COASTAL RECREATIONAL FISHING LICENSE

FINAL PERFORMANCE REPORT

Grant Recipients: Frederick Scharf, J. Wilson White, Chris Batsavage, Chip Collier

Grant Award #: 2012-F-001, contract number 4484

Grant Title: An acoustic tagging study to evaluate migration dynamics and within-estuary habitat use of southern flounder (*Paralichthys lethostigma*) in North Carolina

Grant Award Period: July 1, 2012 – June 30, 2015

Report authors: Frederick S. Scharf, J. Wilson White, Lisa D. Hollensead, and Nathan D. Messenger

Report date: July 21, 2015

Background/Justification

The southern flounder (*Paralichthys lethostigma*) supports fisheries of economic and cultural importance throughout its entire range in the US South Atlantic and northern Gulf of Mexico. In North Carolina, the species represents one of the most sought after inshore finfish resources in the state, with the recreational fishery experiencing rapid growth as human development and population sizes have increased in coastal counties. Since 2000, annual recreational catches have been more than three times higher than those observed during the 1990's, with recent recreational hook and line landings of southern flounder approaching 400,000 pounds per year (NCDMF 2010). The NC stock has recently been categorized as 'depleted', believed to be mainly related to a long period of elevated commercial harvest rates beginning in the early 1990's. In 2005, the NC fishery management plan for southern flounder (NCDMF 2010) established several new commercial and recreational harvest restrictions in an attempt to lessen the impact on the stock by reducing fishing mortality rates. Yet, the most recent stock assessment concluded that while the condition of the NC southern flounder stock has likely improved since 2005, it remains overfished and overfishing is still occurring (Takade-Heumacher and Batsavage 2009). In response, a 2010 amendment to the NC southern flounder fishery management plan (NCDMF 2010) included a reduced bag limit (from 8 fish to 6 fish) and an increased size limit (to 15 inches) to lower harvest rates in the recreational fishery. Large reductions in commercial effort and harvest were projected due to a series of regulatory measures that were put in place in 2010 by proclamation authority to minimize commercial fishery interactions with protected species (primarily sea turtles).

Recovery of the NC southern flounder stock can be promoted through an improved understanding of habitat use and migration dynamics in order to identify essential habitats for conservation and determine the most effective locations and times for harvest restrictions. Throughout their range, southern flounder inhabit riverine, estuarine, and coastal ocean waters. Spawning occurs offshore, primarily during winter months (Dec - Mar), and developing larvae are transported through inlets and then settle toward the head of estuaries during the winter and early spring (Wenner et al. 1990; Monaghan and Armstrong 2000). After achieving rapid growth while occupying estuarine habitats, immature fish (generally < 2 years of age) are thought to remain in estuaries while mature individuals migrate offshore in the fall to spawn. Both commercial and recreational sectors of the fishery are executed throughout upper and lower reaches of estuaries during summer and fall; there is limited fishing pressure (mostly from recreational spear fishers) on southern flounder in offshore or coastal waters.

Although their general life history strategy and migration pattern are known, several aspects of southern flounder habitat use and migration remain poorly understood. For example, historically, it was believed that most adult flounder returned to estuaries in the spring and summer following offshore spawning. However, diving surveys in NC coastal waters (Watterson and Alexander 2004) and analysis of otolith chemical signatures (Taylor et al. 2008) suggest that a portion of mature flounder remain in nearshore coastal waters (and inaccessible to the estuarine fishery) following spawning. These conclusions are based on regular sightings of large, adult flounder in ocean waters during the summer months and chemical signatures that did

not indicate re-entry into lower salinity habitats. It is possible that a contingent of mature flounder exists that utilize primarily high salinity waters adjacent to (just inside or outside of) ocean passes and inlets, making regular excursions into estuarine waters, but not residing in the estuary for considerable time periods. The extent to which these individuals move into and out of estuarine waters, and are thus subject to heavier fishing pressure, is unknown.

The movements of juvenile and sub-adult southern flounder while residing within estuarine habitats, as well as the timing and routes of migration to and from offshore habitats are also not well understood. Other *Paralichthys spp*. (summer flounder, *P. dentatus* and gulf flounder, *P. albigutta*) which co-occur in NC waters, utilize primarily high salinity habitats throughout most of their life history, whereas southern flounder are known to make more extensive use of oligohaline waters during juvenile and sub-adult life stages (Powell and Schwartz 1977; Stokes 1977; Wenner et al. 1990; Burke et al. 1991; Walsh et al. 1999). In fact, recent studies in both Atlantic and Gulf of Mexico estuaries have revealed more extensive use of freshwater habitats by southern flounder than previously believed (Rulifson et al. 2009; Lowe et al. 2011). However, we still know very little about the relative importance of specific habitat regions (e.g., oligohaline, mesohaline, polyhaline) within estuarine systems, or the effects on habitat use of ontogeny and seasonal variation in environmental conditions. Several traditional tag-return studies have been conducted for southern flounder in South Atlantic estuaries during the past three decades (GA: Music and Pafford 1984; SC: Wenner et al. 1990; NC: Monaghan 1996; Craig et al. in press). Collectively, the spatial information obtained from tag returns provides evidence for: (1) limited within-estuary movement, (2) the return of some individuals to their natal estuarine waters after spawning, (3) greater distances moved by larger individuals, (4) earlier migration for larger individuals, (5) a high likelihood that many young fish (age-0 and age-1) overwinter in the estuary, and (6) that nearly all large scale (> 20 km) movement outside of the estuary is in a southerly direction. However, traditional tag-return approaches have several limitations that impede the interpretation of the spatial information obtained. The primary limitation is that only the spatial endpoints (tagging and recapture locations) are known, with no information on routes of movement or durations spent in different habitats. For example, the finding of limited within-estuary movement for most fishes can be biased by short times at large due to heavy fishing pressure in the area of tagging. The evidence for fish returning to their natal estuary after offshore spawning is weakened because it is not known with certainty that an individual actually emigrated from the system. Similarly, the notion that larger individuals may initiate migration earlier is based only on seasonal catch rates of different size classes, rather than true knowledge of fish movement.

Recent advances in acoustic telemetry have proven effective in overcoming many of the limitations associated with using traditional tag-return approaches to infer movement and habitat use. Several examples for a wide diversity of species and systems have demonstrated the advantages of using acoustic approaches for quantifying fish movements, dispersal rates, and habitat use (Arendt et al. 2001; Lowe et al. 2003; Dresser and Kneib 2007; Bacheler et al. 2009a and b). Specifically, the movements and habitat use of many species of flatfishes, including Pacific halibut, North Sea plaice, and summer flounder, have been extensively studied in recent

years using electronic tagging approaches (Hunter et al. 2003; Sackett et al. 2007, 2008; Loher 2008; Loher and Blood 2009). Recently completed acoustic tagging studies of summer flounder, a closely related species which co-occurs with southern flounder in NC waters, have revealed several details about estuarine habitat use and dispersal timing in mid-Atlantic Bight systems. Specifically, Sackett et al. (2007) noted that dispersal from a NJ estuary to offshore spawning grounds was temporally variable and responded to multiple environmental factors (e.g., barometric pressure, dissolved oxygen). Importantly, the authors observed several individuals that exited and re-entered the estuary throughout the summer and fall, prior to offshore migration. They also detected several summer flounder (up to 39% of tagged individuals) returning to the estuary in which they were tagged during the following spring, after spending the winter in offshore habitats. Fabrizio et al. (2007) found that summer flounder showed strong fidelity to specific structured habitats in the lower Chesapeake Bay, and evidence of size-related habitat partitioning at one of the reefs. Within-estuary movements of summer flounder at the scale of 200-400 m exhibited diurnal patterns and were related to tidal stage and other environmental factors (barometric pressure, water temperature). They observed gradual migration throughout the fall, but did not detect differences in movement or dispersal timing between small and large summer flounder. Overall, they found summer flounder to exhibit small home ranges and site fidelity in the estuarine portion of the bay, with evidence that specific habitats may represent preferred nursery sites. To our knowledge, no studies using acoustic telemetry have been conducted to assess the habitat use and migration dynamics of southern flounder.

In addition to acoustic telemetry, the use of archival data storage tags can enable long-term collection of information on temperature, depth, and lat/long of habitats occupied by fishes over very large spatial scales (100's – 1000's km). Data storage tags are being used increasingly in fisheries research to reveal details about migration dynamics for fishes that undertake large scale movements. Successful application of archival tags has been demonstrated for bluefin tuna and other migratory pelagic species (Block et al. 2005, 2011), Atlantic salmon (Reddin et al. 2004), and several flatfish species including Pacific halibut (Loher 2008), North Sea plaice (Hunter et al. 2003), and yellowtail flounder (Walsh and Morgan 2004). Traditional tagging studies involving southern flounder in US South Atlantic states during the past three decades have highlighted strong tendencies for directed movement to the south by tagged individuals recovered at locations more than 20 km from their tagging site (Wenner et al. 1990; Monaghan 1996; Craig et al. in press). The main conclusion drawn from these observations points to southern flounder spawning sites being located at southern latitudes within the South Atlantic Bight (e.g., off the coasts of Georgia and north Florida). However, the exact location of southern flounder spawning has never been verified, either in the South Atlantic or the Gulf of Mexico. It is assumed that southern flounder spawn in relatively deep waters (> 40 m depth) since winter scuba surveys conducted at shallower depths off NC and SC have not recorded observations of aggregations of fish (Watterson and Alexander 2004; Tucker 2011). Knowledge of the spatial distribution of offshore spawning southern flounder would contribute greatly to our understanding of stock structure and the potential for mixing with individuals originating from

estuaries in other states, details which can be crucial to effective fishery management (Stephenson 1999).

The goals of this research project were to examine migration dynamics and within-estuary habitat use for southern flounder in North Carolina. We used acoustic telemetry to broadly quantify estuarine habitat use of southern flounder at different spatial scales across multiple seasons. By deploying a fixed array of acoustic receivers throughout the estuary, we obtained detailed information on migration behavior, timing, and pathways that should prove critically important to any future temporal management strategies (e.g., seasonal closures to increase flounder escapement). Lastly, we fitted a small subset of flounder with archival tags to test the concept that these tags (used in only modest numbers) could yield important information about the location of southern flounder spawning grounds. Our findings contribute new information on estuarine habitat use and migration dynamics that will enhance the ability of the NC Division of Marine Fisheries to effectively manage the southern flounder resource.

Specific study objectives

Objective 1: Estimate southern flounder diel activity space using fine-scale acoustic tracking

a) Estimate daily fine-scale habitat use, activity space, and rates of movement
b) Identify and test for potential diel patterns in movement

Objective 2: Identification of southern flounder essential fish habitat

a) Use acoustic transect methods to quantify southern flounder presence/absence
b) Quantify several biotic and abiotic habitat variables (including sediment characteristics, prey abundance, flow velocity, and bathymetry) to assess their contribution to southern flounder habitat selection

Objective 3: Examine drivers for the initiation of migration behavior, quantify migration timing, and identify migration corridors using acoustic monitoring

a) Test for size-dependence of migration timing
b) Identify several potential abiotic factors that may be associated with the initiation of migration behavior
c) Quantify emigration patterns through the estuary
d) Determine migration timing across multiple study years
e) Identify migration corridors

Objective 4: Test the concept that archival tags can be used to identify southern flounder offshore spawning locations

a) Deploy a small number (n = 25) of archival tags during each study year during late fall to obtain information on depth, temperature, and day length (converted to latitude) of offshore habitats used.
b) Objective dependent on returns (must reacquire the tag to retrieve data) of fish from the offshore environment.

Methodology

Study Area

All telemetry work was completed within the New River estuary located in the southeastern region of North Carolina (Fig 1A). The New River ecosystem has historically supported both commercial and recreational southern flounder fisheries, and also contains several geographic shoreline features, naturally creating narrow regions of the estuary which function effectively as acoustic gates.

Objectives 1 and 2

In order to conduct the fine-scale examination necessary to address questions related to fish diel movement and essential fish habitat identification, data collection for objectives 1 and 2 took place in Northeast Creek, which is a tributary located within the upper region of the New River estuary (see yellow inset box in Fig 1B). Northeast Creek is approximately 7 km long and 1.88 km wide at the mouth and 0.48km wide at its narrowest point. The creek contains shallow banks (< 1 m) which extend out approximately100 m from shore, and depths range from 1.2 m to 2.4 m in the middle of the system. There is an increase in depth with proximity towards the creek mouth. Since Northeast creek is located approximately 20 km from the estuary inlet, observed salinities are generally low (5 – 15 psu). This site was chosen due to historical observations of southern flounder (Smith et al. 2009; Smith and Scharf 2010), and the primarily linear morphology that was ideal for active acoustic transecting. The creek is also moderate in size, which enabled acoustic tracking of the entire system within a single field day.

Objective 3

Questions related to southern flounder migration were addressed at the whole-estuary scale using a system of fixed acoustic receivers (see approximate receiver array in Fig. 1B) which detected tagged flounder as they moved throughout the estuary. This shallow-water wind-influenced system is approximately 25 km long and 5 km wide at its widest point. The system experiences salinities ranging from <10psu in the upper estuary to >30psu near the inlet. Water temperatures vary seasonally from <10 °C during winter to >30 °C during summer. The Intra-Coastal Water Way (ICWW) and the New River Inlet provide three potential migration corridors that fish could use to exit the system. The shoreline is anthropogenically influenced by bridges, concrete bulkheads, and boating ramps, but to a minimal degree. The upper estuary is flanked by the Camp Lejeune U.S. Marine Corps base, which includes shooting and bombing ranges as well as several military air stations. Fishing effort within the system is represented by commercial

shrimp trawling, gill netting, gigging, and tide net fishing. Recreational gigging and hook-and-line fishing also occurs.

Objective 4

During fall 2012, archival tagging was completed using fish captured in the lower New River estuary and the Masonboro Sound area. Fish were released adjacent to New River Inlet and Masonboro Inlet. During fall 2013, we partnered with a commercial pound net fisherman in Core Sound to capture larger individuals, and fish were released outside of Beaufort Inlet.

General methodology for fish capture and tagging

Fish were captured using large (14cm = 5.5 inches) stretched mesh monofilament gillnets set for short durations (< 4 hours). Capture and tagging of fish took place from May through October in most years. Fish with a total length (measured from tip of jaw to tip of caudal fin) exceeding 356 mm (minimum legal size in the commercial fishery) were fitted with an acoustic transmitter (model V9; Vemco Ltd., Nova Scotia, Canada). The V9 transmitters are 29mm in length and weigh 4.7 grams (g) in air and 2.9g in water. Each transmitter had a unique ID code and was programmed to transmit a signal at a frequency of 69 kHz with a nominal (~ ± 50%) delay of 30-90 seconds depending on the specific study objective. Transmitters were surgically implanted within the coelom of each flounder to prevent tag shedding.

Surgical procedures closely followed those outlined by Fabrizio and Pessutti (2007) and used for summer flounder in mid-Atlantic estuaries. Fish were first anesthetized in a saltwater bath/clove oil solution using a concentration of 60 mg/L for ~10 minutes prior to surgery. During the surgical procedure, a reduced (50%) concentration of the clove oil solution was pumped continuously over the gills in order to keep the fish sedated throughout. An approximately 2 cm incision was made between the pectoral fin and the anal fin on the blind side of the fish. The tag was placed into the coelom, and then 3 -4 interrupted surgical stitches using non-absorbent sutures were used to close the incision. An external loop tag (cinch-up model F4, Floy Tag Inc.) was inserted through the caudal peduncle musculature, and contained contact information and the reward amount ($50-$100) for returning both the transmitter and the external tag. After the surgery, each fish was placed in a 100% saltwater bath with an aerator and allowed to recover for 10 – 15 minutes until being released near its capture location.

Methods for specific objectives

Objective 1: Estimate southern flounder diel activity space using fine-scale acoustic tracking

Focal follow approach

A model VH110 directional hydrophone (Vemco Ltd., Nova Scotia, Canada) attached to a model VR100 acoustic receiver (Vemco Ltd., Nova Scotia, Canada) was used for tracking daily movement patterns and estimation of activity space. Active tracking was initiated after a minimum 24-hour period following tagging to allow fish to reacclimatize and return to natural

behavior. During active tracking, fish locations were estimated every 10 minutes, based on latitude and longitude of the tracking vessel, the compass bearing of the directional hydrophone, and the intensity of the acoustic signal. Tracks for individual or groups of fish were conducted for at least 24 continuous hours, and all focal follows took place within Northeast Creek.

Analysis of focal follow data

At each 10 minute listening point, the location of tracked fish was calculated using the following formulae:

$$\text{UTM Fish X} = (\text{UTM Boat X} + (\text{Distance} * (\sin(\text{radians})))$$

$$\text{UTM Fish Y} = (\text{UTM Boat Y} + (\text{Distance} * (\cos(\text{radians})))$$

Fish locations were then visualized in ArcGIS 9.3 (ERSI, Redlands, California, USA), and the extension Home Range Tools (Rodgers and Kie 2011) was used to calculate daily activity space and rates of movement. Daily activity space was estimated using two methods: minimum convex polygons (MCP) and kernel density estimates (KDE). Initially, MCPs were used because they are easy to calculate and have been used in movement studies for decades making estimates of activity space for southern flounder comparable to previous studies. However, MCPs can often overestimate activity space, especially for animals that may not move great distances. Thus, KDEs were also be used as a measure of activity space because this method emphasizes core areas of use, and is less influenced by isolated instances of larger-scale movement (Worton 1989).

KDEs essentially estimate core areas of movement by applying a normal bivariate density distribution to fish locations within an assigned area or kernel. A grid can then be placed over these distributions, and the amount of distribution overlap between the grids can be measured. Areas with high calculated overlap are areas with a large number of points very close together (Worton 1989). However, fitting a distribution function to overdispersed data can result in over fitting of the data and require the use of a smoothing parameter *h* (Gitzen et al. 2006). Selecting *h* is important as over-smoothing or under-smoothing data can lead to misinterpretation of the final estimated activity space results. Different smoothing parameters exist (reference bandwidth, least-square cross-validation, and biased cross-validation; Worton 1995) and the appropriateness of *h* is dependent on the type of data collected (widely dispersed, nested, etc.). Therefore, the selection of this parameter was completed after data collection and visual inspection of patterns of movement.

Comparison of diel patterns in activity space and rates of movement was tested using a Student's paired t-test and performed in R (R team statistical software 2013).

<u>Objective 2</u>: Identification of southern flounder essential fish habitat

Quantifying flounder habitat use

A combination of passive and active acoustic tracking was used to quantify flounder habitat use within Northeast Creek and to identify potential predictors of habitat use. Eight, acoustic hydrophones (model VR2W; Vemco Ltd., Nova Scotia, Canada) were anchored throughout the creek. Several hydrophones were concentrated at the mouth of the creek to ensure that any emigration behavior was detected (Fig 1B). In addition to the passive acoustic monitoring, weekly active tracking was conducted during the summer and fall of 2014. Twenty-three fixed "listening" stations sites separated by roughly 500m (approximate detection range of the tag tested in the system) were arranged along the length of the creek (Fig. 5). During active tracking, the tracking vessel stopped at each site and used a model VH165 omnidirectional (Vemco Ltd., Nova Scotia, Canada) hydrophone connected to a model VR100 (Vemco Ltd., Nova Scotia, Canada) acoustic receiver to listen for tagged flounder. The duration for listening at each site was 3× the longest possible delay (130 s × 3 = 390 s) for signal transmission by the acoustic tag.

Southern flounder (n = 26, average ± SD TL = 397.2 ± 26.5 mm) were tagged between June 3 and September 10, 2014. During active tracking, once one or more fish were detected at a particular listening stations, a model VH110 directional hydrophone (Vemco Ltd., Nova Scotia, Canada) was used to estimate the location of the tagged fish (specific methods for using the directional hydrophone to estimate individual fish locations are outlined above). This enabled the distance from shore for each relocated fish to be measured. Abiotic measurements such as water temperature, salinity, and dissolved oxygen were recorded at each listening station when flounder were detected. In addition, a set of 14 continuous temperature loggers was positioned throughout the creek to estimate variation in water temperature on an hourly time scale. The date and time of day were documented so that corresponding weather conditions (wind speed and direction, precipitation, etc.) could be obtained from the NC State Climate Office and used to refine fish location estimates (detection range is impacted by environmental conditions).

Sediment sampling

A 200 × 200 meter grid was superimposed over a GIS map of the creek (Fig. 6A) dividing the sample area into 146 sample cells. Sediment samples (n = 72) were then taken systematically from every other cell within the experimental strata (Fig. 6B). A Ponar grab was used to retrieve a sample of benthic sediment. Once the grab was raised into the boat, a core sample was then taken from the top 2-3 inches of material using a hollow polyvinyl chloride cylinder. The core sample was pushed into a 50 mL centrifuge tube labeled with the sample site identification number. Any large organic matter, such as grasses or macroscopic benthic organisms, was removed from the centrifuge tube to prevent contamination of the sediment. The tube was then sealed and stored on ice to prevent the decomposition of organic matter before sediment properties were analyzed.

Each sediment sample was then divided into two different containers to allow organic content sampling and sediment size sampling to be conducted separately; 2 to 3 grams of sediment were placed in aluminum weigh boats and set aside for analysis of organic content, and the remainder of the sediment was used for grain size analysis. In the lab, a small amount of

deionized water was added to the sample remaining in the centrifuge tube, and the sample was homogenized by replacing the cap and shaking for about 10 seconds. About 10-20 mL were then transferred into a 50 mL Nalgene container. Since the process of analyzing grain size is sensitive to organic material, all organic content was digested from the sample prior to analysis. A small amount of 30% hydrogen peroxide was first added to the Nalgene container. A cover was then loosely fastened over the top of the container to allow the gasses to vent while also preventing vigorous reactions from bubbling over, and the sample was left in a fume hood for a minimum of 24 hours. The cover was then removed, and more hydrogen peroxide was added. This process was repeated until no reaction occurred, an indication that no organic content remained in the sample. The fully-reacted sample was then transferred into a 50 mL beaker with a stirring rod and placed on a stir plate and mixed for about 10 seconds. While stirring, a pipet was used to transfer a small amount of the sample from the beaker to a Coulter Counter for analysis.

For organic content analysis, the samples in the aluminum weigh boats were incinerated and the weight loss was attributed to organic content as outlined by Evans et al. (1990). Since bicarbonate (CHO_3) would also be incinerated at the temperatures necessary to incinerate organic content, its presence would artificially increase the percent weight loss attributable to organic matter. Therefore, HCl was first used to digest any CHO_3 present within the sample and also to kill any bacteria which could also digest organics before analysis was conducted.

Seven and a half mL of concentrated HCl was first added to 142.5 mL of deionized water to create a 5% HCl solution. In a fume hood, 2-3 mL of 5% HCl were then added to the sediment samples in the aluminum boats. The samples were then left for 2 days to allow the reaction to reach completion. Before incineration, the samples were placed into a drying oven at 75°C for 2-5 days until they were completely dry in order to evaporate excess HCl and water from the sample. The dry samples were then weighed before being placed into a combustion oven at 450°C for 2 hours. The remaining weight after combustion was then taken and subtracted from the initial dry weight to produce the weight of organics combusted. The value of organics was then reported as a percentage of total weight per sample.

Assessing prey fish community spatial distribution

The creek was divided into four strata along latitude or longitude lines for easy distinction in the field (see Fig. 6A). The same 200 × 200 meter grid from the organic sampling protocol outlined above was used to facilitate sampling of the prey community. Each grid square comprised of 50% creek surface area or greater (i.e., <50% land cover) was deemed a suitable sampling area and assigned a unique sample site identification number.

Otter trawls were conducted weekly using a stratified random design to assess the prey community. Before each sampling trip, four grid squares per stratum were randomly selected via their identification numbers using a random number generator and a single trawl tow was completed at each location. Trawling was conducted using a 3.2 m otter trawl, towed at 2-3 knots for 2 minutes. All fishes and macroinvertebrates collected were identified to species, and a subset (up to 20 per species) was weighed and measured (mm fork length).

Morphometric and meristic characteristics were used to identify the species of each fish captured. For each prey fish species, abundance and average size [total length (TL)] were measure for each trawl tow. Data were then aggregated across trawls to calculate metrics such as prey fish species richness, biodiversity, and catch per unit effort (CPUE). Since all trawls were conducted for roughly the same duration (duration SD < 2 seconds), CPUE was calculated as: $CPUE = \frac{n}{trawl}$, where CPUE = catch per unit effort, and n = number of prey fish individuals present.

To ensure that the analysis of prey fishes was conducted on for prey items that could be readily consumed by the southern flounder that were being tracked within the system, we first examined past diet studies (Wenner et al. 1990) to determine which species within the area were most likely to be primary diet items. When comparing past diet studies to each species' relative abundance within the sampled prey community, we concluded that bay anchovy (*Anchoa mitchilli*), spot (*Leiostomus xanthurus*), and spotfin mojarra (*Eucinostomus argenteus*) were most likely to be the primary contributors to southern flounder diet within Northeast Creek. We then adapted regressions from Manderson et al. (2000) relating gape size to total length for the closely related summer flounder (*P. dentatus*), to estimate the gape size of the southern flounder tagged in this study. These regressions were then compared to the distributions of prey fish body depths captured in the trawl surveys in order to determine the vulnerability of sampled prey fishes (see Fig. 13).

Analysis of estuarine habitat use data

The spatial distribution of southern flounder detections was mapped using ArcMap (10.2). Due to their apparent tendency to utilize areas closer to shore than the middle of the creek, and the linear morphology of the creek itself, the creek was divided into strata linearly for statistical analysis. Strata were delineated in 500 meter increments from the creek mouth, resulting in 13 strata, ranging from 0 to 6500 meters from the mouth of the creek. Patterns in southern flounder habitat distribution were examined temporally and spatially, both over the total duration of the surveys (11 weeks), and at a weekly resolution. Environmental sampling data were analyzed with the same resolution as southern flounder distributions. ArcMap was also used to illustrate spatial variation in environmental data, and maps were created depicting the variation in prey fish species richness, prey fish biodiversity, prey fish CPUE, sediment organic content, and mean sediment grain size.

Pair-wise correlation analysis was first used to examine statistical relationships between southern flounder and measured habitat features, and also among habitat features. Both logistic and multiple linear regression models were then fit using either southern flounder presence/absence or the number of southern flounder detections as response variables, and several combinations of habitat variables as predictors. Models were examined for overall significance ($P < 0.05$) and then compared using residual sums of squares to determine the most supported models.

Objective 3: Examine drivers for the initiation of migration behavior, quantify migration timing, and identify migration corridors using acoustic monitoring

Passive acoustic detection of southern flounder movements

An array of acoustic hydrophones (VEMCO model VR2W; n = 38 – 74) was anchored within the New River Estuary in order to determine fish residency within specific areas and to identify migration patterns (see Fig. 1B). For V9 transmitters, the acoustic receivers have a detection range of approximately 300m (100-400m depending on depth and wind conditions) within the study system. For each southern flounder fitted with a transmitter that was detected, the VR2W recorded the date, time, and the unique tag ID. Double gates of hydrophones were positioned at natural "pinch" points in the estuary and at the three possible emigration corridors to confirm direction of movement and increase the probability of fish detection. The hydrophones were downloaded and cleared of fouling approximately every three months (Apr, Jul, Oct, and Dec). Hydrophone batteries were replaced annually (each July) to minimize the risk of missing fish detections due to loss of power.

Analysis of estuarine migration data

For all fish detected emigrating from the New River estuary, the migration corridor was noted, and the migration pathway was traced retrospectively for each fish to determine the start of migration behavior (directed movement towards the ocean on consecutive detections). The date of initiation of emigration behavior was contrasted with the departure date to determine emigration duration. Emigration speed was calculated based on linear distances between detections and time elapsed. Fish TL at time of tagging was regressed against date of departure to examine the size dependence of emigration timing. Quantile regression was used to examine body size as a limiting factor for migration timing. Sizes of fish that emigrated were also compared with sizes of fish that remained in the estuary for the winter period using a t-test. The timing of individual migration events was used to determine windows of time when most migration events occurred.

Objective 4: Test the concept that archival tags can be used to identify southern flounder offshore spawning locations

Fish capture, tagging, and data analysis

For archival tagging, fish were captured using either overnight gill net soaks or pound nets, depending on the area being fished. After capture, the procedure for surgical implantation of the archival tags were generally the same as outlined above for acoustic transmitters, with the exception of the need to make an additional opening in the dorsal musculature to extend the light-sensing stalk through. The ability to detect changes in day length via the light-sensing stalk would, in theory, allow latitude and longitude to be estimated for the duration of time at large for each fish. In addition to implantation of an archival tag in the coelom, each fish was fitted with the same external loop tags as outlined for telemetry fish. The external tags contained all contact

information and indicated a $200 reward for the return of the fish. After tagging was completed, fish were transported to the nearest ocean inlet for release. This was done to ensure that fish fitted with archival tags would not be captured immediately after tagging by NC fisheries. For all recaptured fish, the archival tag was retrieved and shipped to the tag manufacturer (Lotek, Inc.) for data retrieval.

Results and conclusions

Objective 1: Estimate southern flounder diel activity space using fine-scale acoustic tracking

Focal follows were completed for seven southern flounder during 2013 and 2015 (Table 1). Individual southern flounder ranged in size from 338-482 mm TL (mean ± SD =393.3 ± 49.8 mm), and fish were released between mid-May and mid-August. Five individuals were followed for 36 hours and two fish were followed for 48 hours. Estimates of activity space ranged between 0.002 and 0.007 km^2, with higher estimates coming from the minimum convex polygons (Table 1; Figs. 2 and 3). In most cases, estimated core areas based on kernel density were 2 to 3 times smaller in size than activity space estimates generated by the minimum convex polygons (Fig. 4). For two individuals (ID 9285 and 9286) followed during the same event, estimates of area used were similar between the two metrics; an indication of very limited movement by these two fish. Rates of movement for individual fish were found to range within 0.018 - 0.030 m/sec. No statistical differences in activity space (paired t-test: $t = 0.95$, $df = 5$, $P = 0.39$) or rate of movement (t-stat = 0.47, df = 5, P = 0.66) were detected between day and night. All habitat tracks occurred within 100 meters of shore, in water less than 2 meters depth. Given the season during which this work was completed, the physicochemical attributes in Northeast Creek were typical of summer conditions (mean ± SD: temperature = 28.7±1.2°C, dissolved oxygen = 4.9±1.6 mg/L, salinity = 10.1±2.1 psu). During the main period of effort for our focal follows (summer 2013), a total of 20 individuals were fitted with transmitters and released into Northeast Creek. During the summer/fall, five individuals were captured in recreational or commercial fisheries, five individuals emigrated from the creek based on detections by the directional gate at the creek mouth, and ten individuals remained in the creek into early winter (last transect in January). Estimates of residency within the creek ranged from 1-246 days.

Our findings suggest very limited movement during summer nursery residency by southern flounder in North Carolina. Similarly, the closely related summer flounder has been demonstrated to exhibit highly localized movement within the estuary, with large-scale (100s of meters) movements uncommon until fish begin to emigrate. During active hydroacoustic tracking, Sackett et al. (2008) found that individual summer flounder remained in limited areas (0.18 km^2), but demonstrated a high level of activity within those areas. At the whole-estuary scale, no telemetry studies of southern flounder habitat use have been conducted; however, Furey et al. (2013) used a fine-scale positioning system to quantify southern flounder habitat use within a confined area of a Texas estuary. They found that fish also tended to remain within relatively

small areas for extended periods, but that some individuals did show considerable movement (e.g., > 8 km over ~ 8 d) within their study area. Their study fish showed slightly higher rates of movement (avg. = 0.07 m/sec), driven largely by occasional movements of larger distances (Furey et al. 2013). While generally in agreement with the observations of Furey et al. (2013), we did not observe southern flounder to make any extensive movements during our focal follows. Differences in fine-scale habitat features or specific seasonal effects may have contributed to these disparities.

A recent study documenting several years of conventional mark-recapture efforts also indicates limited movement by southern flounder during estuarine residency. In a similar tidal creek, Craig et al. (in press) reported that the average distance between release and relocation sites was 0.22km, with the majority of recaptures occurring within < 0.1km of the release site. This was despite many of the fish being at large for more than 100 days. Our telemetry results align closely with the mark-recapture findings, and we conclude that southern flounder are likely to display only limited movement, as the scale of 100's of meters, while residing in estuarine nurseries.

Objective 2: Identification of southern flounder essential fish habitat

In total, 26 southern flounder ranging between 342-439 mm total length (average ± SD TL = 397.2 ± 26.5 mm) were tagged and monitored between June and Nov 2014. Gape size versus total length relationships showed that southern flounder tagged within this study possessed mouth heights ranging from 42.7 to 54.5 mm, mouth widths ranging from 38.7 to 49.4 mm, and esophageal widths ranging from 25.3 to 30.6 mm, and were capable of consuming nearly all sizes of prey fishes captured in the trawl surveys (see Fig. 13).

Acoustic transects were conducted weekly between June 27 and November 24, 2014. There were 107 positive detections of southern flounder in total, with only 2 tagged fish which were never detected throughout the duration of the study. The number of detections per tagged fish ranged between 0 and 11 (average ± SD detections = 4.12 ± 2.13). Flounder were detected from 0 to 5500 meters from the mouth of the creek, with about 70% of detections occurring in mid-creek, between 2000 and 4500 meters from the creek mouth (Fig. 7).

Trawls to sample the prey fish community were completed biweekly from July 10 to October 24, 2014, resulting in the completion of 204 trawls. Of the 18 fish species recovered, spot, bay anchovy, and spotfin mojarra collectively comprised about 74% of the total catch. Prey fish CPUE ranged from 0 to 284 individuals per trawl, and species richness ranged from 0 to 9. Both species richness and prey fish abundance were observed to peak in the middle of the creek length, with about 55% of prey fish catch occurring between 2000 and 4500 meters from the creek mouth (Figs. 8-10), an area which comprises about 38% of the overall sampling area.

Sediment samples were taken from 71 locations. Analysis of organic content revealed a range from 3.1 to 34.5% of sediment weight (average ± SD organics = 17.69 ± 9.64%). A steep transition, from lower organic content toward the creek mouth to higher organic content farther

upstream, occurred between 2500 and 3500 meters from the creek mouth (Fig. 11). Variation in organic content occurred both along the creek length, as well as across the creek width, with sediment closer to shore and farther downstream tending to contain a lower percentage of organic content by weight than sites upstream or toward the middle of the creek width (Fig. 11).

Sediment grain size analysis revealed a slight increase in grain size toward the middle of the creek in both length and width, but mostly sediment grain size was highly variable along the length of the creek (Fig. 12). The values upstream, however, could be unrepresentative as they were comprised of relatively few samples compared to the number of samples in other strata. The effect of low sample size is indicated by the elevated standard deviations in grain size toward the upper regions of the creek.

Pair-wise correlations were positive between southern flounder detections and both prey fish species richness ($r = 0.5691$) and prey fish cpue ($r = 0.4643$) (Table 2). The pattern was statistically significant for prey fish species richness ($P = 0.04$), and marginally significant ($P = 0.11$) for prey fish cpue. Neither of the sediment characteristics were strongly correlated with southern flounder habitat use, although a modest positive association ($r = 0.3113$) was observed between flounder detections and sediment grain size.

Logistic regression models using presence/absence of southern flounder as a response performed poorly and could not identify any significant predictors of habitat. Multiple linear regression models using the number of southern flounder detections as a response variable performed better. The best model included only prey fish species richness as a predictor ($P = 0.042$; $R^2 = 0.324$); however a model that included prey fish species richness, prey fish CPUE, and their interaction also received some support ($R^2 = 0.44$; $P = 0.14$). Neither sediment grain size nor organic content were strong contributors to the model.

This research identified several environmental variables that may influence fine-scale southern flounder habitat selection. Positive associations were identified between southern flounder habitat use and prey fish species richness, as well as prey fish abundance. An association between southern flounder habitat use and areas with moderate sediment organic content and grain size was also identified. This study also developed a method of acoustic transecting that successfully allowed the tracking and quantification of habitat use in an aquatic species. This procedure could provide a method for fisheries managers to quantify shallow-water habitat use by fishes which is free from the biases associated with active capture gears, such as beach seines or otter trawls, where catchability can vary among habitat types.

Within estuarine systems, areas that retain southern flounder may be defined by a suite of habitat characteristics that promote growth and survival. Several studies have indicated the importance of structured habitats at higher salinities, as well as specific physicochemical conditions (temperature, salinity, dissolved oxygen) for newly settled southern flounder, with increasing importance of freshwater habitats and fine-grained sediments during the post-settlement period (Burke et al. 1991; Allen and Baltz 1997; Walsh et al. 1999; Glass et al. 2008; Nañez-James et al. 2009; Froeschke et al. 2013a and 2013b; Furey and Rooker 2013). In a study focused on sub-adult individuals in a Texas estuarine nursery, southern flounder primarily used deeper estuarine habitats, avoided areas with high summer water temperatures (> 30° C), and

preferred sandy substrates as opposed to more complex benthic habitats (Furey et al. 2013). More recently, extensive use of oligohaline/freshwater habitats during part or all of the estuarine residency period has been documented from otolith elemental signatures (Lowe et al. 2011; Farmer et al. 2013; Nims and Walther 2014). Furthermore, as southern flounder increased in size during the first year, Furey and Rooker (2013) found that the probability of fish occurrence increased in habitats located further from tidal inlets and closer to sources of freshwater. While estuarine habitat preferences of southern flounder have been shown to vary seasonally and ontogenetically in these recent studies, the patterns of limited movement and positive associations with prey fish communities observed in this study indicate a high potential for habitat fidelity during the sub-adult life stages. Continued focused efforts to identify factors that determine southern flounder distributional patterns during estuarine residency prior to offshore emigration would be valuable, especially if spatial management strategies (e.g., partial closures of southern flounder nursery habitats) are considered.

Objective 3: Examine drivers for the initiation of migration behavior, quantify migration timing, and identify migration corridors using acoustic monitoring

Patterns of emigration

Southern flounder were fitted with transmitters and migration behavior monitored during 2012, 2013, and 2014 (Table 3). In 2012, 41 fish (average ± SD = 384.1 ± 44.5mm TL) were tagged between September and December in the New River estuary. Of those, seventeen were determined to have emigrated from the system, and two emigrants were recaptured as far south as the southern portion of South Carolina. The New River Inlet served as the emigration corridor for 71% of the fish, with the remaining emigrants leaving the New River estuarine system via the ICWW north corridor. In 2013, 40 fish (average ± SD = 410.4 ± 45.2mm TL) were tagged between September and October in the New River estuary. Of those, eighteen fish were determined to have emigrated from the system. Sixty-one percent of the fish emigrated via the New River inlet with the rest exiting through the northern ICWW. During 2012 and 2013, there was a significant difference in size between emigrants and fish that remained in the estuary at the start of winter, with emigrating fish being larger ($t = 3.29$, $df = 61$, $P = 0.02$). In 2014, 94 fish were tagged (average ± SD = 393.2 ± 29.2mm TL) between June and October in the New River estuary. Twenty-two flounder were determined to have emigrated from the system. with 55% of emigrants leaving through the New River inlet and the remainder via the northern ICWW. In each year, several tagged fish were harvested in commercial or recreational fisheries operating within the New River estuary, and thus were not available to emigrate. For instance, 48 transmitters were deployed during summer (prior to Sep 1) in 2014, and only six of those fish were determined to have emigrated successfully. Twenty-one of those fish were harvested in the estuary.

When emigration data were pooled for all three study years (2012-2014), several patterns emerged. First, roughly half of all southern flounder that were determined to have emigrated

from the New River estuary had exited the system by Nov 1st. Moreover, approximately 90% of the emigrating fish had left by Nov 21st (Fig. 14). There was a clear window of time when the vast majority of emigration occurred from the New River estuary, with roughly 85% of the emigration activity occurring between Oct 19th and Nov 16th (Fig. 15). We only observed two fish emigrating from the New River estuary after Dec 1st (Fig. 15). Approximately, two-thirds of the fish determined to emigrate, did so through the New River Inlet. The remaining fish followed the ICWW to the north. We suspect that this may occur for two reasons. First, Traps Bay appears to serve as a staging area, with many fish aggregating in the bay during fall. Traps Bay is positioned on the north side of the main river channel, with several large openings leading up the northern ICWW corridor. Second, Brown's Inlet is located 5-10 km north of the New River and is, by far, the next closest exit point to the ocean if fish do not exit directly through the New River Inlet. The closest exit point to the south is Topsail Inlet, which is more than 20km away. As such, we observed no southern flounder emigrating through the southern ICWW corridor during the study period.

Generally, southern flounder exhibited two distinct behaviors: a resident behavior with low rates of movement confined to a specific region of the estuary, and a migratory behavior with increased rates of directed movement towards emigration corridors. Fish were observed to initiate migration behavior between Sep 28th and Nov 24th, and about 75% of southern flounder exited the estuary within ten days of the initiation of migration behavior (Fig. 16). Therefore, southern flounder estuarine movements could be characterized as salutatory, with long periods of limited movement followed by brief periods of extensive movement. We did detect a tendency for larger southern flounder to exit the estuary earlier relative to smaller fish, but the relationship was highly variable (Fig. 17). We expected that we might observe a stronger relationship between emigration timing and body size based mainly on anecdotal information from commercial fisherman. It is possible that a stronger size-dependence in migration timing does exist for female southern flounder, but the pattern is confounded by smaller mature males that may also begin to emigrate early to coincide their arrival on the spawning grounds with the larger females.

Some evidence was observed for southern flounder movement rates during emigration to be driven by the passage of cold fronts during the fall. While we did not detect strong relationships between average flounder movement rates and temperature, we did note that movement rates were much more variable following sharp drops in temperature (Fig. 18). Similarly, flounder movement rates were more variable during high wind events (Fig. 19). During fall along the North Carolina coast, weather patterns are driven largely by the passage of cold fronts that originate in the Northwest U.S. The severity and duration of cold fronts can cause considerable drops in air temperature, and thus, shallow estuarine water temperatures. It is likely that southern flounder respond to these events with more extensive movements during the migration season. Our observation of the variation in flounder movement being associated with wind and temperature changes is likely to due to the heterogenous effects of cold fronts throughout the estuary, due mainly to differences in water depth and other geographic features. Our temperature logger data revealed much greater variability in daily water temperatures in

shallower regions of Northeast Creek (Fig. 20). We expect that southern flounder occupying habitats with variable depths and other geographic features may respond differently to the passage of cold fronts.

Objective 4: Test the concept that archival tags can be used to identify southern flounder offshore spawning locations

During 2012-2013, we recovered two archival tags in the spring that had been placed in southern flounder the previous fall. One fish was tagged in Traps Bay (New River estuary) and was recovered in Traps Bay, having overwintered in the estuary. The second fish was tagged in the upper part of the New River estuary, emigrated in late fall, and was recaptured near Oak Island in the lower Cape Fear River estuary. The fish had moved about 80km to the south during the winter. Temperature data indicated that the fish stayed within ~10°C isotherm during the winter. Pressure data indicated that the pressure experienced by the fish was approximately 1 atmosphere (~30-40 ft. in depth) for the winter. Collectively, the data retrieved suggest that this fish stayed in the near shore ocean off the southern North Carolina coast for the winter period and returned to a different estuarine system in spring as water temperatures began to warm. It is unlikely, but unknown, that this individual participated in spawning.

During 2013-14, we also recovered only two archival tags. The first was recovered only one week after the fish were released outside of Beaufort Inlet. The fish apparently returned to the estuarine waters and was captured by a recreational angler near Fort Macon. The second fish was recaptured the following spring in the Newport River, and also appeared to have returned inshore after release and spent the winter in the Newport River.

The use of archival tags has thus far, not provided any new information on potential offshore spawning locations for southern flounder. We remain hopeful that a subset of the tagged fish from either 2012 or 2013 will be recaptured in future years and that we can retrieve data on temperature, pressure, and latitude/longitude for those fish. We suspect that to have a reasonable expectation for a sufficient number of returns (e.g., 10 fish), at least 200 tags may need to be released.

Future work:

Southern flounder migration data collected during 2012-2014 will be used to construct a logistic regression model in order to assess the contribution of several abiotic factors to the initiation of emigration behavior, defined as consistent movement down-estuary. These factors will include air temperature (°C), wind speed (km/hr), wind direction (degrees), photoperiod (hours), tidal amplitude (meters), and barometric pressure (Pa). Several iterations of the model will be run that incorporate time lags and interaction affects, and the resulting models will be ranked using Akaike Information Criterion (AIC) with the best model having the lowest AIC value. The model constructed from this analysis will then be tested for performance using an independent migration data set that will obtained in 2015 as part of a separate study.

Literature cited

Allen, R. L., and D. M. Baltz. 1997. Distribution and microhabitat use by flatfishes in a Louisiana estuary. Environmental Biology of Fishes 50(1):85-103.

Arendt, M.D., Lucy, J.A., and Munroe, T.A. 2001. Seasonal occurrence and site-utilization patterns of adult tautog, *Tautoga onitis* (Labridae), at manmade and natural structures in lower Chesapeake Bay. Fishery Bulletin 99:519-527.

Bacheler, N.M., Paramore, L.M., Burdick, S.M., Buckel, J.A., and Hightower, J.E. 2009a. Variation in movement patterns of red drum (*Sciaenops ocellatus*) inferred from conventional tagging and ultrasonic telemetry. Fishery Bulletin 107:405-419.

Bacheler, N.M., Paramore, L.M., Buckel, J.A., and Hightower, J.E. 2009b. Abiotic and biotic factors influence the habitat use of an estuarine fish. Marine Ecology Progress Series 377:263-277.

Block, B.A., Teo, S.L.H., Walli, A., Boustany, A., Stokesbury, J.W., Farwell, C.J., Weng, K.C., Dewar, H., and Williams, T.D. 2005. Electronic tagging and population structure of Atlantic bluefin tuna. Nature 434:1121-1127.

Block, B.A., Jonsen, I.D., Jorgensen, S.J., et al. 2011. Tracking apex marine predator movements in a dynamic ocean. Nature 475: 86-90.

Burke, J.S., J.M. Miller, and Hoss, D.E. 1991. Immigration and settlement pattern of *Paralichthys dentatus* and *P. lethostigma* in an estuarine nursery ground, North Carolina, USA. Netherlands Journal of Sea Research 27:393-405.

Craig, J.K., W.E. Smith, F.S. Scharf, and J.P. Monaghan. (in press). Estuarine residency and migration of southern flounder (*Paralichthys lethostigma*) inferred from conventional tag returns at multiple spatial scales. Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science.

Dresser, B.K. and Kneib, R.T. 2007. Site fidelity and movement patterns of wild subadult red drum, *Sciaenops ocellatus* (Linnaeus) within a salt marsh-dominated estuarine landscape. Fisheries Management and Ecology 14:183-190.

Evans, K. M., Gill, R. A., and Robotham, P. W. J. 1990. The PAH and organic content of sediment particle size fractions. Water, Air, and Soil Pollution 51(1-2):13-31.

Fabrizio, M.C., Henderson, M.J., and Lucy, J.A. 2007. Understanding localized movements and habitat associations of summer flounder in Chesapeake Bay using passive acoustic arrays. Final report to the Virginia Marine Resources Commission, December 2007. http://www.mrc.virginia.gov/vsrfdf/pdf/RF06-11_Dec07.pdf

Fabrizio, M.C. and J.P. Pessutti. 2007. Long-term effects and recovery from surgical implantation of dummy transmitters in two marine fishes. Journal of Experimental Marine Biology and Ecology 351:243-254.

Farmer, T. M., D. R. DeVries, R. A. Wright, and J. E. Gagnon. 2013. Using seasonal variation in otolith microchemical composition to indicate largemouth bass and southern flounder residency patterns across an estuarine salinity gradient. Transactions of the American Fisheries Society 142:1415-1429.

Froeschke, B. F., G. W. Stunz, M. M. Reese Robillard, J. Williams, and J. T. Froeschke. 2013. A modeling and field approach to identify essential fish habitat for juvenile bay whiff (*Citharichthys spilopterus*) and southern flounder (*Paralichthys lethostigma*) within the Aransas Bay complex, TX. Estuaries and Coasts 36(5):881-892.

Froeschke, B. F., P. Tissot, G. W. Stunz, and J. T. Froeschke. 2013. Spatiotemporal predictive models for juvenile southern flounder in Texas estuaries. North American Journal of Fisheries Management 33:817-828.

Furey, N. B., M. A. Dance, and J. R. Rooker. 2013. Fine-scale movements and habitat use of juvenile southern flounder *Paralichthys lethostigma* in an estuarine seascape. Journal of Fish Biology 82(5):1469-1483.

Furey, N. B., and J. R. Rooker. 2013. Spatial and temporal shifts in suitable habitat of juvenile southern flounder (*Paralichthys lethostigma*). Journal of Sea Research 76:161-169.

Gitzen, R. A., Millspaugh, J. J., and Kernohan, B. J. 2006. Bandwidth selection for fixed-kernel analysis of animal utilization distributions. Journal of Wildlife Management 70(5):1334-1344.

Glass, L. A., J. R. Rooker, R. T. Kraus, and G. J. Holt. 2008. Distribution, condition, and growth of newly settled southern flounder (*Paralichthys lethostigma*) in the Galveston Bay Estuary, TX. Journal of Sea Research 59:259-268.

Hunter, E., Metcalfe, J.D., and Reynolds, J.D. 2003. Migratory route and spawning area fidelity by North Sea plaice. Proceedings of the Royal Society of London 270:2097-2103.
Jonsen, I.D., Mills Flemming, J., and Myers, R.A. 2005. Robust state-space modeling of animal movement. Ecology 86: 2874-2880.

Loher, T. 2008. Homing and summer feeding site fidelity of Pacific halibut (*Hippoglossus stenolepis*) in the Gulf of Alaska, established using satellite-transmitting archival tags. Fisheries Research 92:63–69.

Loher, T. and Blood, C. L. 2009. Dispersion of Pacific halibut (*Hippoglossus stenolepis*) summering off British Columbia and the US Pacific Northwest, evaluated via satellite archival tagging. Canadian Journal of Fisheries and Aquatic Sciences 66:1409–1422.

Lowe, C.G., Topping, D.T., Cartamil, D.P., and Papastamatiou, Y.P. 2003. Movement patterns,

home range, and habitat utilization of adult kelp bass *Paralabrax clathratus* in a temperate no-take marine reserve. Marine Ecology Progress Series 256:205-216.

Lowe, M.R., DeVries, D.R., Wright, R.A., Ludsin, S.A., and Fryer, B.J. 2011. Otolith microchemistry reveals substantial use of freshwater by southern flounder in the Northern Gulf of Mexico. Estuaries and Coasts 34:630–639.

Manderson, J.P., B.A. Phelan, A.W. Stoner, and J. Hilbert. 2000. Predator–prey relations between age-1 1 summer flounder (*Paralichthys dentatus*, Linnaeus) and age-0 winter flounder (*Pseudopleuronectes americanus*, Walbaum): predator diets, prey selection, and effects of sediments and macrophytes. Journal of Experimental Marine Biology and Ecology 251:17-39.

Monaghan, J.P., Jr. 1996. Life history aspects of selected marine recreational fishes in North Carolina: Migration of paralichthid flounders tagged in North Carolina. Completion Report Grant F-43. Segments 1-5. North Carolina Department of Environment and Natural Resources, Division of Marine Fisheries, Morehead City, NC. P. 2.1-2.44.

Monaghan, J.P., Jr. and J.L. Armstrong. 2000. Reproductive ecology of selected marine recreational fishes in North Carolina: Southern Flounder, *Paralichthys lethostigma*. Completion Report Grant F-60. Segments 1-2. North Carolina Department of Environment and Natural Resources, Division of Marine Fisheries, Morehead City, NC. pp. 1.1-1.17.

Music, J.L. and Pafford, J.L. 1984. Population dynamics and life history aspects of major marine sportfishes in Georgia's coastal waters. Georgia Department of Natural Resources, Coastal Resources Division Contribution Series Number 38, 175 pp.

Nanez-James, S. E., G. W. Stunz, and S. A. Holt. 2009. Habitat use patterns of newly settled southern flounder, *Paralichthys lethostigma*, in Aransas–Copano Bay, Texas. Estuaries and Coasts 32:350-359.

Nims, M.K. and B.D. Walther. 2014. Contingents of southern flounder from subtropical estuaries revealed by otolith chemistry. Transactions of the American Fisheries Society 143:721-731.

NCDMF. 2010. Draft Amendment 1 to the North Carolina Fishery Management Plan for Southern Flounder. September 2010. 359pp.

Powell, A.B. and Schwartz, F.J. 1977. Distribution of Paralichthid Flounders (Bothidae: *Paralichthys*) in North Carolina Estuaries. Chesapeake Science 18:334-339.

R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/

Reddin, D.G., Friedland, K.D., Downton, P., Dempson, J.B., and Mullins, C.C. 2004. Thermal habitat experience by Atlantic salmon (*Salmo salar* L.) kelts in coastal Newfoundland waters. Fisheries Oceanography 13:24-35.

Rodgers, A.R. and J.G. Kie. 2011. User's Manual for HRT: Home Range Tools for ArcGIS. Centre for Northern Forest Ecosystem Research, Ontario, Canada. http://flash.lakeheadu.ca/~arodgers/hre/HRT%20Users%20Manual%20Draft%20August%2010%202011.pdf

Rulifson, R.A., VanSalisbury, C., and Spidel, M.R. 2009. Critical habitat for southern flounder, *Paralichthys lethostigma*: Do coastal watersheds play an important role in life history and growth? Final Report to the NC Sea Grant Fishery Resource Grant Program http://www.ncseagrant.org/images/stories/ncsg_pdf/documents/research/FRG_08-EP-03_FlounderHabitat.pdf

Sackett, D.K., Able, K.W., and Grothues, T.M. 2007. Dynamics of summer flounder, *Paralichthys dentatus*, seasonal migrations based on ultrasonic telemetry. Estuarine, Coastal and Shelf Science 74:119-130.

Sackett, D.K., Able, K.W., and Grothues, T.M. 2008. Habitat dynamics of summer flounder *Paralichthys dentatus* within a shallow USA estuary, based on multiple approaches using acoustic telemetry. Marine Ecology Progress Series 364:199-212.

Smith, W.E., Scharf, F.S. and Hightower, J.E. 2009. Fishing mortality in North Carolina's southern flounder fishery: Direct estimates of instantaneous fishing mortality from a tag return experiment. Marine and Coastal Fisheries 1:283-299.

Smith, W.E. and Scharf, F.S. 2010. Demographic characteristics of southern flounder, *Paralichthys lethostigma*, harvested by an estuarine gillnet fishery. Fisheries Management and Ecology 17:532-543.

Stephenson, R.L. 1999. Stock complexity in fisheries management: a perspective of emerging issues related to population sub-units. Fisheries Research 43:247-249.

Stokes, G.M. 1977. Life history studies of Southern Flounder (*Paralichthys lethostigma*) and Gulf Flounder (*P. albigutta*) in the Aransas Bay Area of Texas. Texas Parks and Wildlife Department Technical Series, No. 25, 37 pp.

Takade-Heumacher, H. and Batsavage, C. 2009. Stock status of North Carolina southern flounder (*Paralichthys lethostigma*). NC Div. Mar. Fish., Morehead City, NC. 101pp.

Taylor, J.C., Miller, J.M. and Hilton, D. 2008. Inferring southern flounder migration from otolith microchemistry. NC Sea Grant, Fishery Resource Grant 05-FEG-06 Final Report, 27pp.

Tucker, C. R. 2011. Reproductive analysis of southern and gulf flounders (*Paralichthys lethostigma* and *P. albigutta*) in South Carolina based on scuba surveys. MS thesis. College of Charleston, Charleston, SC.

Walsh, H.J., Peters, D.S., and Cyrus, D.P. 1999. Habitat utilization by small flatfishes in a North Carolina estuary. Estuaries 22:803-813.

Walsh, M.J. and Morgan, S.J. 2004. Observations of natural behavior of yellowtail flounder derived from data storage tags. ICES Journal of Marine Sciences 61:1151-1156.

Watterson, J.C. and Alexander, J.L. 2004. Southern flounder escapement in North Carolina. Final Performance Report Grant F-73, Segments 1-3, 39pp.

Wenner, C.A., Roumillat, W.A., Moran, Jr., J.E., Maddox, M.B., Daniel, III, L.B., and Smith, J.W. 1990. Investigations on the life history and population dynamics of marine recreational fishes in South Carolina: Part 1. Marine Resources Research Institute, South Carolina Wildlife and Marine Resources Department, Charleston,SC. 2.6-2.11.

Worton, B. J. 1989. Kernel methods for estimating the utilization distribution in home-range studies. Ecology 70(1):164-168.

Worton, B. J. 1995. Using Monte Carlo simulation to evaluate kernel-based home range estimators. The Journal of Wildlife Management 59(4):794-800.

Table 1. - Date of track start, fish total length (in mm), track duration (in hours), and activity space estimates (95% MCP and KDE in km^2) for seven southern flounder that were tracked continuously in Northeast Creek. Average (± SD) for physicochemical attributes of the water were based on samples taken every two hours during tracking.

ID	Date	TL	Duration	95% MCP	95% KDE	Water temp (°C)	Dissolved oxygen (ppm)	Salinity (psu)
9272	6/27/13	482	36	0.007	0.003	28.4 ± 1.2	5.3 ± 0.5	9.7 ± 0.9
9276	6/27/13	382	36	0.006	0.003	28.4 ± 1.2	5.3 ± 0.5	9.7 ± 0.9
9278	7/30/13	338	48	0.007	0.002	29.2 ± 0.8	5.1 ± 1.0	8.6 ± 0.9
9279	7/30/13	366	48	0.007	0.003	29.2 ± 0.8	5.1 ± 1.0	8.6 ± 0.9
9285	8/14/13	397	36	0.002	0.002	28.1 ± 1.4	4.0 ± 1.2	12.2 ± 0.8
9286	8/14/13	355	36	0.002	0.002	28.1 ± 1.4	4.0 ± 1.2	12.2 ± 0.8
2561	5/19/15	433	36	0.007	0.003	28.2 ± 1.2	4.0 ± 0.8	6.1 ± 0.4

Table 2. - Correlation matrix illustrating association between flounder presence and several measured environmental factors. Correlation coefficients (r) are displayed in the lower non-shaded area; P-values are displayed in the upper shaded area with significant ($P < 0.05$) correlations in bold.

Factors	flounder	sediment size	sediment organics	prey spp. richness	prey CPUE
flounder	1.0000	0.3246	0.7773	**0.0424**	0.1100
Sediment size	0.3113	1.0000	0.0895	0.1732	0.1611
Sediment organics	-0.0871	0.5111	1.0000	0.5852	0.2641
Prey spp. richness	0.5691	0.4207	0.1671	1.0000	0.0000
Prey CPUE	0.4643	0.4317	0.3344	0.9396	1.0000

Table 3. - Summary information for southern flounder fitted with acoustic tags during summer/fall of 2012-2014 in the New River estuary. Any fishery removals (n harvested) occurred prior to emigration, so those fish were not available to emigrate. All emigrating fish either exited through the New River Inlet (% using NR inlet) or followed the ICWW corridor to the north, likely entering the ocean via Brown's Inlet.

Year	n tagged	n emigrants	n harvested	Avg±SD TL (mm)	% using NR inlet
2012	41	17	4	384.1±44.5	71%
2013	40	18	7	410.4±45.2	61%
2014	94	23	34	393.2±29.2	55%

Figures

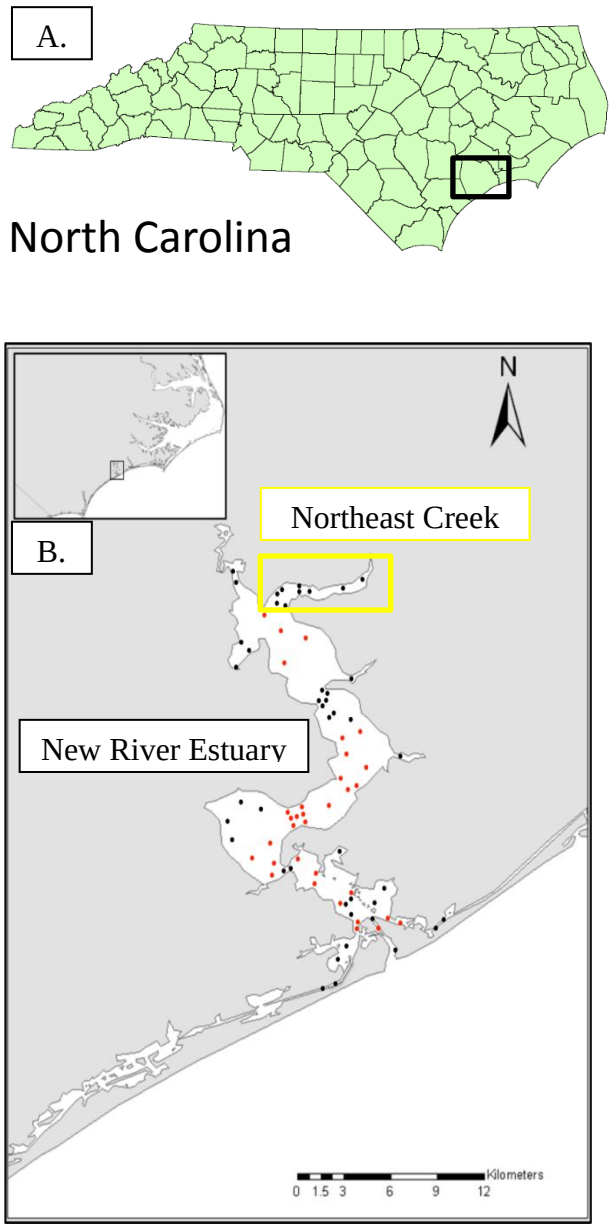


Figure 1: (A) A map of the state of North Carolina. The black box indicates the location of the New River Estuary. (B) A map of the New River Estuary. The yellow box indicates the location of the upper tributary, Northeast Creek, where fine-scale tracking and habitat mapping for southern flounder were completed. The colored dots indicate the location of anchored acoustic hydrophones. Locations indicated in black were present during 2012 and 2013. The red dots indicate locations of an additional 31 hydrophones installed during the summer of 2014 as part of a separate study.

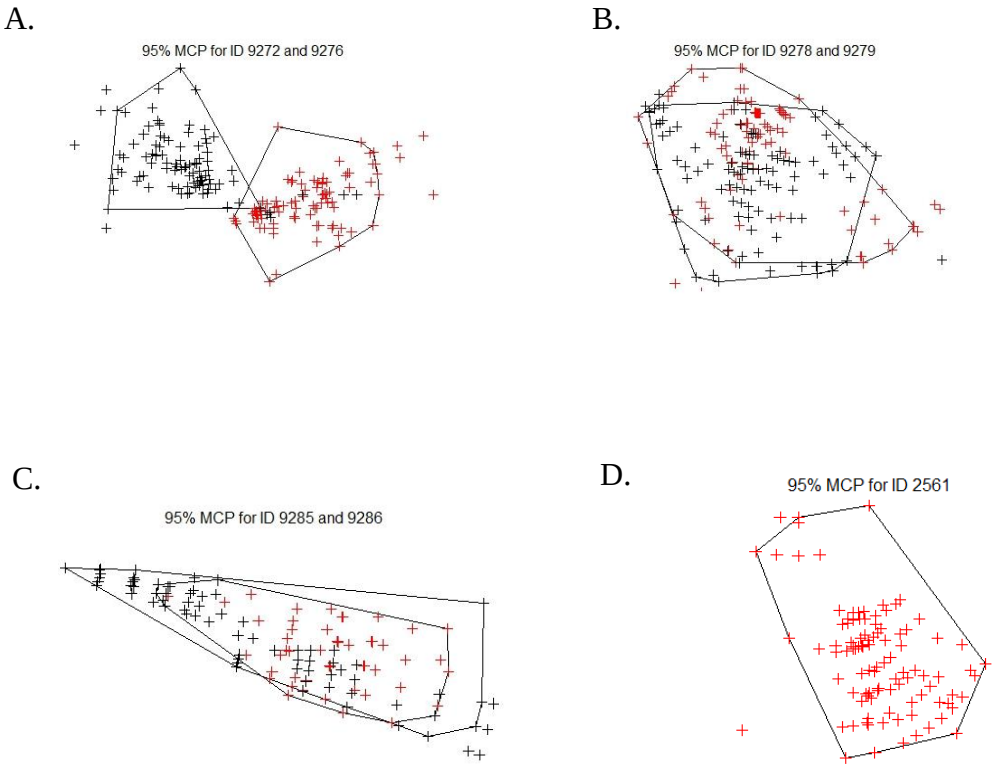


Figure 2: Ninety-five percent minimum convex polygons (MCPs) calculated based on focal follows for seven southern flounder (ID 9272, 9278, 9285 in black and 9276, 9279, 9286 and 2561 in red). Two fish were tracked simultaneously for each focal follow event during summer 2013 (A, B and C), and one lone individual was tracked during spring 2015 (D). Focal follows were conducted for 36 hours for tracks A, C, and D and 48 hours for track B. Each symbol represents a positional estimate in time.

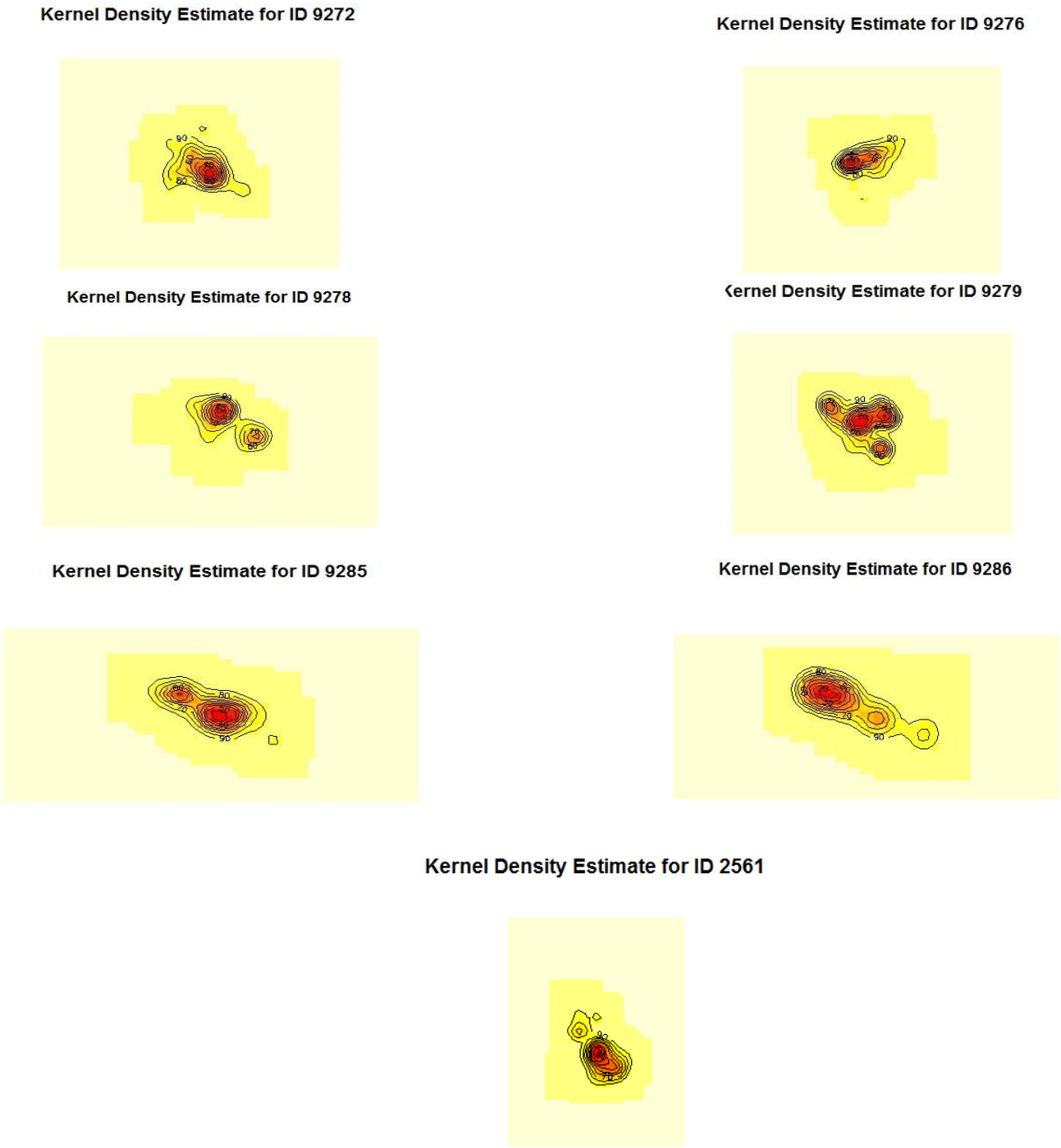


Figure 3: Visualization of core area use kernel density estimates for the seven southern flounder shown in Fig. 2.

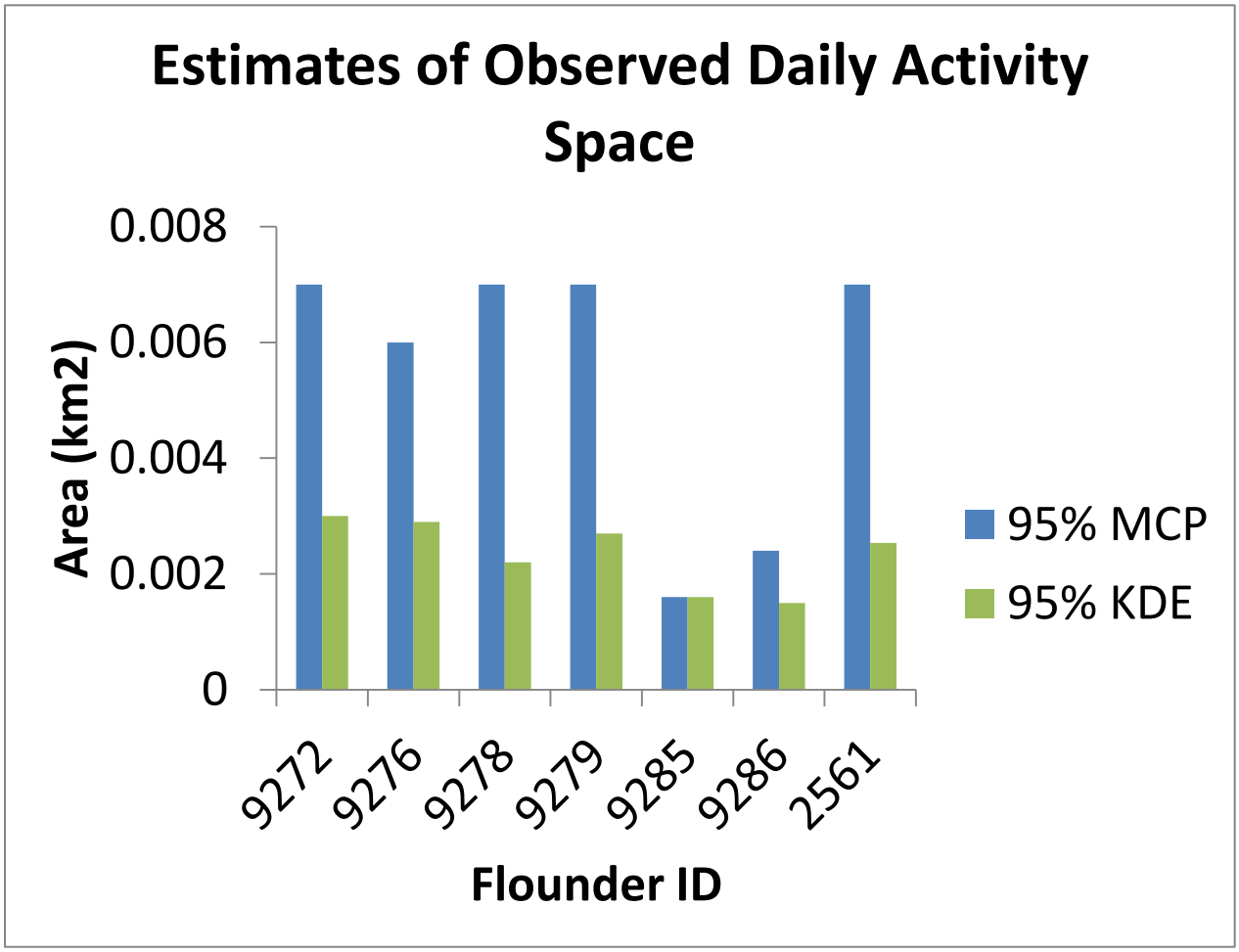


Figure 4: Daily activity space area estimates for seven southern flounder based on 36-48 hour focal follows using 95% minimum convex polygons (blue) and 95% kernel density estimates (green). Daily activity space estimates for southern flounder were small and ranged from 0.002 – 0.007 km^2, with kernel density estimates of core area use being smaller for each fish.

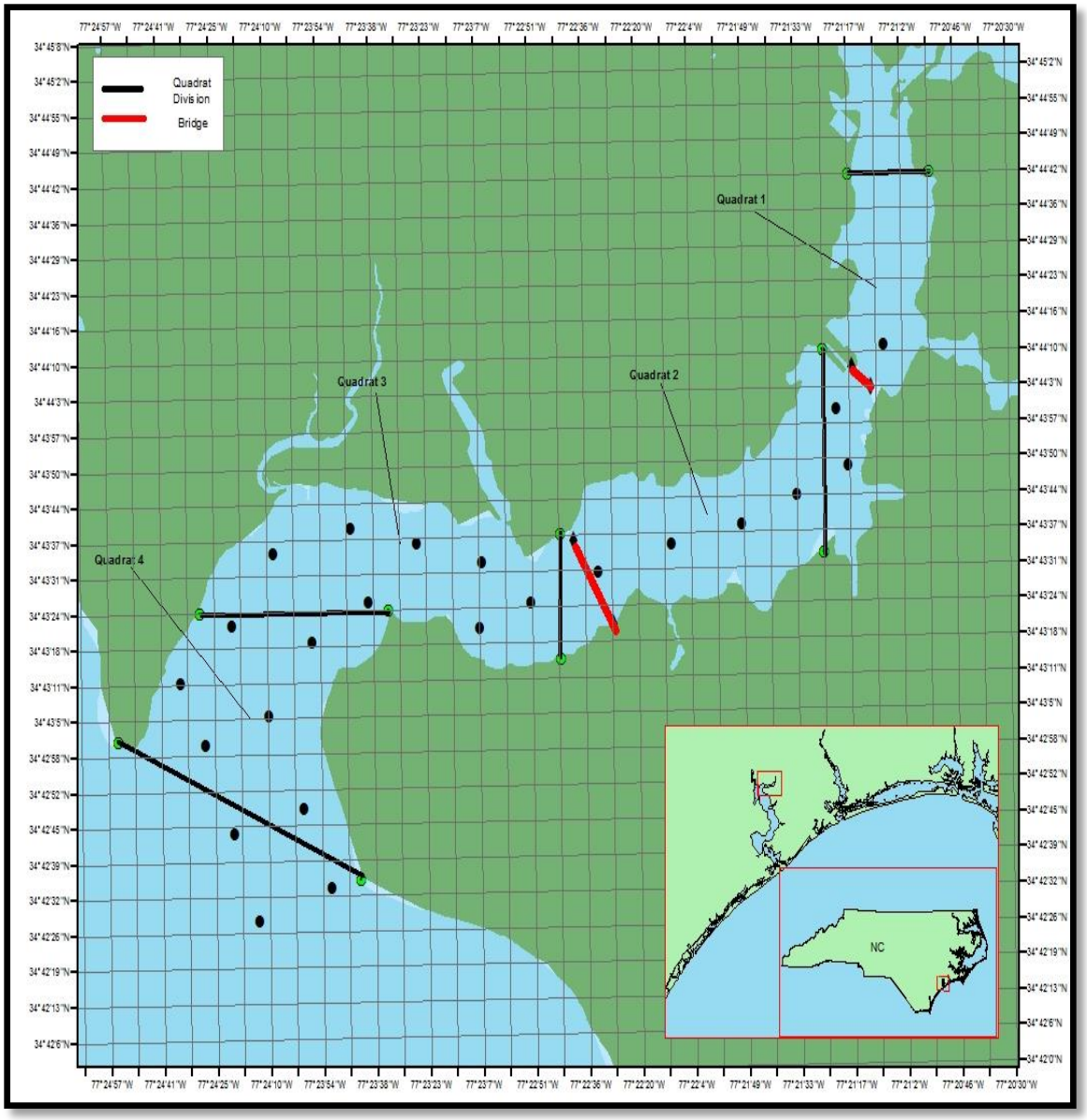
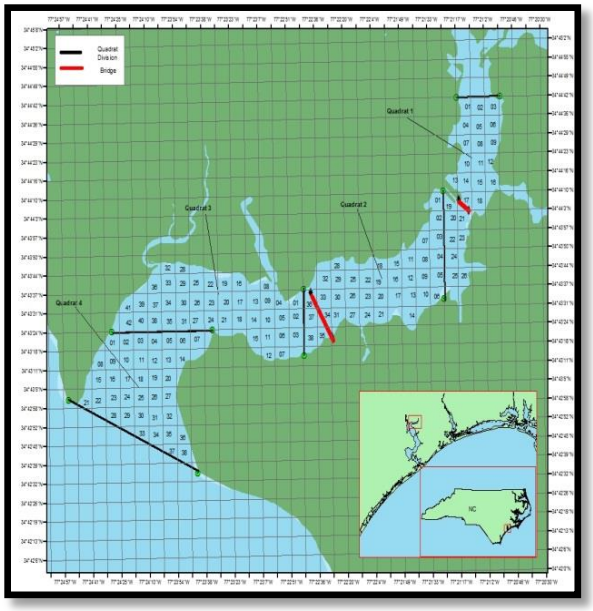


Figure 5: A map of Northeast Creak, within the New River estuary. Black dots indicate acoustic transect listening stations. Black lines indicate division of the creek into 4 strata for prey fish trawling and red lines indicate man-made bridges. A 200 × 200m grid was superimposed over the creek map to select random sites for sediment and prey fish sampling.

A.



B.

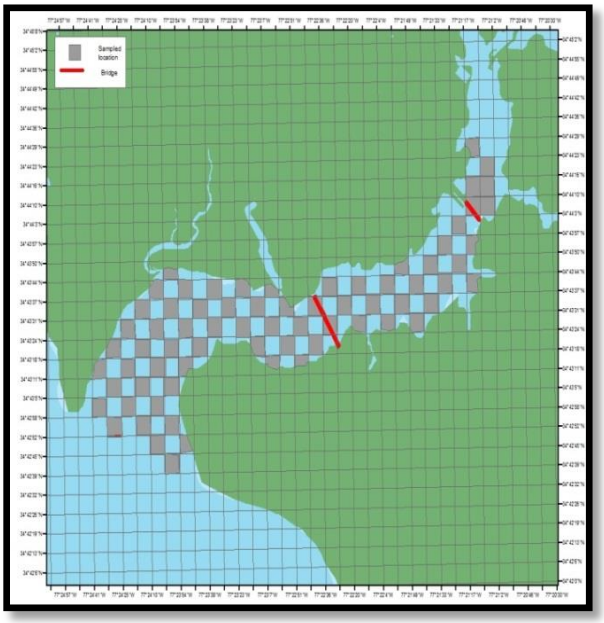


Figure 6: (A) Stratified random sampling grid used to conduct prey fish trawls in Northeast Creek. During 2014, four trawls were conducted in each strata (n = 16) weekly to assess temporal variation in the spatial patterns in flounder prey fish communities. (B) Red grids indicate sites of core samples to quantify sediment grain size and organic content.

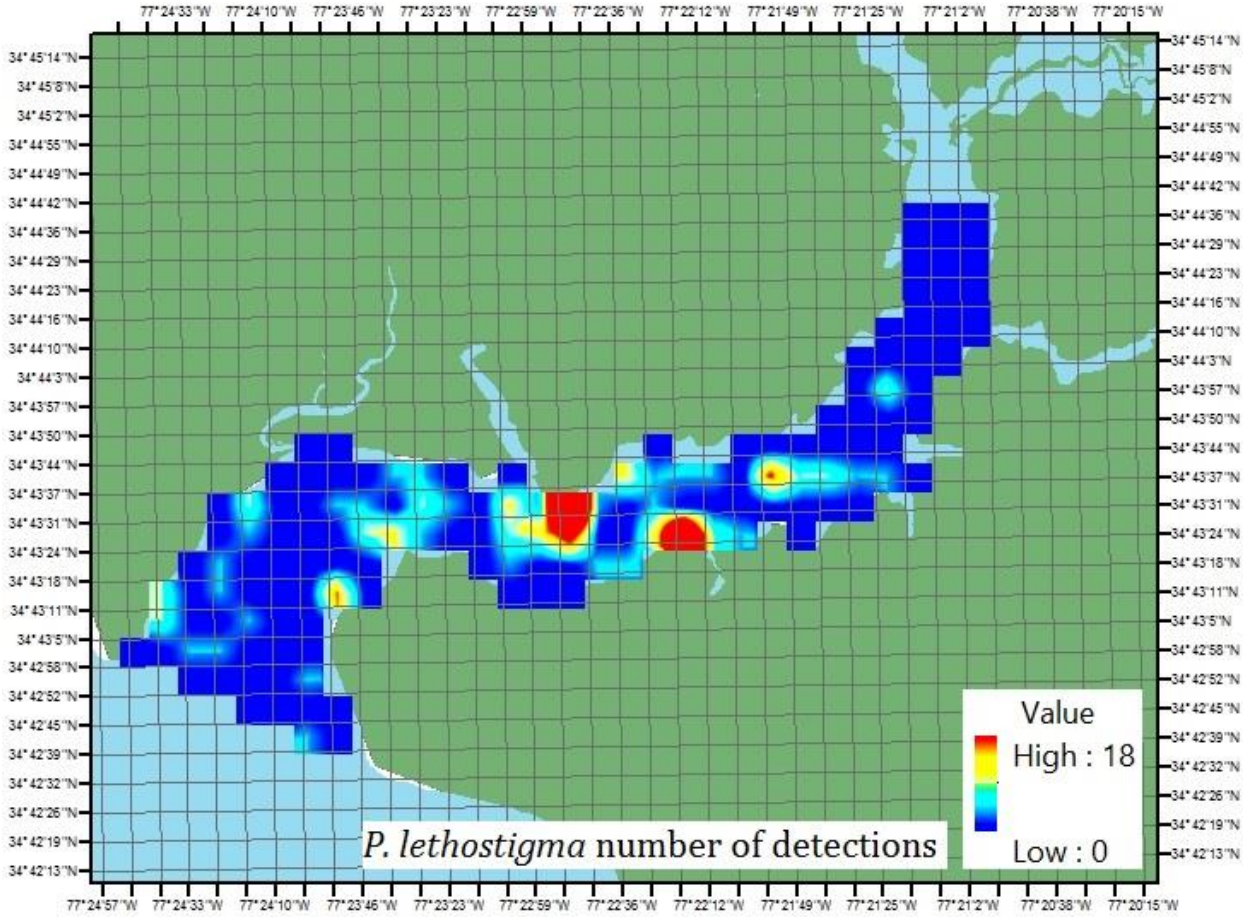


Figure 7: Detections of southern flounder in Northeast Creek during weekly transects in summer and fall 2014. Areas in red indicate high numbers (up to 18) of detections, and dark blue shading represents areas with no flounder detections. Intermediate values are represented by the colors in the sliding scale depicted in the legend.

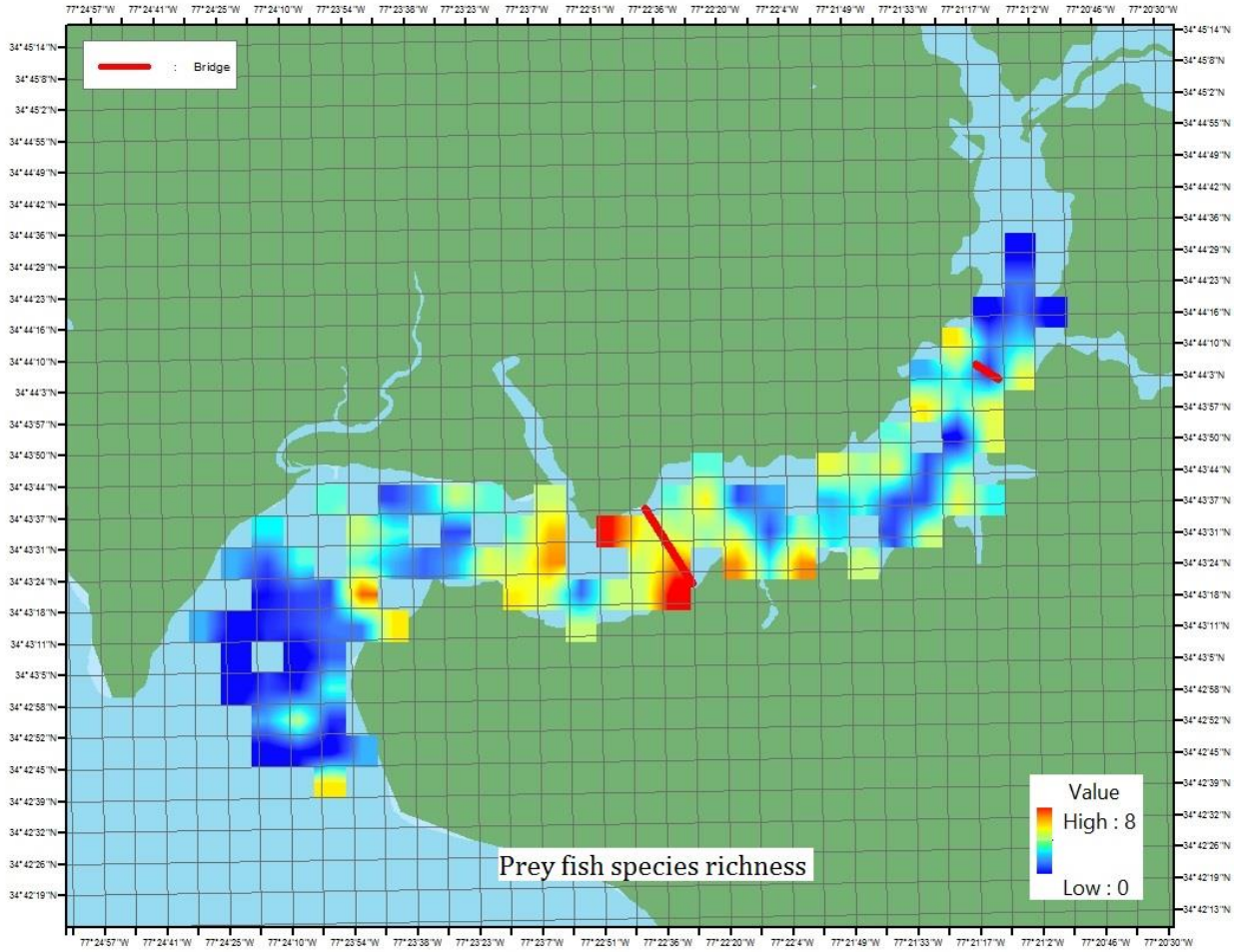


Figure 8: Map of Northeast Creek with colors representing mean prey species richness. Red represents a mean species richness of 8, and dark blue represents a mean species richness of 0. Intermediate values are represented by the colors in the sliding scale depicted in the legend.

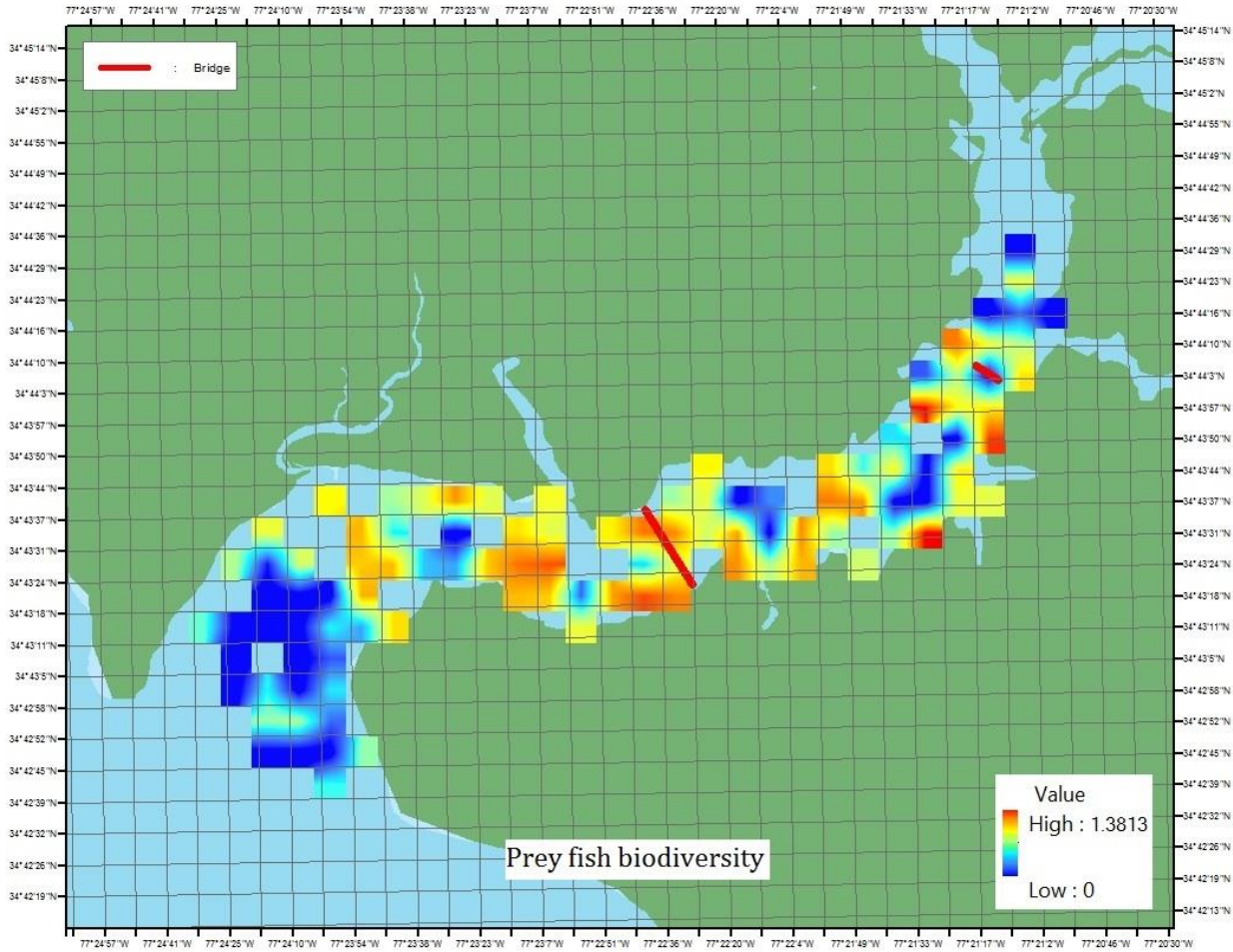


Figure 9: Map of Northeast Creek with colors representing average prey fish biodiversity as determined by the Shannon-Wiener Index. Red represents a mean biodiversity of 1.38 and dark blue represents a mean biodiversity of 0. Intermediate values are represented by the colors in the sliding scale depicted in the legend.

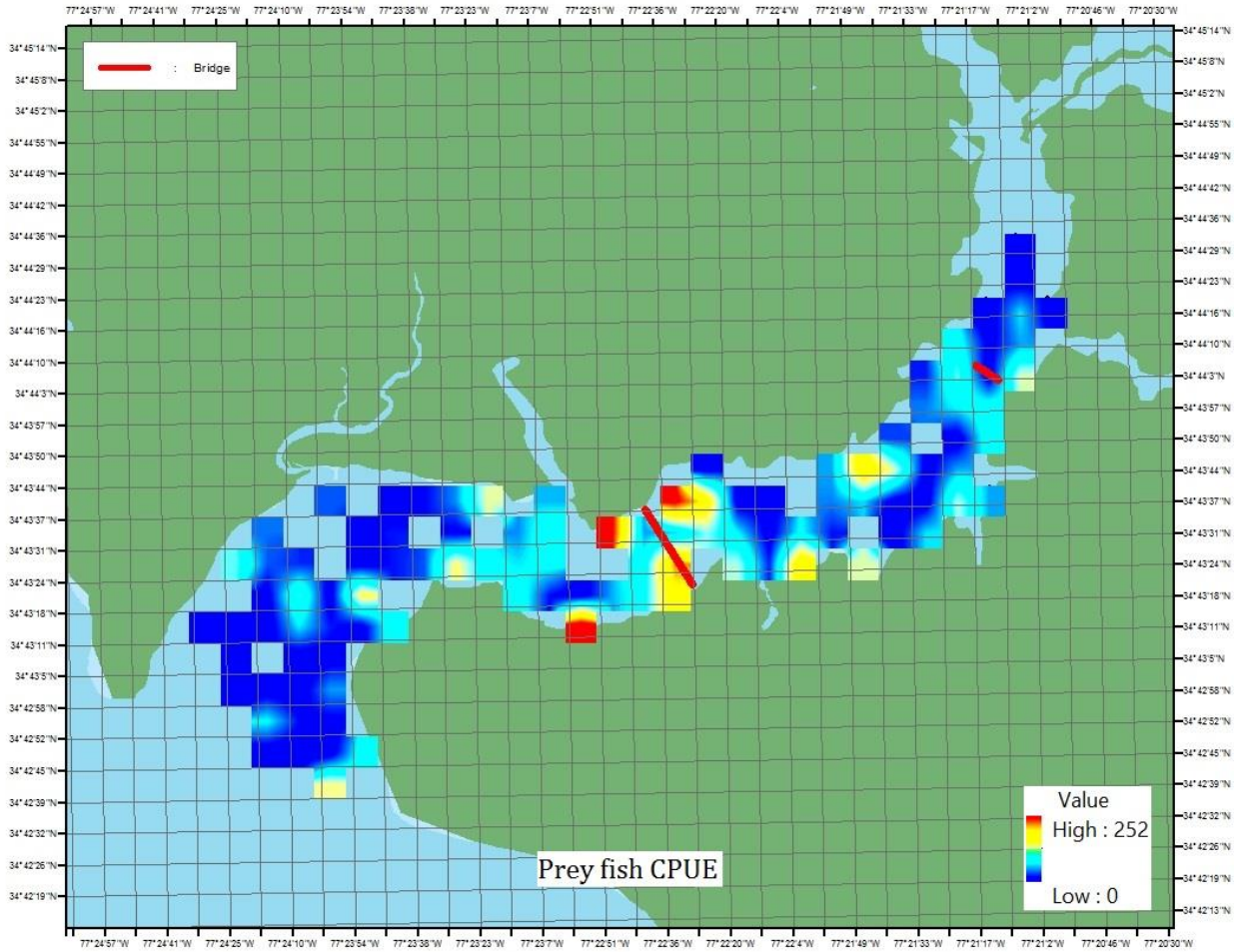


Figure 10: Map of Northeast Creek with colors representing average prey fish CPUE. Red represents a mean abundance per trawl of 252 and dark blue represents a mean abundance per trawl of 0. Intermediate values are represented by the colors in the sliding scale depicted in the legend.

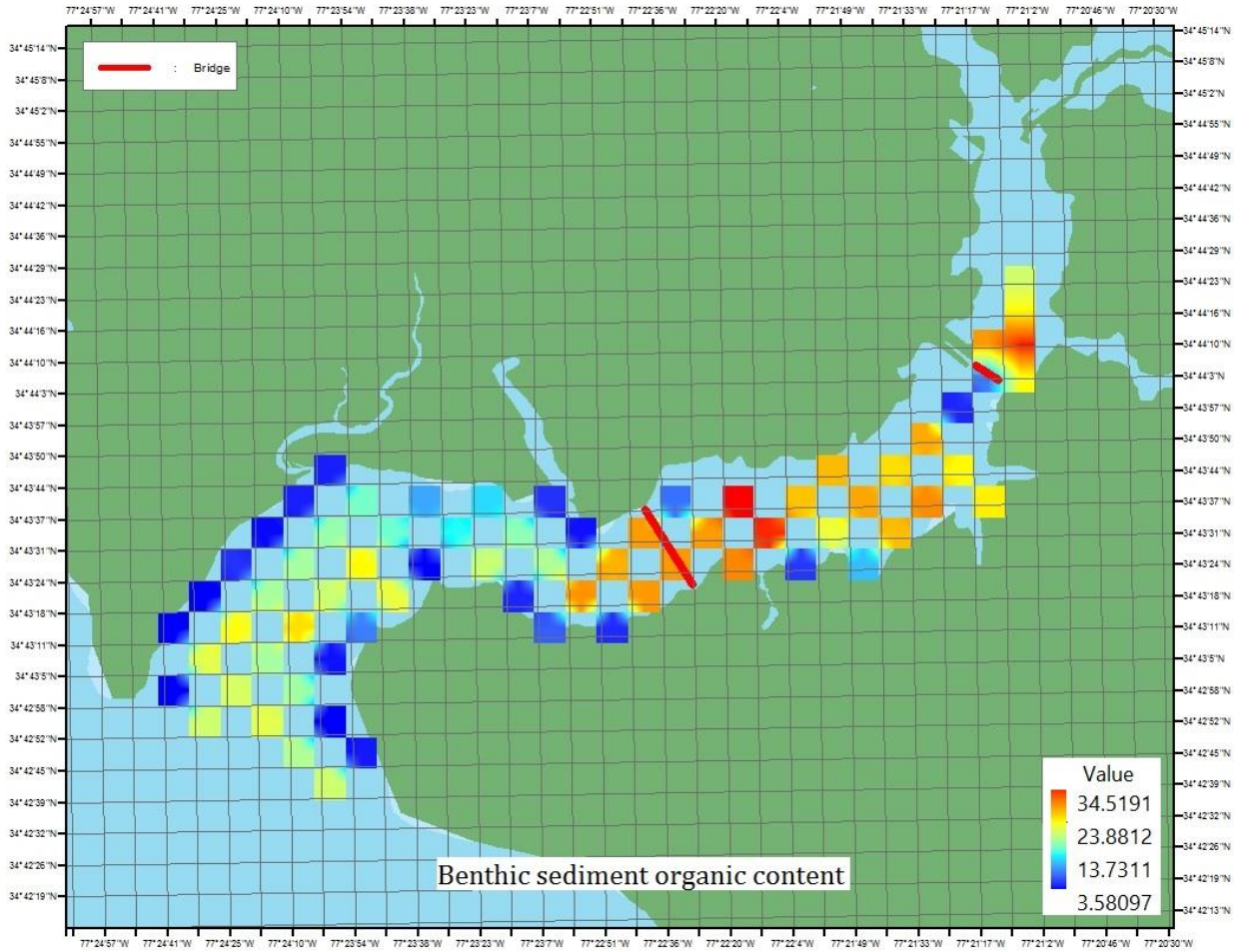


Figure 11: Map of Northeast Creek with colors representing sediment organic content. Red represents an organic content of 34.5% (by weight) and dark blue represents an organic content of 3.6%. Intermediate values are represented by the colors in the sliding scale depicted in the legend.

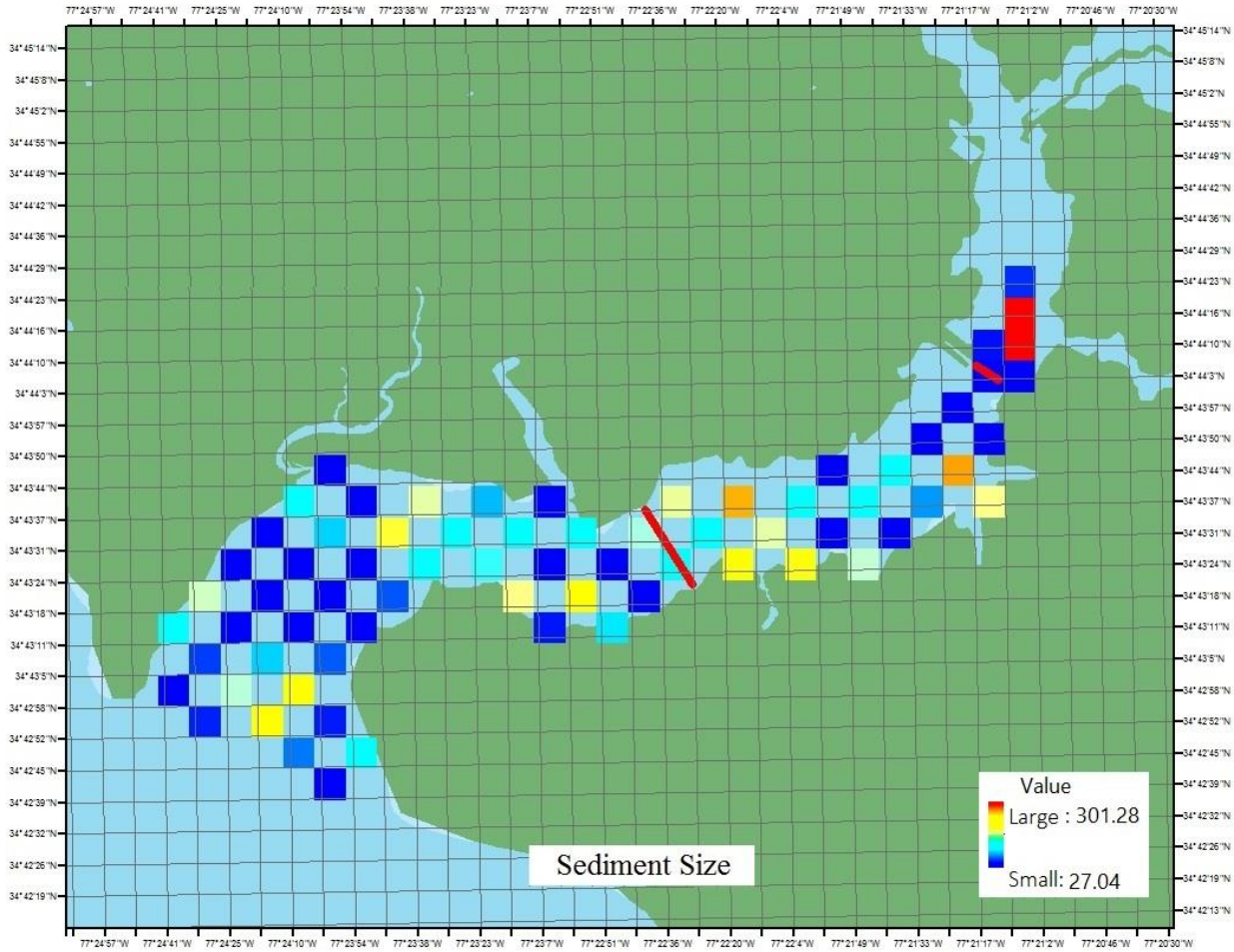


Figure 12: Map of Northeast Creek with colors representing mean sediment grain size. Red represents a mean grain size of 301.3 µm and dark blue represents a mean grain size of 27.0 µm. Intermediate values are represented by the colors in the sliding scale depicted in the legend

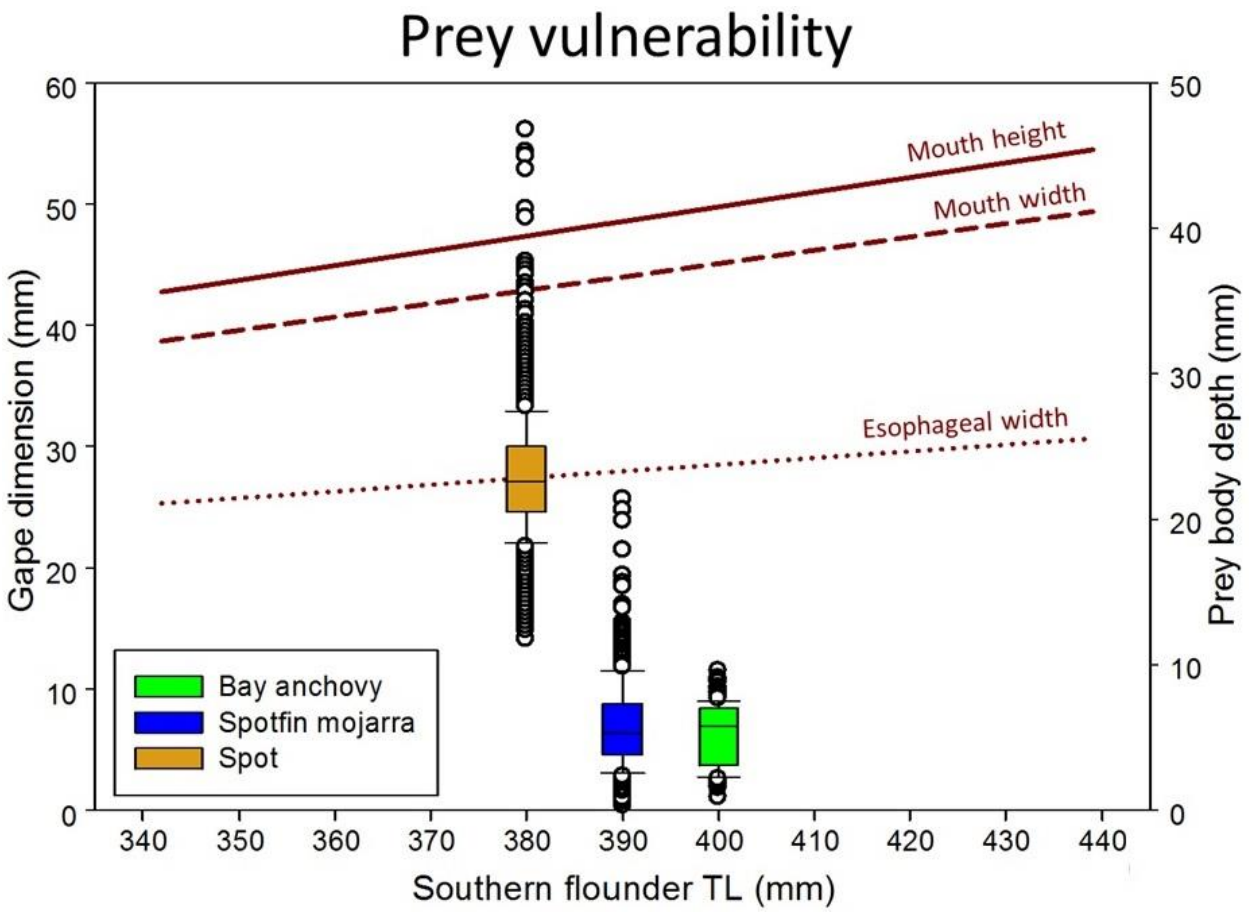


Figure 13: Box plots show values of prey body depth for the three primary prey items in the system in relation to regressions of southern flounder total length (TL) to gape size relationship for mouth height, mouth width, and esophageal width to illustrate vulnerability of the prey fishes available in the estuary. Gape information from the closely related and morphologically similar summer flounder was used as a proxy for southern flounder gape allometry.

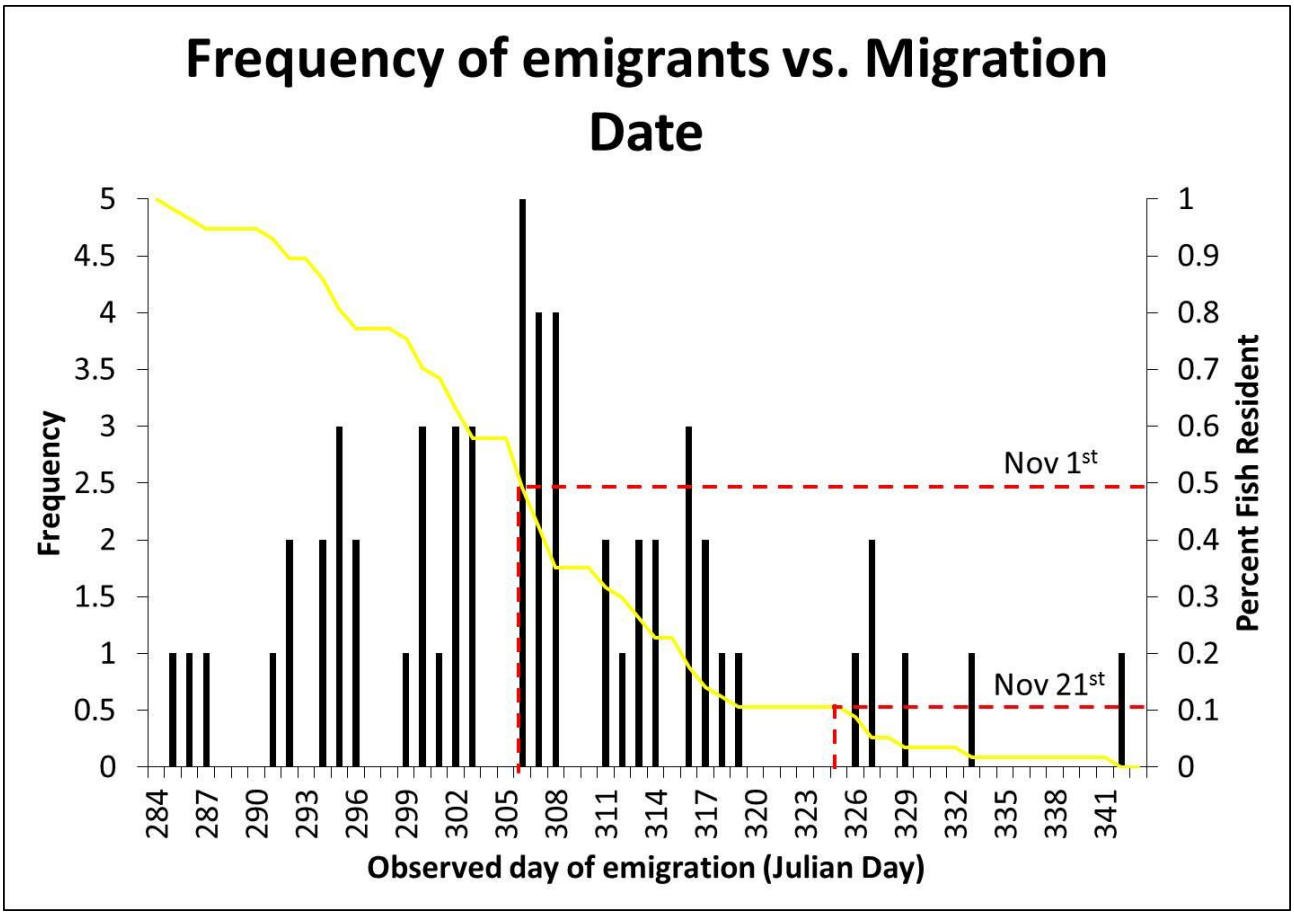


Figure 14: A histogram of emigrant frequency and observed migration timing (Julian Day) pooled for 2012-2014. Cumulative frequency distributions indicated that 50% of emigrating southern flounder left the New River estuary by Nov 1st and that 90% of emigrating fish had exited by Nov 21st. One additional fish was determined to have emigrated on Dec 27, 2014, and is not shown on this plot.

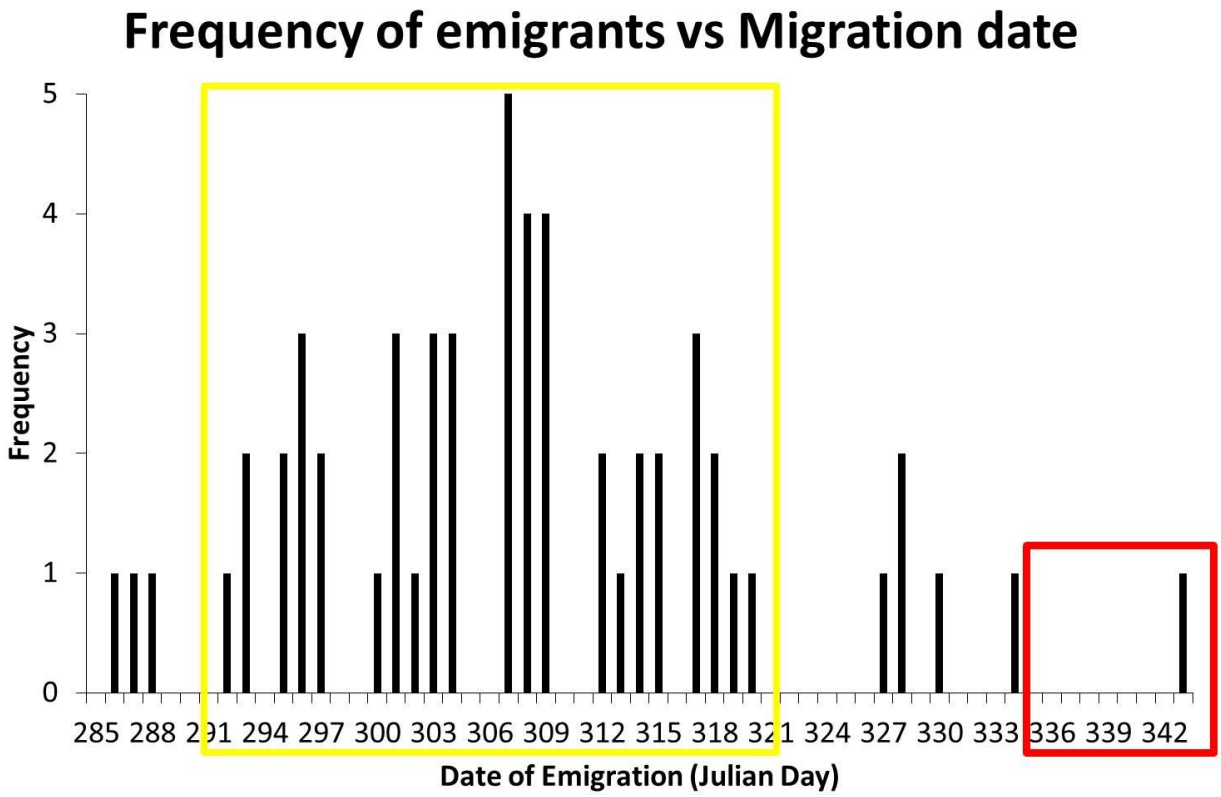


Figure 15: A histogram of emigrant frequency and observed migration timing (Julian Day) pooled for 2012-2014. The yellow box encompasses roughly 85% of observed migration activity, which occurred between Oct 19th and Nov 16th. The red box highlights the timing of the commercial closure (month of Dec) in North Carolina. One additional fish was determined to have emigrated on Dec 27, 2014, and is not shown on this plot.

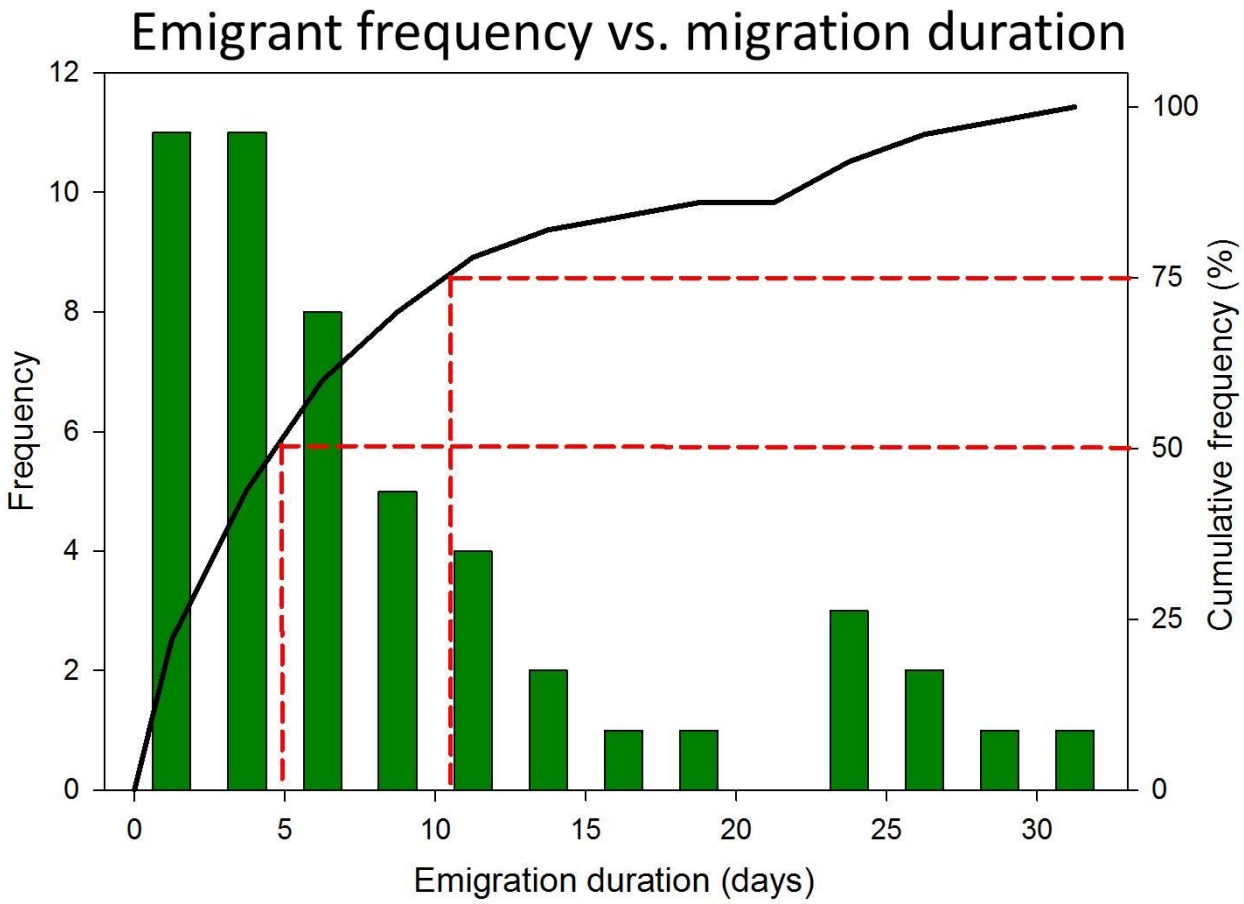


Figure 16: A frequency histogram showing emigration duration for southern flounder which exited the New River estuary. The cumulative frequency distribution indicated that 50% of emigrants left the system within 5 days after initiation of migration behavior, while 75% of emigrants exited within about 10 days of first showing emigration behavior.

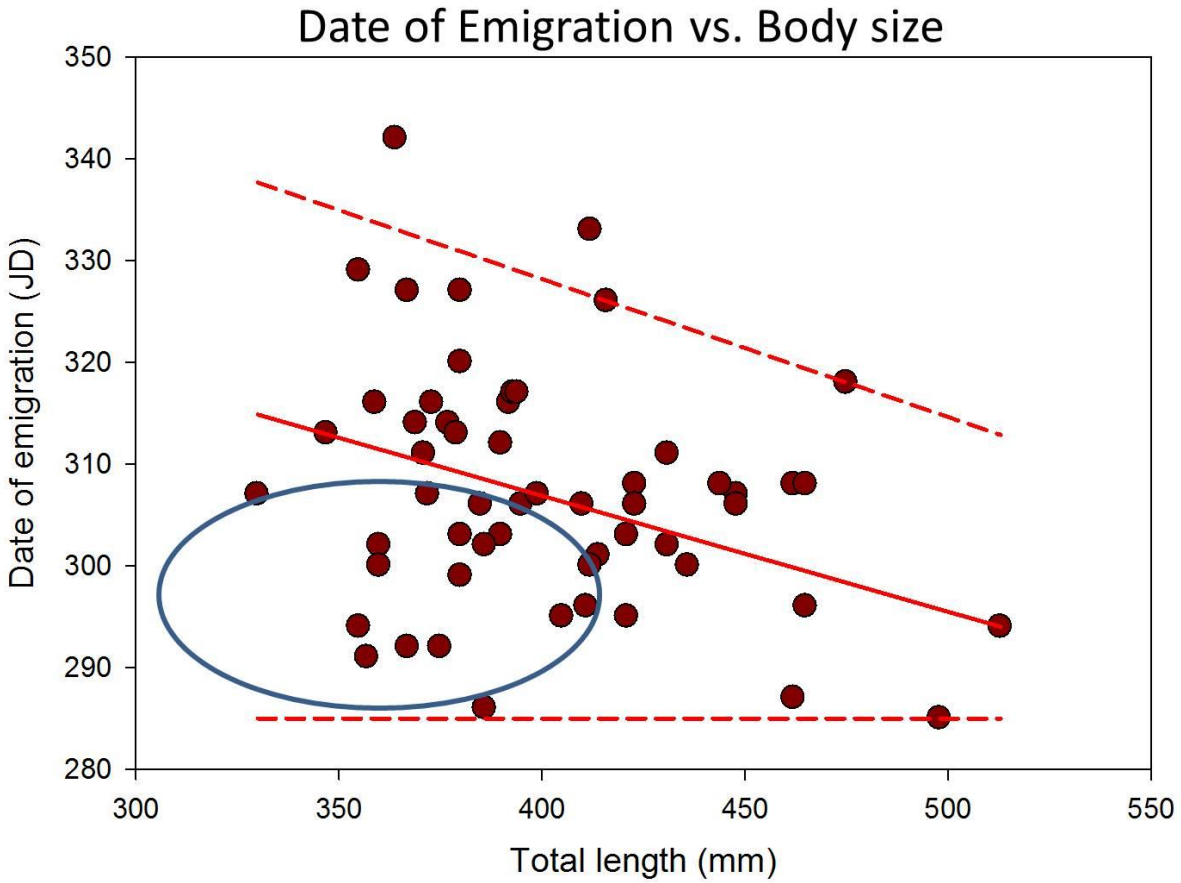


Figure 17: Bivariate scatter plot of migration date as a function of southern flounder TL. Regession quantiles (5th, 50th, 95th) are shown with red lines (dashed lines = upper and lower boundaries of the distribution). The negative slope of the median and the upper bound reveal a tendency for larger flounder to have earlier emigration dates, however much variation exists. Some of the smaller flounder with early departure dates (highlighted by the blue circle) could possibly be males, which confounds our ability to detect a strong pattern for females.

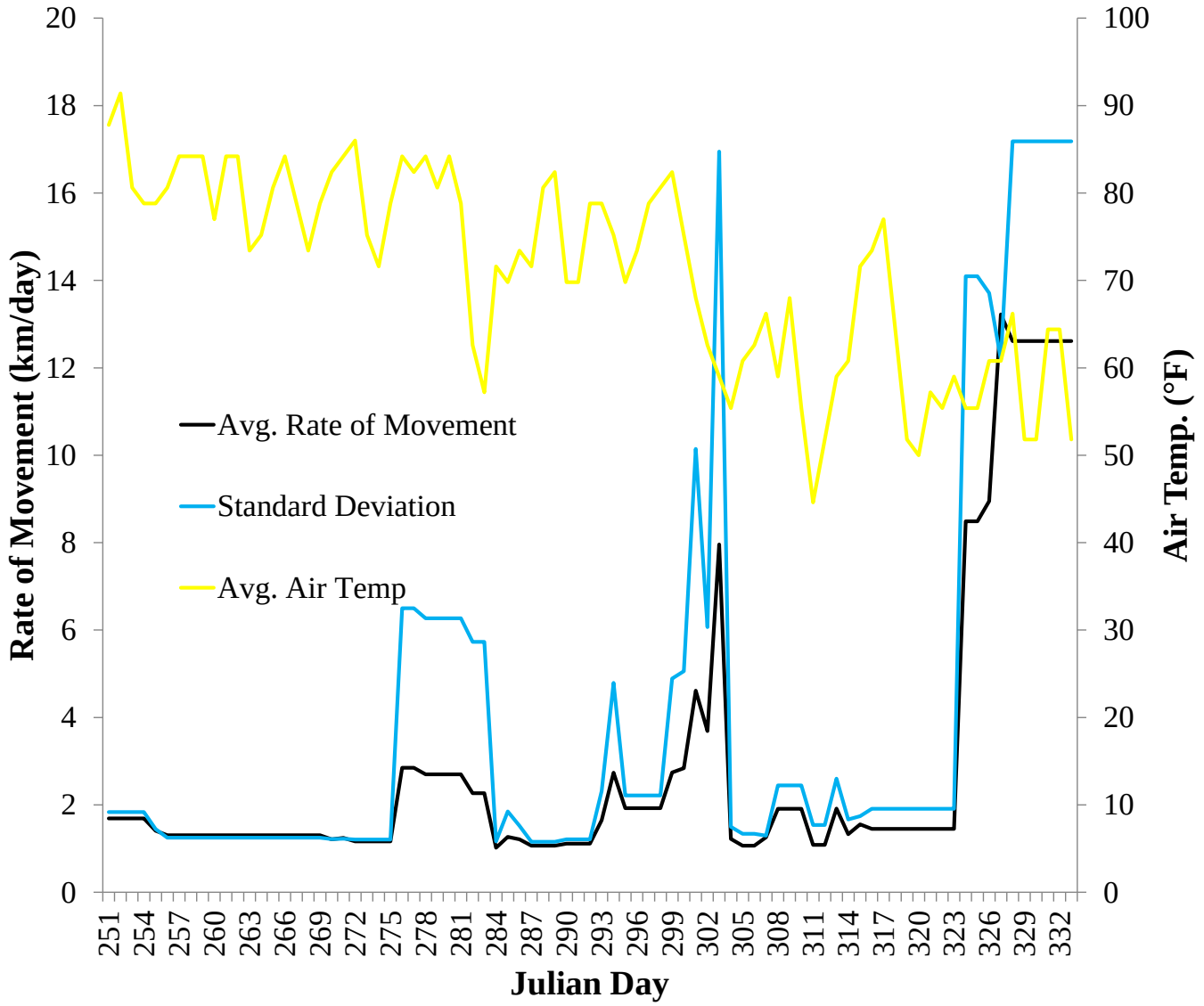


Figure 18: Southern flounder movement rate (km/day) versus day of the year plotted along with mean daily air temperature (in °F, solid yellow line) in the vicinity of the New River (Ellis airport, Richlands, NC). Solid black line = average rate of southern flounder movement; solid blue line = standard deviation of southern flounder movement. Variation in southern flounder movement was more pronounced following sharp drops in temperature.

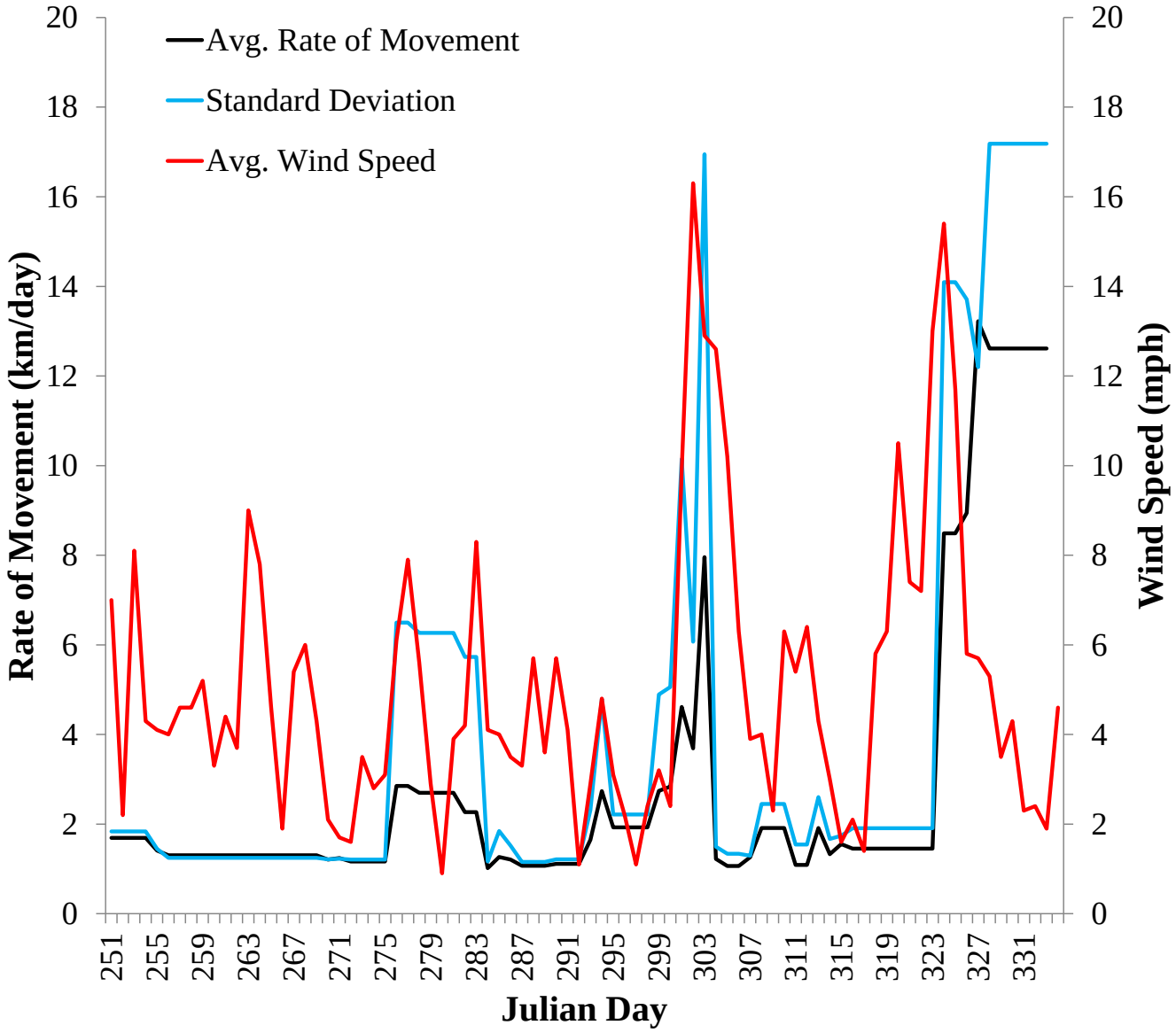


Figure 19: Southern flounder movement rate (km/day) versus day of the year plotted along with mean daily wind speed (mph, solid red line) in the vicinity of the New River (Ellis airport, Richlands, NC). Solid black line = average rate of southern flounder movement; solid blue line = standard deviation of southern flounder movement. Variation in southern flounder movement was more pronounced during high wind events.

Temporal and spatial variation in water temperature

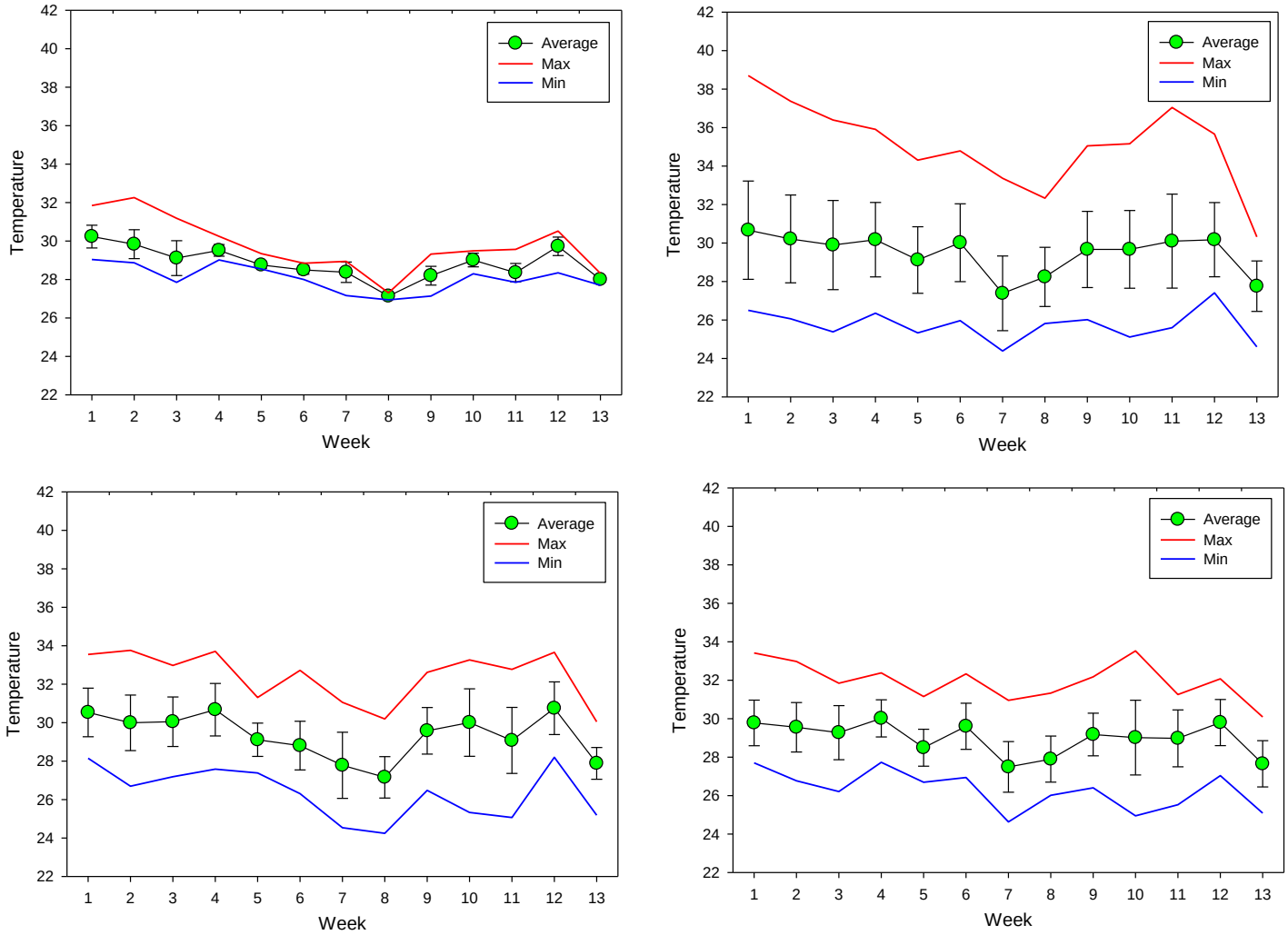


Figure 20: Variation in water temperature at four different locations in Northeast Creek during summer/fall 2014. The data in each panel was generated by a temperature logger that was moored in the creek. Water temperatures remained mostly steady across weeks, but some sites showed much greater variation within weeks than others. Most of the variation was related to water depth and diurnal changes in temperature, with shallow sites (< 1m depth) showing greater variability than deeper (1m < depth < 3m) sites.