplitude or vice versa) among individuals that ultimately resulted in similar thrust production (i.e. total tail displacement). However, as Fundulus spp. are typically found along the water surface in low flow habitats (Alldredge et al. 2011; Clark & Schaefer 2016), sustained swimming capacity may be under weaker selective pressures compared to alternative swimming performance metrics (e.g. burst-start performance) needed to complete life-history or physiological demands (e.g. prey capture, predator avoidance) (Langerhans 2009). Further studies testing for effects across
multiple movement types may be able isolate potential tagging consequences or potential tag related trade-offs between modes of swimming.

Interestingly, I observed only slight differences between the sexes in swimming performance metrics. Male *F. olivaceus* bear exaggerated morphological structures (elongated dorsal and anal fins) that would presumably impose physiological or functional performance costs (Oufiero & Garland 2007). My results suggest the elongated fins did not induce a cost on swimming as males did not compensate by altering swimming kinematics compared to females, or alternatively, other physiological or compensatory mechanisms functioned to decrease the cost (e.g. aerobic capacity; Oufiero et al. 2014b). It is important to note however, that I quantified swimming performance during the non-spawning season, thus any performance trade-offs associated with female reproductive status is unknown. The increased mass associated with developing or ripe ovaries (Helfman et al. 2009), coupled with a concomitant increase in cross-sectional body area, may hydrodynamically alter swimming mechanics, and consequently the performance of gravid females (Plaut 2002). Furthermore, as a greater proportion of energy must be allocated to reproductive tissues, energy for routine metabolic processes may not be readily available, consequently resulting in reduced growth and swimming performance (Billerbeck et al. 2001).

Perhaps one of the most beneficial uses of PIT tags and other biologging devices moving forward may be in application to species of concern (Cooke 2008). Most conservation attempts are confounded by the large proportion of rare species among diverse communities, and little is known of the general biology and life history characteristics of these threatened taxa (Dudgeon et al. 2006). While these factors are
undoubtedly fundamental in conservation and management planning, the development of
effective conservation strategies for taxa worldwide must be based on strong scientific
assessments that include factors associated with the ecology, demographics and
structuring of local populations. PIT tags offer a novel avenue to pursue such studies,
offering the ability to simultaneously collect a multitude of data with distinct advantages
over alternative methods (reviewed in Gibbons & Andrews 2004). Perhaps, most
importantly, PIT tags offer the ability to study organisms in their natural environment,
exposed to natural biotic and abiotic cues not easily reproducible in controlled settings.
Secondly, as PIT tags will generally stay within an individual for the remainder of its life,
they offer the ability to collect long-term datasets; data that are often logistically and
monetarily difficult to obtain. This may be especially important in highly variable and
complex environments (e.g. aquatic systems) where focal individuals are often difficult to
locate or capture due to their varying degrees of size, mobility or behaviors (Cooke et al.
2013; Hussey et al. 2015). Furthermore, collection of more detailed data also reduces the
need for extensive sample sizes, especially important when conducting studies with
species of concern (Cooke 2008). While PIT tags and other telemetry or logging
techniques certainly pose logistic limitations and ethical challenges, especially in regards
to threatened taxa (Gibbons & Andrews 2004; Cooke 2008), continued exploration of
their utility as a monitoring tool may yield innovative approaches to simultaneously
collect suites of ecological and life-history data.

The results from this study suggest that both 8 and 12 mm PIT tags can be reliably
used in adult *F. olivaceus*, and presumably other *Fundulus* spp. of similar or greater size.
Furthermore, these results indicate that PIT tags may be applicable to natural settings as
the experimental mesocosm fish were exposed to quasi-natural abiotic conditions, over an ecologically-relevant time period, rather than laboratory-based evaluations in highly controlled environments. Moreover, I was able to document normal life-history patterns (i.e. spawning) in conjunction with the presence of PIT tag implantation. Assuredly, other factors not evaluated in this study (e.g. predation rates on tagged versus untagged fish) have the potential to affect behavior or survivorship; and studies addressing such factors are warranted. While PIT tags and other telemetry methods have only become viewed as viable tools in conservation actions within the last few decades (Cooke 2008), data on the feasibility of using such an approach with threatened fishes seems promising (Archdeacon et al. 2009; Archdeacon & Remshardt 2012; Bangs et al. 2013; Ward et al. 2015). Obviously any study attempting to employ the use of PIT tags, or any other logging or telemetry method, must first assess the appropriateness of a particular method in terms of cost, logistics and possible consequences in relation to the focal species and study objectives. Similar studies to this one, across a broader range of taxa, that evaluate not only tag retention and mortality, but also suites of performance, physiological, phenotypic and fitness-related traits, may allow for a more holistic assessment of potential tagging consequences on target species.
Table 3

Results from studies examining effects of PIT tag implantation on growth, tag retention and survival across 13 families of fish.

<table>
<thead>
<tr>
<th>Species</th>
<th>Body Length (mm)</th>
<th>PIT Tag Size (mm)</th>
<th>Study Duration</th>
<th>Growth Effects</th>
<th>Retention (%)</th>
<th>Survival (%)</th>
<th>Survival Affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anguillidae</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anguilla australis</td>
<td>39 ± 1.8 (TL)</td>
<td>11</td>
<td>108 days</td>
<td>No</td>
<td>96%</td>
<td>94%</td>
<td>No</td>
</tr>
<tr>
<td>Catostomidae</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Catostomus commersoni</td>
<td>100-158 (TL)</td>
<td>12</td>
<td>30 days</td>
<td>—</td>
<td>100%</td>
<td>32%</td>
<td>Yes</td>
</tr>
<tr>
<td>Catostomus commersoni</td>
<td>102-172 (TL)</td>
<td>23</td>
<td>30 days</td>
<td>—</td>
<td>100%</td>
<td>44%</td>
<td>Yes</td>
</tr>
<tr>
<td>Deltistes luxatus</td>
<td>61-85 (SL)</td>
<td>12</td>
<td>34 days</td>
<td>—</td>
<td>98%</td>
<td>90%</td>
<td>—</td>
</tr>
<tr>
<td>Cottidae</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cottus bairdii</td>
<td>56-83 (TL)</td>
<td>12</td>
<td>28 days</td>
<td>ST</td>
<td>&gt;96%</td>
<td>&gt;96%</td>
<td>No</td>
</tr>
<tr>
<td>Cottus gobio</td>
<td>50-94 (TL)</td>
<td>12</td>
<td>49 days</td>
<td>No</td>
<td>95%&lt;sup&gt;a&lt;/sup&gt;</td>
<td>95%&lt;sup&gt;a&lt;/sup&gt;</td>
<td>No</td>
</tr>
<tr>
<td>Cyprinidae</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gila cypha</td>
<td>40-79 (TL)</td>
<td>8</td>
<td>60 days</td>
<td>No</td>
<td>90%&lt;sup&gt;a&lt;/sup&gt;</td>
<td>95%&lt;sup&gt;a&lt;/sup&gt;</td>
<td>BSD</td>
</tr>
<tr>
<td>Gila cypha</td>
<td>40-79 (TL)</td>
<td>12</td>
<td>60 days</td>
<td>No</td>
<td>83%&lt;sup&gt;a&lt;/sup&gt;</td>
<td>89%&lt;sup&gt;a&lt;/sup&gt;</td>
<td>BSD</td>
</tr>
<tr>
<td>Leuciscus cephalus</td>
<td>113 ± 6.4 (FL)</td>
<td>12</td>
<td>182 days</td>
<td>—</td>
<td>100%</td>
<td>97.5</td>
<td>No</td>
</tr>
<tr>
<td>Leuciscus cephalus</td>
<td>125 ± 6.4 (FL)</td>
<td>23</td>
<td>182 days</td>
<td>—</td>
<td>100%&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&gt;98%&lt;sup&gt;b&lt;/sup&gt;</td>
<td>No</td>
</tr>
<tr>
<td>Leuciscus leuciscus</td>
<td>115 ± 3.7 (FL)</td>
<td>12</td>
<td>182 days</td>
<td>—</td>
<td>100%</td>
<td>96.3%</td>
<td>No</td>
</tr>
<tr>
<td>Leuciscus leuciscus</td>
<td>121 ± 7.4 (FL)</td>
<td>23</td>
<td>182 days</td>
<td>96.6%</td>
<td>72.5%</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Oregonichthys crameri</td>
<td>55 ± 0.7 (TL)</td>
<td>8</td>
<td>150 days</td>
<td>—</td>
<td>95%</td>
<td>94%</td>
<td>No</td>
</tr>
<tr>
<td>Oregonichthys crameri</td>
<td>55 ± 0.7 (TL)</td>
<td>9</td>
<td>150 days</td>
<td>—</td>
<td>89%</td>
<td>82%</td>
<td>Yes</td>
</tr>
<tr>
<td>Platygobio gracilis</td>
<td>100-149 (TL)</td>
<td>12</td>
<td>30 days</td>
<td>—</td>
<td>100%</td>
<td>100%</td>
<td>No</td>
</tr>
<tr>
<td>Platygobio gracilis</td>
<td>97-140 (TL)</td>
<td>23</td>
<td>30 days</td>
<td>—</td>
<td>100%</td>
<td>100%</td>
<td>No</td>
</tr>
<tr>
<td>Rutilus rutulus</td>
<td>116 ± 5.3 (FL)</td>
<td>12</td>
<td>182 days</td>
<td>—</td>
<td>100%</td>
<td>100%</td>
<td>No</td>
</tr>
<tr>
<td>Rutilus rutulus</td>
<td>123 ± 6.8 (FL)</td>
<td>23</td>
<td>182 days</td>
<td>98.7%</td>
<td>97.5%</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Rutilus rutulus</td>
<td>15-30 g</td>
<td>23</td>
<td>37 days</td>
<td>No</td>
<td>100%</td>
<td>96%</td>
<td>No</td>
</tr>
<tr>
<td>Scardinus erythrophthalmus</td>
<td>17-47 g</td>
<td>23</td>
<td>37 days</td>
<td>No</td>
<td>100%</td>
<td>94%</td>
<td>No</td>
</tr>
<tr>
<td>Semotilus atromaculatus</td>
<td>105-155 (TL)</td>
<td>12</td>
<td>30 days</td>
<td>—</td>
<td>95%</td>
<td>68%</td>
<td>Yes</td>
</tr>
<tr>
<td>Semotilus atromaculatus</td>
<td>94-163 (TL)</td>
<td>23</td>
<td>30 days</td>
<td>—</td>
<td>100%</td>
<td>63%</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Table 3 (continued).

<table>
<thead>
<tr>
<th>Family</th>
<th>Species Name</th>
<th>Length (range)</th>
<th>Age</th>
<th>Mortality</th>
<th>Loss</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Centrarchidae</td>
<td><em>Lepomis macrochirus</em></td>
<td>75-162 (TL)</td>
<td>12</td>
<td>42 days</td>
<td>No</td>
<td>&gt;80%</td>
</tr>
<tr>
<td></td>
<td><em>Micropterus salmoides</em></td>
<td>180–404 (TL)</td>
<td>12</td>
<td>12 months</td>
<td>—</td>
<td>98%</td>
</tr>
<tr>
<td>Esocidae</td>
<td><em>Esox masquinongy</em> (Illinois)</td>
<td>302 ± 0.8 (TL)</td>
<td>12</td>
<td>153 days</td>
<td>No</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td><em>Esox masquinongy</em> (Wisconsin)</td>
<td>284 ± 1.1 (TL)</td>
<td>12</td>
<td>210 days</td>
<td>No</td>
<td>99.5%</td>
</tr>
<tr>
<td>Gadidae</td>
<td><em>Lota lota</em></td>
<td>65-92 (TL)</td>
<td>9</td>
<td>28 days</td>
<td>No</td>
<td>97%</td>
</tr>
<tr>
<td>Goodeidae</td>
<td><em>Crenichthyes baileyi moapae</em></td>
<td>41-67 (TL)</td>
<td>9</td>
<td>41 days</td>
<td>—</td>
<td>100%</td>
</tr>
<tr>
<td>Percidae</td>
<td><em>Perca flavescens</em></td>
<td>75-162 (TL)</td>
<td>12</td>
<td>42 days</td>
<td>No</td>
<td>&gt;80%</td>
</tr>
<tr>
<td></td>
<td><em>Perca fluviatilis</em></td>
<td>55-96 (FL)</td>
<td>11</td>
<td>126 days</td>
<td>ST</td>
<td>&gt;80%</td>
</tr>
<tr>
<td></td>
<td><em>Sander lucioperca</em></td>
<td>(\bar{x} = 188) (SL)</td>
<td>12</td>
<td>56 days</td>
<td>No</td>
<td>100%</td>
</tr>
<tr>
<td>Petromyzontidae</td>
<td><em>Entosphenus tridentatus</em></td>
<td>120-165 (TL)</td>
<td>12</td>
<td>40 days</td>
<td>—</td>
<td>96%</td>
</tr>
<tr>
<td></td>
<td><em>Entosphenus tridentatus</em></td>
<td>120-165 (TL)</td>
<td>9</td>
<td>180 days</td>
<td>No</td>
<td>95%</td>
</tr>
<tr>
<td>Pomacentridae</td>
<td><em>Pomacentrus amboinensis</em></td>
<td>45-65 (SL)</td>
<td>11.5</td>
<td>47 days</td>
<td>No</td>
<td>100%</td>
</tr>
<tr>
<td>Salmonidae</td>
<td><em>Oncorhynchus mykiss</em></td>
<td>73-97 (FL)</td>
<td>23</td>
<td>30 days</td>
<td>ST</td>
<td>97%</td>
</tr>
<tr>
<td></td>
<td><em>Oncorhynchus tsawytscha</em></td>
<td>Not Reported</td>
<td>12</td>
<td>28 days</td>
<td>—</td>
<td>&gt;99%</td>
</tr>
<tr>
<td></td>
<td><em>Oncorhynchus tsawytscha</em></td>
<td>40-69 (FL)</td>
<td>8</td>
<td>28 days</td>
<td>No</td>
<td>95-98%</td>
</tr>
<tr>
<td></td>
<td><em>Oncorhynchus tsawytscha</em></td>
<td>40-69 (FL)</td>
<td>9</td>
<td>28 days</td>
<td>No</td>
<td>97-99%</td>
</tr>
<tr>
<td></td>
<td><em>Oncorhynchus tsawytscha</em></td>
<td>50-69 (FL)</td>
<td>12</td>
<td>28 days</td>
<td>No</td>
<td>93-99%</td>
</tr>
<tr>
<td></td>
<td><em>Salmo salar</em></td>
<td>46-182 (FL)</td>
<td>12</td>
<td>9 months</td>
<td>—</td>
<td>&gt;99%</td>
</tr>
<tr>
<td></td>
<td><em>Salmo trutta</em></td>
<td>41-70 (FL)</td>
<td>12</td>
<td>28 days</td>
<td>No</td>
<td>BSD</td>
</tr>
<tr>
<td></td>
<td><em>Salmo trutta</em></td>
<td>50-63 (TL)</td>
<td>12</td>
<td>60 days</td>
<td>BSD</td>
<td>BSD</td>
</tr>
<tr>
<td>Sparidae</td>
<td><em>Chrysophrys auratus</em></td>
<td>132 ± 13 (FL)</td>
<td>14</td>
<td>70 days</td>
<td>No</td>
<td>&gt;92%</td>
</tr>
</tbody>
</table>

*a* denotes significant difference from the control group.
Table 3 (continued).

<table>
<thead>
<tr>
<th>Species</th>
<th>Weight (g) ± Error</th>
<th>Age/Condition</th>
<th>Treatment</th>
<th>BSD</th>
<th>ST</th>
<th>FL</th>
<th>SL</th>
<th>TL</th>
<th>BSD Ind.</th>
<th>ST Ind.</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Sparus aurata</em> [27]</td>
<td>3.5 ± 0.03 g</td>
<td>11</td>
<td>26 &amp; 66 days</td>
<td>ST</td>
<td>&gt;86% b</td>
<td>97% b</td>
<td>No</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


* All size classes evaluated were pooled; *b* Experiments were pooled; FL = fork length; SL = standard length; TL = total length; BSD indicates body size dependent; ST indicates short term; ‘—’ indicates metric not evaluated.
Table 4

Results from studies examining the effects of PIT tag implantation on swimming performance metrics (burst-start and sustained) across four families of fish

<table>
<thead>
<tr>
<th>Species</th>
<th>PIT Tag Size (mm)</th>
<th>Performance Metric</th>
<th>Performance Differ from Control</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Catostomidae</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Catostomus commersoni</em> [1]</td>
<td>12</td>
<td>Sustained</td>
<td>Yes (lower)</td>
</tr>
<tr>
<td><em>Catostomus commersoni</em> [1]</td>
<td>23</td>
<td>Sustained</td>
<td>Yes (lower)</td>
</tr>
<tr>
<td><strong>Cyprinidae</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Platygobio gracilis</em>  [1]</td>
<td>12</td>
<td>Sustained</td>
<td>No</td>
</tr>
<tr>
<td><em>Platygobio gracilis</em>  [1]</td>
<td>23</td>
<td>Sustained</td>
<td>No</td>
</tr>
<tr>
<td><em>Semotilus atromaculatus</em> [1]</td>
<td>12</td>
<td>Sustained</td>
<td>No</td>
</tr>
<tr>
<td><em>Semotilus atromaculatus</em> [1]</td>
<td>23</td>
<td>Sustained</td>
<td>No</td>
</tr>
<tr>
<td><strong>Cottidae</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Cottus gobio</em> [2]</td>
<td>12</td>
<td>Burst-start</td>
<td>No</td>
</tr>
<tr>
<td><strong>Petromyzontidae</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Lampetra tridentate</em>  [3]</td>
<td>12</td>
<td>Burst-start</td>
<td>Yes (lower)</td>
</tr>
<tr>
<td><em>Lampetra tridentate</em>  [3]</td>
<td>12</td>
<td>Sustained</td>
<td>No</td>
</tr>
</tbody>
</table>

Table 5

Results from MANCOVA models testing effects of tag treatment, sex and standardized SL on the standardized intraperitoneal metrics quantified

<table>
<thead>
<tr>
<th>Effect</th>
<th>Pillai</th>
<th>DF</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tag Treatment</td>
<td>0.053</td>
<td>8,342</td>
<td>1.16</td>
<td>0.32</td>
</tr>
<tr>
<td>Sex</td>
<td>0.0003</td>
<td>4,170</td>
<td>0.01</td>
<td>0.99</td>
</tr>
<tr>
<td>SL</td>
<td>0.314</td>
<td>4,170</td>
<td>19.47</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tag Treatment × Sex</td>
<td>0.033</td>
<td>8,342</td>
<td>0.71</td>
<td>0.68</td>
</tr>
<tr>
<td>Tag Treatment × SL</td>
<td>0.030</td>
<td>8,342</td>
<td>0.64</td>
<td>0.74</td>
</tr>
<tr>
<td>Sex × SL</td>
<td>0.100</td>
<td>4,170</td>
<td>4.71</td>
<td>0.001</td>
</tr>
<tr>
<td>Tag Treatment × Sex × SL</td>
<td>0.025</td>
<td>8,342</td>
<td>0.54</td>
<td>0.83</td>
</tr>
</tbody>
</table>
Table 6

*Summary of tag treatment effects of sex and treatments. Sample sizes reflect individuals that were retained for analysis*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Condition</th>
<th>GSI</th>
<th>HSI</th>
<th>Gut Content Mass (g)</th>
<th>Number of Mature Eggs</th>
<th>Proportion of Mature Eggs</th>
<th>Mean Egg Mass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Males</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>32</td>
<td>0.121 ±</td>
<td>0.0050 ±</td>
<td>0.0131 ±</td>
<td>0.0293 ±</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.006</td>
<td>0.0009</td>
<td>0.0018</td>
<td>0.0110</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Small PIT</td>
<td>36</td>
<td>0.118 ±</td>
<td>0.0048 ±</td>
<td>0.0131 ±</td>
<td>0.0270 ±</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.007</td>
<td>0.0011</td>
<td>0.0015</td>
<td>0.0090</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Large PIT</td>
<td>31</td>
<td>0.121 ±</td>
<td>0.0046 ±</td>
<td>0.0135 ±</td>
<td>0.0307 ±</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.008</td>
<td>0.0008</td>
<td>0.0018</td>
<td>0.0108</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><strong>Females</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>31</td>
<td>0.121 ±</td>
<td>0.0648 ±</td>
<td>0.0226 ±</td>
<td>0.0191 ±</td>
<td>8.87 ±</td>
<td>0.247 ±</td>
<td>0.0047 ±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.006</td>
<td>0.0220</td>
<td>0.0033</td>
<td>0.0056</td>
<td>6.83</td>
<td>0.144</td>
<td>0.0004</td>
</tr>
<tr>
<td>Small PIT</td>
<td>28</td>
<td>0.121 ±</td>
<td>0.0605 ±</td>
<td>0.0226 ±</td>
<td>0.0185 ±</td>
<td>7.68 ±</td>
<td>0.225 ±</td>
<td>0.0049 ±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.006</td>
<td>0.0218</td>
<td>0.0030</td>
<td>0.0081</td>
<td>7.81</td>
<td>0.163</td>
<td>0.0005</td>
</tr>
<tr>
<td>Large PIT</td>
<td>29</td>
<td>0.119 ±</td>
<td>0.0630 ±</td>
<td>0.0231 ±</td>
<td>0.0200 ±</td>
<td>7.62 ±</td>
<td>0.230 ±</td>
<td>0.0050 ±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.008</td>
<td>0.0278</td>
<td>0.0028</td>
<td>0.0070</td>
<td>6.18</td>
<td>0.140</td>
<td>0.0004</td>
</tr>
</tbody>
</table>

Note: Values represent means ± 1 SD.
Table 7

*Summary of the size (standard length; SL), mass (g) and body condition of fish used in performance and endurance trials.*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>SL (mm)</th>
<th>Mass (g)</th>
<th>Condition</th>
<th>Tag:Body Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Males</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>14 (13)</td>
<td>58.4 ± 4.6</td>
<td>2.57 ± 0.73</td>
<td>0.126 ± 0.007</td>
<td>—</td>
</tr>
<tr>
<td>Small PIT</td>
<td>15 (13)</td>
<td>55.9 ± 4.0</td>
<td>2.19 ± 0.55</td>
<td>0.124 ± 0.009</td>
<td>0.014 ± 0.003</td>
</tr>
<tr>
<td>Large PIT</td>
<td>12 (10)</td>
<td>59.2 ± 4.4</td>
<td>2.74 ± 0.67</td>
<td>0.130 ± 0.012</td>
<td>0.037 ± 0.010</td>
</tr>
<tr>
<td><strong>Females</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>12 (12)</td>
<td>55.1 ± 2.2</td>
<td>2.00 ± 0.28</td>
<td>0.119 ± 0.007</td>
<td>—</td>
</tr>
<tr>
<td>Small PIT</td>
<td>11 (11)</td>
<td>55.2 ± 3.5</td>
<td>2.06 ± 0.39</td>
<td>0.121 ± 0.005</td>
<td>0.015 ± 0.003</td>
</tr>
<tr>
<td>Large PIT</td>
<td>10 (10)</td>
<td>56.3 ± 2.6</td>
<td>2.23 ± 0.31</td>
<td>0.124 ± 0.004</td>
<td>0.044 ± 0.007</td>
</tr>
</tbody>
</table>

Note: Sample size for kinematic analyses are in parentheses. Values represent means ± 1 SD.
Figure 15. Schematic diagram of swimming flume used in performance trials.
Figure 16. Sex-specific proportional growth rates across tagging treatments.

Mean ± 2 SE proportional growth of males (closed circles) and females (open circles) across tagging treatments. The control treatment is visualized for purposes of comparison and was not included in analyses. Control values represent the mean growth change pooling all control individuals within each mesocosm array (n = 3).
Figure 17. Swimming kinematics of tagging treatments across flow speeds.

Mean ± 1 SD (A) tail-beat beat frequency (beats/sec), (B) tail-beat amplitude (mm), and (C) total tail displacement (mm × beats/sec) among experimental tagging treatments at the four test speeds.
Figure 18. Endurance swimming across tagging treatments.

Endurance times (log, transformed seconds) of the control (open), small (black) and large (grey) PIT fish at 30 cm/s against body mass (grams).
References


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APPENDIX A – IACUC Approval Letter

THE UNIVERSITY OF SOUTHERN MISSISSIPPI
Institutional Animal Care and Use Committee

118 College Drive #5147
Hattiesburg, MS 39406-0001
Phone: 601-266-4063
Fax: 601-266-4377

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE NOTICE OF COMMITTEE ACTION

The proposal noted below was reviewed and approved by The University of Southern Mississippi Institutional Animal Care and Use Committee (IACUC) in accordance with regulations by the United States Department of Agriculture and the Public Health Service Office of Laboratory Animal Welfare. The project expiration date is noted below. If for some reason the project is not completed by the end of the three year approval period, your protocol must be reactivated (a new protocol must be submitted and approved) before further work involving the use of animals can be done.

Any significant changes (see attached) should be brought to the attention of the committee at the earliest possible time. If you should have any questions, please contact me.

PROTOCOL NUMBER: 13041102
PROJECT TITLE: An experimental study of convergent evolution and species fusion in replicate Fundulus hybrid zones
PROPOSED PROJECT DATES: April 2013 – September 2015
PROJECT TYPE: New
PRINCIPAL INVESTIGATOR(S): Jake Schaefer
DEPARTMENT: Biological Sciences
FUNDING AGENCY/SPONSOR: NSF-Pending
IACUC COMMITTEE ACTION: Designated Member Review
PROTOCOL EXPIRATION DATE: September 30, 2015

Jodie M. Jawor, Ph.D.
IACUC Chair

6/23/2012
Date