Habitat Use by Telemetered Alabama Shad during the Spawning Migration in the Lower Flint River, Georgia

by

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A thesis submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the degree of
Master of Science

Auburn, AL
August 6, 2016

Keywords: *Alosa alabamae*, diadromous, GIS, substrate, migration

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Abstract

The Alabama Shad *Alosa alabamae* is an anadromous fish which lives in the northern Gulf of Mexico and ascends freshwater rivers in during springtime to spawn. Populations have experienced substantial range-wide declines due to habitat alteration, including the construction of dams which block access to historical spawning sites. The largest known population of Alabama Shad is found in the Apalachicola River in northwest Florida. To assess movement during the spawning run, 250 Alabama Shad were outfitted with radio and acoustic transmitters and transported from the Apalachicola River upstream to the lower Flint River near Bainbridge, Georgia during 2010-2014. The 153 relocations from 126 individual fish revealed congregation areas that were suspected to be spawning locations. To investigate substrate use by Alabama Shad, relocation data was combined with a detailed substrate map of the lower Flint River to determine whether Alabama Shad were using substrates in proportion to its availability. To assess movement during the spawning run, relocation data was used to determine temporal displacement from the stocking locations during March 15-May 31. Daily river discharge for the lower Flint River for March 15-May 31 2010-2014 was used to determine the timing of large (≥ 20-km) upstream movements in relation to river discharge. Analyses showed that Alabama Shad were selecting for Limerock Boulder substrate, avoiding Limerock Fine and Rocky substrates, and using Sandy substrate in the same proportion as its availability. Alabama Shad upstream movement was greatest during April-mid May, and appeared to be influenced by declining river discharge after

periods of high discharge. Movements ≥ 20-km generally occurred during April-mid May and were clustered together over a period of a few days. X-Y coordinates for all Limerock Boulder substrate areas on the lower Flint River were calculated to focus future efforts to determine exact spawning locations of Alabama Shad on the lower Flint River. Future telemetry efforts on the lower Flint River and elsewhere should collected water temperature data reliably in order to investigate the possible interactions between water temperature and river discharge that influence Alabama Shad behavior during the spawning migration. Icthyoplankton sampling conducted in the vicinity of Limerock Boulder substrates, combined with boat electrofishing, during periods of declining river discharge April-mid-May is recommended as a sampling approach for identifying exact spawning locations for Alabama Shad in the lower Flint River. The development of a Habitat Suitability Index for Alabama Shad using flow, depth, temperature and substrate measurements from identified spawning locations will aid in locating additional spawning locations on the lower Flint River and in other drainages where this species still persists.

Acknowledgements

I would like to thank Dr. Steve Sammons for both the opportunity to work in his lab as a Researcher and to attend Graduate School at Auburn University. Without his continued support, belief in second chances, and expertise this project would not have been possible. Committee members Dr. Jim Stoeckel and Dr. Terry Hanson have been supportive throughout this and other processes. I would like to thank Dr. Mike Maceina for his support, willingness to share his extensive fisheries knowledge, and good company at the lab and in the field. This thesis is but a small part of a much larger project aimed at understanding Alabama Shad in the ACF basin that began in 2005 and is ongoing. Travis Ingram with the Georgia Department of Natural Resources, Dr. Shawn Young, who completed a doctoral dissertation with Clemson University working on this project, and Dr. Steve Sammons were all instrumental in making this project a multi-year success. I would like to thank Chase Katechis and David Belkoski from Auburn University, along with others, for their efforts in tracking Alabama Shad on the Lower Flint River. Finally, I would like to thank Steve Rider of the Alabama Department of Conservation and Natural Resources for the funding which allowed this thesis to be completed.

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Introduction

The family Clupeidae contains nearly 200 species of fish collectively referred to as the herrings (Metee et al. 1996; Boschung and Mayden 2004). Clupeidae contains seven subfamilies, including the subfamily Alosinae, which in turn contains seven genera (Nelson 2006). The genus *Alosa* comprises 14 species and has representatives in North America, Europe, Southwest Asia and the Mediterranean (Waldman 2003). Six species of *Alosa* are found in North America, and all but Skipjack Herring *Alosa chyrsochloris* have an anadromous reproductive strategy involving adults migrating from marine environments into freshwater lakes and streams to spawn, with age-0 juveniles returning to marine environments in their first year to feed until reaching sexual maturity (Laurence and Yerger 1966; Kissil 1974; Loesch and Lund 1977; Mettee and O'Neil 2003; Harris et al. 2007). Homing behavior (adults returning to their natal rivers to spawn) has been documented for American Shad *Alosa sapidissima* (Dodsen and Leggett 1973; Hendricks et al. 2002), Alewife *Alosa psuedoharengus* (Messieh 1977) and Alabama Shad *Alosa alabamae* (Bowen 2005; Kreiser and Shaefer 2009) and is suspected to be prevalent in the genus *Alosa*. The cited authors conclude that a combination of olfactory and visual cues coupled with orientation to tides/ocean currents is likely important.

Alewife and Blueback Herring *Alosa aestivalis* can forgo the anadromous life stage and are capable of completing their life history in freshwater (Loesch 1987). However, Alewife has established more self-sustaining landlocked populations than Blueback Herring (Hildebrand et al. 1963; Scott and Crossman 1973; Prince and Berwick 1981). Whether this is due to intrinsic biological traits or is a result of Alewife stocking efforts to bolster forage for sport fishes (Kohler and Ney 1982) remains unknown.

Several populations of Blueback Herring have become established in Alabama reservoirs in recent years (Grove 2016); this and landlocked populations elsewhere suggest that Blueback Herring may readily establish self-sustaining landlocked populations when introduced (Owens et al. 1998). Perhaps the best known (and most extensively studied) landlocked population of Alewife is found in the Laurentian Great Lakes of North America, where this species invaded via the Erie Canal soon after its construction in the 1860s and has subsequently had a profound effect on the ecology and fisheries of the entire system (Smith 1970; Madenjian et al. 2008).

Members of the genus *Alosa* have long been exploited commercially and recreationally in North America. Commercial and recreational fisheries have principally focused on spawning runs of the various species as this offers the opportunity to collect large numbers of fish at highly predictable times (Waldman 2003). The American Shad is the most famously exploited *Alosa* species, with fishing rights along streams leased as early as the Colonial Period (Loesch and Atran 1994). Total landings in the United States for American Shad was more than 22,000 mt in 1896 (Kocik 2000), and in 1908 the American Shad fishery was considered the worlds' second largest by volume and third largest by value (Walburg and Nichols 1967).

However, dam construction to provide the hydropower necessary to fuel the Industrial Revolution was already taking a toll on the American Shad fishery by blocking access to ancestral spawning sites, and entire spawning runs of American Shad were eliminated as early as the mid-to-late 19th Century (Stillwell et al. 1874; Loesch and Atran 1994). An estimated 5,300 river km were lost as American Shad habitat by 1898 due to dam building and water pollution; roughly 1,000 river km had been restored by

2003 (Limburg et al. 2003). The combined effects of large harvests, dam building, and pollution led to a precipitous decline in American Shad abundance after 1900, with total landings declining to around 5,000 mt by 1925 (Walburg and Nichols 1967) and having stagnated around 1,000 mt for the period of 1980-2000 (Kocik 2000). Alewife and Blueback Herring, the other commercially important members of *Alosa* and often referred to collectively as “river herring”, have undergone a similar decrease in abundance. From 1950-1970 landings fluctuated between 20,000-30,000 mt. Following two decades of steady decline, landings had decreased to around 800 mt by 1993 and have remained about the same since (National Marine Fisheries Service (NMFS) 2015).

Conservation of *Alosa* stocks, particularly for American Shad and river herring, has been a priority for both state and federal natural resource-management agencies for decades, with the Atlantic States Marine Fisheries Commission (ASMFC) adopting a management plan for the anadromous alosid stocks of the eastern United States in 1985 (ASMFC 1985). Amendments, supplements and addendums to the plan have since been generated as needed to reflect changing priorities and data. Conservation efforts for river herring and American Shad have focused primarily on fish passage, stocking, and habitat protection/restoration (NMFS 2009). Currently, priorities for alosine management and conservation have shifted to other concerns, including improving fish passage success, investigating factors affecting riverine productivity, assessing mortality at hydropower facilities, quantifying impacts of various fisheries upon populations, and acquiring data on factors affecting movement and survival while in the marine environment (ASMFC 2014).

Alabama Shad *Alosa alabamae* is the only anadromous alosine in the Gulf of Mexico (Ross 2001; Metee and O'Neil 2003), having likely been separated from American Shad with the closure of the Suwannee Straits in what is now northern Florida, in the Miocene Epoch (Nolan et al. 2003). The historic distribution of Alabama Shad ranged from the Suwannee River in northwest Florida westward to the Mississippi River. Alabama Shad were reported to have traveled as far inland as the lower Ohio and Missouri rivers and the Mississippi River in central Iowa (Coker 1930; Lee et al. 1980; Patillo et al. 1997). There are no known estimates of historical abundance, although commercial landing data and observations indicate that Alabama Shad populations were once large enough to support commercial and recreational fisheries. However, the Alabama Shad has experienced substantial range-wide declines over the past century. Collection data over the past 30 years revealed that this species has become restricted to small portions of its former range, with most records consisting of a few individuals collected at sporadic intervals (Smith et al. 2011). The Alabama Shad is currently considered a Species of Concern by National Oceanographic and Atmospheric Association Fisheries due to a lack of available information (Federal Register 15, Vol 69, No. 73, 15 April 2004). Limburg and Waldman (2009) provide an extensive review on the declines and current status of anadromous fishes of the North Atlantic Ocean, and concluded that Alabama Shad are the most imperiled North American member of *Alosa*.

Dam construction that limited access to historical spawning grounds is likely the primary reason behind the observed range-wide population declines (Boschung and Mayden 2004). The largest known population of Alabama Shad occurs in the

Apalachicola River in northwestern Florida (Mettee et al. 1996; Ross 2001; Boschung and Mayden 2004).

Comparatively little information in general has been collected on Alabama Shad compared to American Shad (Adams et al 2000; Federal Register 19975, Vol 69, No. 73, 15 April 2004; Bowen 2005; Smith et al. 2011). Despite this, research efforts have been sufficient to describe basic spawning behavior and reproductive ecology of this species. Alabama Shad spawn March-May at temperatures of 18-23°C (Laurence and Yerger 1967; Mills 1972). Males are the first to arrive in rivers, with females arriving later as water temperatures increase (Mills 1972; Mettee and O'Neil 2003).

The primary spawning age-classes in the Apalachicola River, Florida are composed of age 2-3 males and age 2-4 females, with almost all fish age-4 or older being female (Laurence and Yerger 1967; Mills 1972; Ingram 2007). However, Mettee and O'Neil (2003) reported that males age 1-5 and females age 2-6 were collected from the Choctawhachee River in southern Alabama and northwestern Florida. Varying numbers of eggs present in sampled female Alabama Shad, coupled with fluctuating ratios of mature to immature eggs, indicates that Alabama Shad are heterochronal spawners, releasing eggs in batches as the eggs mature (Mills 1972; Mettee and O'Neil 2003; Ingram 2007; Grice et. al 2015). Mills (1972) and Laurence and Yerger (1967) reported spawning marks on scales in 35-38% of sampled fish from the Apalachicola River, FL; however, Ingram (2007) did not find spawning marks on sampled fish from the Apalachicola River.

There are no known records describing spawning habitat for Alabama Shad. However, the closely related American Shad broadcasts eggs over benthic substrates

(Walburg and Nichols 1967; Jenkins and Burkhead 1994) composed of sand, gravel and/or limestone in areas with moderate current (Laurence and Yerger 1967; Mills 1972; Fox et al. 2000; Mettee and O’Neil 2003. Alabama Shad may share similar characteristics of spawning biology with American Shad, but the lack of information regarding specific spawning habitat of Alabama Shad represents one of several substantial knowledge gaps in the life history of this species, and must be investigated further to aid management and conservation efforts.

A number of studies have characterized substrate types associated with American Shad spawning activity, although these methods have typically involved point sampling of habitat in locations where spawning activity was visually observed (Beasley and Hightower 2000), transect sampling in reaches where eggs were collected (Hightower and Sparks 2003) or transect sampling in reaches occupied by fish outfitted with transmitters (Bowman 2001). No effort at reach-scale substrate characterization has been attempted for American Shad due to time and personnel constraints. However, advances in side-scan sonar technology over the past decade have led to this technology now being utilized for fast, affordable and effective substrate characterization in lotic habitats (Kaeser and Litts 2010). This method shows promise to allow identification and quantification of alosine spawning habitat at the reach scale, but currently this has not been attempted.

Similarly, there is a relative lack of information regarding how American Shad respond to variations in river discharge during the spawning run. In 2009, the ASMFC completed a review of habitat requirements and preferences for a number of diadromous fishes in the Atlantic Ocean, including American Shad. The section on spawning flow/velocity for American Shad was generally restricted to flow measurements at the site of spawning activity and at fish passage structures (Greene et al. 2009).

In 2005 an ongoing, multi-faceted study involving numerous collaborators was undertaken to better understand the Alabama Shad population in the Apalachicola-Chattahoochee-Flint (ACF) river basin in southwestern Georgia and northwestern Florida (Figure 1). Primary objectives were to assess the population size of migrating Alabama Shad, assess voluntary passage success utilizing the lock chamber at Jim Woodruff Lock and Dam (JWLD), and to identify potential Alabama Shad spawning locations upstream of Jim Woodruff Lock and Dam in the lower Flint and Chattahoochee rivers (Ely et al. 2008; Young 2010, 2011; Sammons and Young 2012; Young et al. 2012; Sammons 2013, 2014). Results from the telemetry portion of this study revealed groupings of Alabama Shad at several locations in the lower Flint River across different years, leading investigators to postulate that these fish may be spawning in these discrete river reaches. Further, Schaffler et. al (2015) used otolith microchemistry to determine that 86% of adult Alabama Shad returning to JWLD were spawned in the Lower Flint River. My study seeks to characterize habitat use by and movement of Alabama Shad in the Lower Flint River during the spawning run.

Study Area

The ACF river basin has its headwaters in northern Georgia, with the Chattahoochee River originating in mountainous areas of northeast Georgia while the Flint River originates underneath Hartsfield-Jackson Airport in Atlanta, Georgia. Both rivers flow south and west and join at Lake Seminole, forming the Apalachicola River JWLD near the Florida-Georgia-Alabama state lines (Figure 1). While the Chattahoochee River has been heavily impounded and fragmented, with ten dams upstream of JWLD, the Flint River has only two dams upstream of JWLD, both near Albany, Georgia. The approximately 145-km reach of the Flint River from Albany Dam downstream to JWLD (Figure 1) is commonly referred to as the lower Flint River, and is the study area for my study.

Methods

Objectives

1. Describe habitat use by Alabama Shad in reaches within the lower Flint River and investigate whether or not Alabama Shad are using certain substrate types selectively.
2. Describe Alabama Shad temporal movements during the spawning season and in relation to river discharge fluctuations.

Telemetry

From 2010-2014, a total of 250 Alabama Shad were tagged with either acoustic tags (2010) or radio transmitters (2011-2014), (Ely et al. 2008; Young 2010, 2011; Sammons and Young 2012; Young et al. 2012; Sammons 2013, 2014). Fish were collected from the tailwaters of JWLD using boat electrofishing and angling. After tagging fish were transported to and immediately released from one of two locations upstream of JWLD: the Flint River near Bainbridge, Georgia (2010-2012) and at Chattahoochee Park ~ 5 km upstream of JWLD (2013-2014, Figure 3). This change in stocking locations was aimed at giving tagged fish a more equitable choice between ascending the Flint or Chattahoochee Rivers.

Manual telemetry using boat or aircraft-mounted directional YAGI antenna was conducted upstream of JWLD from mid-March-early June, depending on year. Despite the change in stocking location to give fish a more equitable choice of tributaries to ascend, Alabama Shad used Lake Seminole and the lower Flint River almost exclusively. Of the 250 tagged fish, 126 were eventually relocated, with the vast majority (~97%) of these relocations occurring in the Lower Flint River and Lake Seminole (Appendix 1, Figure 2). Relocated fish ranged in length from 270-475 mm, and ranged in weight from 183-1342 g (Figure 4). Suspected fish mortalities (i.e., additional locations for a fish that were less than 50-m apart from previous relocations) were removed from analysis. Repeat locations composed of the same day and waypoints were pared down to a single location for that day and fish. There were 153 total locations after removing mortality and repeat locations.

Substrate Map

Kaeser et al (2013) developed a high-resolution, landscape-level inventory of aquatic habitat in the lower Flint River to aid future conservation efforts of lower Flint River fishes, including Alabama Shad. Side-scan images and associated waypoints were collected using a small single-person boat during periods of high flow to allow for capture of the entire river channel in a single pass. Images were geo-referenced in ArcGIS (unknown version), and substrate types in the resulting mosaic were delineated by hand to create polygons of each substrate type. Accuracy of substrate classification was investigated using an underwater camera to view the substrate at randomly assigned points after image processing was complete. The resulting product was a detailed map of the lower Flint River broken into substrate types. I used these data, with the results of Alabama Shad telemetry activities, to investigate substrate use by Alabama Shad in the lower Flint River during the spawning run.

Analysis

For GIS analysis, the substrate types Unsure Sandy and Unsure Rocky in Kaeser et al. (2013) were assumed to be Sandy and Mixed Rocky substrate types, respectively, due to the likely composition of these areas despite the difficulty in classification from sonar images. Similarly, the substrate types No Data and Sonar Shadow were included in a single Missing Data substrate type. Substrate types were combined in this manner in order to increase the number of fish locations per substrate type. The substrate types used in my study, along with percent area and classification definitions, may be found in Table 1.

GIS-based analysis (ESRI 2013) of Alabama Shad telemetry data and substrate was based upon recently developed techniques used in the upper Flint River for three species of black bass (Goclowski et al. (2013). This analysis is principally a distance-based approach that was designed to be more robust to positional error inherent in data collected with GPS equipment (Conner et al. 2003; Kaeser and Litts 2010) than classification-based methodologies while also preserving the spatial complexity resulting from census-type habitat measurements as performed by Kaeser et al. (2013) on the lower Flint River. Only those waypoints that fell within the boundaries of the Lower Flint River substrate between Bainbridge, Georgia and Albany Dam were used. Likely fish mortalities (i.e., additional locations for a fish that were less than 50-m apart from previous relocations) were removed from analysis. Repeat locations composed of the same day and waypoints were pared down to a single location for that day and fish. This resulted in 109 fish location waypoints from 70 different fish used in the GIS analysis (Appendix 2).

An index of substrate complexity in the vicinity of each fish location was generated by placing a 15-m buffer around each waypoint, and then extracting the substrate information contained within the buffers using the Geoprocessing tools within ArcMap (Goclowski et al. 2013). Total perimeter (m) of the substrate types contained within the buffer was then calculated. Buffers containing multiple substrate types will have multiple shapes, conferring a larger aggregate perimeter than a buffer containing a single substrate (which will have a perimeter of a 15-m circle). An index of habitat complexity throughout the entire study area was then generated by placing a regular grid of 15-m buffers spaced 45-m apart over the substrate map (Goclowski et al. 2013).

Perimeters for substrate complexity within these buffers were calculated as with individual fish locations. Those buffers for fish location and study area substrate complexity that spilled over the boundaries of the substrate map were left intact to avoid artificially deflating perimeter calculations for locations near the river’s edge. The amount of buffer spilling over the substrate map was generally small (< 20% of the buffer) when this did occur. Perimeter values for both fish location and study area buffers were pooled and averaged to describe the relative substrate complexity of Alabama Shad locations compared to substrate complexity within the study area.

Finally, Euclidian distance (m) from fish locations to the nearest polygon of each substrate type was calculated to describe the average distance of Alabama Shad to different substrate types (Goclowski et al. 2013). Distances were derived using the Model Builder function and NEAR tool in ArcGIS. The substrate polygon containing the fish location was assigned a distance value of 0. A regular grid of points spaced 20-m apart was then placed over the map, and distances from each point to the nearest polygon of each substrate type was calculated in a similar manner as with fish locations. Distance to nearest polygon of each substrate type for both fish locations and points within the study area were pooled and averaged to compare the average distance of Alabama Shad to different substrate types to average distance of regular points within the study area to different substrate types.

Chi-square tests are commonly used for quantifying habitat associations of telemetered fish when the amount of available habitat is known or can be readily estimated. Rogers and White (2007) favored chi-square log-liklihood test statistics over the simpler Pearson chi-squared test statistic as more sophisticated models may be built although both approaches often yield comparable results. However, the use of log-liklihood test statistics is dependent upon the number of observations being large enough to support analysis using the fish as the primary sampling unit. Ott (1988) recommended expected frequencies (# of observations for a habitat per fish times the proportion of that habitat avaialble) of greater than five for each fish/habitat combination, with a minimum of 90% of expected frequencies greater than two. This is usually not a concern when many observations come from a few fish and the individual fish are treated as the sampling unit, which is typically the case in telemetry studies. However, when few observations come from many fish, as in the case with this study (109 locations from 70 fish; ~ 3.5% of expected frequencies greater than two), observations may be pooled and treated as the sampling unit (Rogers and White 2007).

McDonald (2014) cautioned against using chi-square tests when overall sample size is < 1000 observations due to artificial deflation of *P*-values at lower sample sizes. This is of particular concern when a test result is marginally significant. In these cases, exact goodness-of-fit tests, which are similar to chi-square test, are recommended. Exact goodness-of-fit tests examine the likelihood of the observed distribution against all possible combinations of distributions (~200,000 with this dataset) while being robust to small datasets.

To examine whether Alabama Shad are using substrates in proportion to their availabilty, the number of fish locations per substrate type was tested using the same dataset as for GIS analysis (Appendix 2) and two exact goodness-of-fit tests. The substrate types Rocky Boulder, Rocky Fine and Mixed Rocky were combined into a single “Rocky” substrate due to the low numbers of observations in each of those substrate categories (McDonald 2014). An exact multinomial goodness-of-fit test was performed using the EMT package (McDonald 2014; Menzel 2015) in the statistical software R (R Core Team 2014). An additional multinomial goodness-of-fit test using the XNomial package in R was run as this package provides a *P*-value for both the likelihood ratio and the multinomial probability (Engels 2013). The likelihood ratio is the probability of the observed result under the null hypothesis over its probability given the alternative (best fitting distribution under the multinomial distribution), and is is given by the following equation:

$$latexLLR = \sum_{i=1}^{k} m_i \; ln\left(\frac{np_i}{m_i}\right)$$

where m_i is the number of objects in category *i* and p_i is the hypothesized probability of that category (Engles 2013). The multnomial probability is the probability of the observed outcome under the null hypothesis, and is given by the following equation:

$$latexP(m_1, m_2,..., m_k) = \frac{n!}{m_1!m_2!...,m_k!} \; p_1^{\,m1}p_2^{\,m2}\ldots p_k^{\,mk}$$

where *m* is the number of objects in category *i* and *p* is the hypothesized probability of that category (Engels 2013). In the event that the EMT test returns a significant overall test, exact binomial post-hoc tests will be used to determine which individual substrates are being used differently than expected given the proportion of substrates avaialble. Post-hoc testing was not done for the XNomial multinomial test as it would be the same test as with the EMT multinomial test. A Bonferroni correction for multiple comparisons will be computed for the *P*-values resulting from post-hoc testing.

Finally, Manley's Selection Ratios were calcuated in the R package adehabitatHS (Calenge 2015) to determine what, if any, substrates Alabama Shad are selecting for or against (Rodgers and White 2007; Manly et. al (1993, 2002). Manley's Selections Ratios for substrate use by Alabama Shad were calculated using techniques for Type II data (where habitat availability is the same for all individuals and use by each individual is recorded). Manley's Selection Ratios is given by the following equation:

$$\widehat{w_i} = u_i / (\pi_i u_{++})$$

where U_{i+} is the amount of habitat type *i* used by all fish and U_{++} is the total number of habitat units used by all fish. Test values >1 indicate selection for a particular habitat, while test values <1 indicate avoidance of a particular habitat. All programming code used for statistical analysis was generated using guidelines found in Mangiafico (2015), and all programming code used may be found in Appendix 4.

If statistical analysis indicated that Alabama Shad were showing a preference for certain substrates (i.e., significant post-hoc tests for each substrate and a Manly Selection Ratio >1) centroids (X, Y coordinates for the center of a feature) for all polygons of that substrate type(s) were calculated in ArcGIS to direct future research efforts. Emphasis was placed upon those substrate polygons that contained a fish location or were within 500-m of a fish location.

Temporal patterns of movement by Alabama Shad were investigated by measuring displacement from stocking locations for all Alabama Shad locations within Lake Seminole and the lower Flint River (Appendix 1). Displacement was calculated using ArcGIS, pooled across years, and grouped into 20-km bins from -40 km (i.e., downstream of stocking location) to 160 km, as measured from stocking locations. These pooled data were then placed into 5 different temporal categories as appropriate: March 15-31, April 1-15, April 16-30, May 1-15, and May 16-31. Movement data was then plotted as percentage of all movement per 20-km movement bin for a given temporal period.

Alabama Shad movement in relation to river discharge variation was investigated by obtaining daily flow data for the period March 15-May 31 from USGS stream gauge # 02353000 near Newton, Georgia for 2010-2014. Those Alabama Shad locations which represented a ≥20-km movement were then plotted, by day and year, against the daily discharge data to identify flow patterns that triggered larger-scale movements. With yearly discharge regimes during the study period established, Alabama Shad movements were plotted in a manner similar to temporal displacement discussed above to investigate variation in upstream movement by year. Movement data was calculated using ArcGIS, pooled by year, and grouped into 20-km bins from -40 km (i.e., downstream of stocking location) to 160 km, as measured from stocking locations.

Results

GIS analysis showed that average substrate complexity within a 15-m circle of fish locations was slightly higher (169.0 m) than for the study area point grid (162.4 m, Table 2). Complexity values for fish locations ranged from 94.1 m to 395.7 m. On average, Alabama Shad were farther away from all substrate classes compared to the point grid, with the exception of Limerock Boulder, which Alabama Shad were more more than twice as close to than the point grid. On average, Sandy was the closest substrate to Alabama Shad locations wheras Island and Rocky Fine were the furthest from Alabama Shad locations. Alabama Shad locations and the point grid were similar distances from Sandy, Mixed Rocky, Rocky Boulder and Limerock Fine substrates.

A plot of observed/expected values of Alabama Shad substrate use suggested that Alabama Shad were using Sandy substratres in accordance with its availability (i.e., "expected"), Limerock Fine and All Rocky substrates less than their availability, and Limerock Boulder greater than its availabilty (Figure 5). The overall multinomial test in the EMT package was highly significant (P<0.0001). Exact binomial *post-hoc* testing gave significant results for AllRocky (P=0.0052) and Limerock Boulder (P<0.0001) substrates. Exact binomial *post-hoc* testing for Sandy and Limerock Fine substrates were not significant (P > 0.05). The likelihood ratio in the multinomial package XNomial was highly significant (P<0.0001), while the multinomial probability was also highly significant (P<0.0001). Manley's Selection Ratios indicated selection against Limerock Fine substrate (w_i=0.87, 95% C.I. = 0.33-1.42), selection against All Rocky substrate (w_i=0.52, 95% C.I. = 0.21-0.85), and selection for Limerock Boulder substrate (w_i=4.86, 95% C.I. = 2.29-7.44). There was neither selection for nor against for Sandy substrate

(w_i= 1.0, 95% C.I. = 0.96-1.11). Given the apparent selection for Limerock Boulder substrates by Alabama Shad, X-Y coordinate centroids were created for all Limerock Boulder substrate polygons within the study area (n = 195), with emphasis given to those Limerock Boulder polygons that either contained a fish location or were within 500-m of a fish location (Appendix 3).

Analysis of Alabama Shad temporal displacement showed that peak upstream movement during 2010-2014 generally occurred in April, with approximately 50% of relocated fish exhibiting upstream movement during the period of April 16-30 over the study period (Figure 6). Lesser upstream movement was observed during May, while no upstream movement was observed during March. Timing of upstream movement appeared to be influenced by increased river discharge, with almost all observed movements ≥ 20-km closely following periods of increased river discharge, generally clustered together over a period of a few days, and typically occurring at river discharges of 5,000-10,000 cubic feet per second (Figure 7). Number of relocations and extent of upstream migration also appeared to be influenced by river discharge, with the lowest number of relocations and least amount of upstream movement occuring during years with relatively high river discharges (2010 and 2014; Figure 8).

Discussion

Results from my study indicated that Alabama Shad in the Lower Flint River were selecting against Rocky substrates and selecting for Limerock Boulder substrates, with Sandy and Limerock Fine substrates generally being used in accordance with their availabilty. The closely related American Shad has been shown to select for larger, coarser substrates such as gravel, cobble and boulder/bedrock during spawning activities while avoiding finer substrates such as silt and sand (Hightower et al. 2012). This selection for larger substrates has also been demonstrated in other members of the genus *Alosa* (Caswell and Aprahamian 2001; Hightower and Sparks 2003; Harris and Hightower 2011). Having X-Y coordinates for the location of preferred substrates should prove valuable in focusing future efforts on the lower Flint River aimed at positiviely identifying discrete areas used by spawning Alabama Shad.

The patterns of upstream movement by Alabama Shad occurred during the general spawning period of March-May reported for Alabama Shad (Laurence and Yerger 1967; Mills 1972), although these results suggest that the period of greatest upstream movement in the lower Flint River may be concentrated during April-mid May most years. River discharge appeared to have an effect on Alabama Shad movement during the spawning migration, with Alabama Shad upstream movements ≥ 20 km generally clustered together over a period of a few days closely following periods of increased river discharge. The overall extent of upstream movement also appeared to be influenced by river discharge, with fewer fish moving shorter distances upstream during high discharge years than in lower discharge years. River discharge has been shown to be an important variable affecting the timing of spawning and spawning-associated migrations in other anadromous fishes. Shortnose Sturgeon *Acipenser brevirostrum* in the Connecticut River spawned over a short period coinciding with decreasing river discharge (Buckley and Kynard 1985). Migration timing of spawning Chinook Salmon *Oncorhynchus tshawytscha* in the Columbia River has been linked to river discharge, with later-than-usual upstream migrations at high discharge and earlier-than-usual migrations at low discharge (Keefer et. al 2008).

However, river discharge alone is likely insufficient to explain movement of Alabama Shad in the lower Flint River. Water temperature has long been regarded as an important variable affecting the spawning migration of fishes, although understanding the exact role temperature plays can be complex. The initiation of the spawning migration of Alewife in six different New England streams began 13 days earlier over the course of a 30-year period beginning in the 1970's (Ellis and Vokoun 2009). This coincided with stream water temps reaching 13°C (considered a useful predictor for run initiation of Alewife in the study streams) about 12 days earlier over the same period. Richkus (1974) found a complex relationship between river discharge, water temperature, and numbers of spawning Alewife arriving at a fishway. Water temperature was an important variable for defining initiation and cessation of the spawning run as well as daily fish movement through the fishway. However, patterns of daily counts of Alewife at the fishway were practically identical across study years despite differences in daily stream temperature between years. In terms of river discharge, the numbers of alewife arriving at the fishway reliably increased approximately two days following a period of increased river discharge. The author postulated that this delay represented travel time necessary for the fish to travel from a downstream estuary after receiving environmental cues associated with increased river discharge that initiated upstream migration.

Unfortunately, I was unable to investigate the effect of water temperature on Alabama Shad spawning migration behavior as these data are unavailable for the lower Flint River due to USGS streamflow gauge operation, equipment breakdowns, and shifting study objectives during 2010-2014. It seems likely that water temperature and river discharge work together in a complex manner to cue upstream movement of Alabama Shad in the lower Flint River. Svedsen et al. (2004) observed that there was an increased probability of anadromous female Brown Trout *Salmo trutta* making an upstream migration in the increased discharge, but did not see this relationship with water temperature. However, there was a significant interaction between water temperature and river discharge where the effect of river discharge affected the probability of a fish making an upstream migration differently depending on temperature. Higher water temperatures were associated with a greater probability of upstream migration than cooler water temperatures at a given river discharge.

Future studies examing Alabama Shad movements in the lower Flint River and elsewhere during the spawning migration should ensure that water temperature data are collected reliably over the study period. Characterizing the roles that river discharge, water temperature, and the possible interactions between them would further knowledge of movement by Alabama Shad in the lower Flint River, and would likely aid future management efforts.

Dutterer et al. (2011) found that the extent of upstream movement of American Shad was affected by river discharge in the St. Johns River, Florida, with American Shad migrating shorter distances upstream during low-discharge years compared to high-discharge years. Alabama Shad in the lower Flint River showed the opposite behavior, with fewer movements ≥ 20-km during higher-discharge years (2010 and 2014). These movements also occurred in mid-late May, which was generally later than during the other three years. This may reflect a reduced ability to orient in the swirling, turbulent currents present in the Lower Flint River at higher flows. Leggett (1976) reported that American Shad needed steady, direct flow to locate spawning habitat and would begin meandering or moving downstream in the presence of tidally-induced flow turbulence or flow reversals. Similarly, Katz (1986) found that high river discharge could overcome the swimming ability of American Shad and flush fish downstream.

Low relocation rates of Alabama Shad throughout the study, even in low discharge years, limited my ability to assess habitat use and movement patterns. Telemetry of Alabama Shad within the lower Flint River and lower Alabama River has proven to be difficult, with low relocation rates and suspected high fallback rates being a concern in both systems (Sammons 2014; Kern and Sammons 2015). "Fallback" is a term used to describe anadromous fishes that abandon their spawning migration as a result of stress from handling, transmitter implantation, or environmental conditions (Moser and Ross 1993). It is typically manifested as tagged fish that are not subsequently detected or are detected downstream of the tagging location and do no return upstream.

Fallback rates for Alabama Shad tagged downstream of JWLD for the voluntary passage study ranged from negligible (<2%) to >30% (Sammons and Young 2012). Fallback rates of Alabama Shad in the Alabama River were much higher, as 87-92% of tagged fish rapidly moved downstream of the stocking area in both study years (2014-2015). While fallback certainly accounts for some of the tagged Alabama Shad that were never relocated in the lower Flint River, it does not explain the large gaps between relocations observed for some of the relocated fish.

In telemetry studies, “Detection Efficiency” is defined as the probability of transmitter detection (Melnychuk 2012). Many factors affect detection efficiency, including user-controlled factors, tag-to-receiver distance, and environmental/behavioral factors. Individuals performing boat telemetry on the lower Flint River were given proper instruction on the use of the tracking equipment, while transmitter operation was checked prior to insertion into Alabama Shad, thus greatly reducing the chance of user-controlled error. Given that the lower Flint River generally does not exceed a width of 150 m (a much shorter distance than what I have detected radio-tagged fish at during other boat telemetry projects and aerial surveys) and the ease with which those fish that were relocated were detected, it appears that tag-to-receiver distance was not problematic during this study.

Some combination of environmental and behavioral factors is likely the cause of low relocation rates of Alabama Shad in the lower Flint River. The lower Flint River passes through the Floridian Aquifer, which is characterized by highly erodible limestone bedrock. As a consequence, there are many “blue hole” springs along the river’s length that are substantially deeper than the surrounding river. While Shoal Bass *Micropterus cataractae* telemetry efforts in major tributaries to the lower Flint River have not encountered issues with telemetered fish “disappearing” into these geologic features, it is possible that Alabama Shad may use these features as refugia or for other reasons during the spawning migration. Radio transmitter frequencies (commonly 8.000 kHz-152.00 kHz) experience significant signal attenuation with increasing fish depth. Freund and

Hartman (2002) measured signal strength attenuation of radio transmitters at different depths in the Ohio River, and found a significant reduction in detection range with increasing water depth. Transmitters were detected at a distance of 800 m at a depth of 1 m, while the detection distance decreased to 150 m at a depth of 9 m. Transmitters were generally undetectable at depths greater than 10.3 m.

American Shad have been found to occupy the lower half of the water column during the spawning migration (Witherell and Kynard 1990), thus, a combination of increased water depth during periods of high river discharge, swimming depth of Alabama Shad, and the presence of significantly deeper habitats than what is available in the rest of the river could lead to decreased detection probability.

Results of my study indicated that Alabama Shad in the lower Flint River were capable of large, rapid movements that may have also have hindered detection by telemetry. Telemetry efforts on the lower Alabama River provide additional insight into how rapidly these fish are capable of moving (Kern and Sammons 2015). In the lower Alabama River, one fish moved approximately 72 km downstream 5 h post-stocking and eventually 97 km downstream 19 h post-stocking. Another fish moved downstream into the Mobile-Tensaw Delta, and returned to Claiborne Lock and Dam within 6 weeks for a distance travelled of approximately 338 km. A third fish moved approximately 60 km upstream and back downstream over the course of 2 days. These rapid, long-distance movements bring into question the possibility of a “leap-frogging” effect, where fish may move into river reaches previously surveyed during overnight periods or during other periods when boat telemetry is not occurring.

Despite the difficulty in relocating Alabama Shad, efforts on the lower Flint River were successful in that we were able to identify patterns of substrate use and movements within the system. While high rates of movement can help fish evade detection, useful inferences may be made about general behavioral patterns if data are collected over multiple years (Winter 2012). Future telemety studies involving Alabama Shad on the Lowel Flint River and elsewhere should consider the difficulties involved and plan accordingly, including multi-year study designs, more frequent tracking efforts, and more powerful tags.

Hightower et al. (2012) identified three primary strategies for confirming American Shad spawning areas: itchtyhoplankton sampling, telemetry, and spawning splashes. Spawning splashes are probably the best direct indicator of where spawning activity is occuring, but drawbacks include this activity being possibly more prevalent in shallow habitats (Layzer 1974), and occuring at night, which makes observations over a large area difficult (Leim 1924). It is unknown whether Alabama Shad engage in such activity despite being closely related to American Shad. Ichthyoplankton sampling was seen as an efficient and simple method to sample many locations within a river reach. The primary disadvantage with this method is that the points of successful collection may not coincide with the spawning location due to downstream drift and other variables (Chittendon 1969; Marcy 1972; Layzer 1974).

Efforts to identify spawning sites for Alabama Shad in the lower Flint River using ichthyoplankton sampling would be greatly aided by basing study design around the locations given for Limerock Boulder in this study. Utilizing boat electrofishing to sample for adult Alabama Shad in the vicinity of reaches sampled with ichtyhyoplankton gear will aid in reducing complications associated with downstream drift. Sampling should begin within a few days after the arrival of gravid, adult female Alabama Shad at JWLD with an emphasis on increasing sampling intensity during periods of declining river discharge. Given a sufficient return of adult Alabama Shad to JWLD in a given year, this general strategy should prove useful to efforts aimed at identifying exact spawning locations for Alabama Shad in the lower Flint River.

The U.S. Fish and Wildlife Service began developing a habitat-based methodology for understanding relationships between species and their habitats and environmental impact assessment in 1974 (USFWS 1980) that eventually lead to the development of the Habitat Suitability Index (HSI). A HSI can be used to characterize a species' habitat requirements at a particular life stage by obtaining a range of values for pertinent habitat variables, calculating the likelihood of a species being present at a given variable value, and developing a Suitability Curve. The Suitability Curve is a relatively simple graph with the range of the habitat variable values on the x-axis and the suitability on the y-axis, with a value of 1.0 indicating optimum habitat suitability and a value of 0.0 indicating no suitability (USFWS 1981). The result is a graph with a peak at 1.0 for optimum values for a given habitat variable with scores between 0.0 and 1.0 for variable values outside the optimum habitat values.

The first HSI for American Shad was developed by Stier and Crance (1985), and included models for both riverine and estuarine habitats with variable values based on a literature review and expert opinion. The riverine model focused on habitat variables for spawning adults and egg/larval stages, with water velocity and temperature comprising the HSI. Ross et al. (1993) expanded upon this initial effort with a riverine model that included HSI's for spawning adults, egg/larval stages, and pre-migratory juveniles. The model for spawning adults used five variables (water temperature and velocity, dissolved oxygen, depth, and turbidity) that were collected at the site of observed spawning activity. Habitat type at locations where spawning activity was observed was also included in their analysis. Bilkovich et al. (2002) used ichthyoplankton sampling to generate HSI's for egg/larval stages of American Shad as a proxy for measurements taken at the location of observed adult behavior. Microhabitat parameters (water temperature and velocity, dissolved oxygen, and secchi depth) at collection sites were combined with macrohabitat features including watershed land use, river width, woody debris cover, and shoreline erosion to explore the possible influence of previously uninvestigated parameters.

Hightower et al. (2012) developed the most recent HSI for American Shad. They sought to expand upon the literature review and expert opinion utilized by Stier and Crance (1985) by basing suitability estimates of habitat variables on habitat data collected in the field for a number of rivers. The studies mentioned previously had generally used a combination of principal components analysis, multiple regression, and logistic regression in the development of an HSI for spawning American Shad. Hightower et al. (2012) utilized resource selection functions as described by Boyce et al. (2002) and Manly et al. (2002) to generate an updated HSI for spawning American Shad. The framework provided by Thomas et al. (2004) was used to develop resource selection functions analyzed by Bayesian statistical methods as described by McCarthy (2007), with water temperature and velocity, depth and substrate type being the habitat variables modeled. The updated Suitability Curves for each habitat variable and resulting HSI did not differ substantially from the Stier and Crance (1985) results, with the exception of sand substrate being much less important in the updated HSI.

Hightower et al. (2012) recommended using resource selection functions in the development of updated HSI's for spawning American Shad as well as HSI's for other species due to the data-driven, objective nature of the model. Resource selection functions describe the probabilty of an organism using resource characteristics over a range of possible conditions (Boyce et al. 2002; Manley et al. 2002) by comparing actual use of a resource to that resources's availability. Hightower et al. (2012) modeled four different resource varialbles (depth, temperature, velocity, and substrate) using a multinomial distribution (Thomas et al. 2004), which is preferred in instances where a trial (ie, fish location) could result in more than one outcome (ie, substrate type) (Hightower et al. 2012). Additionally, new data on habitat variables can be easily incorporated to provide a model than can be adjusted and refined as new data is collected. OpenBUGS (Spiegenthaler et al. 2010) software can be used build the multinomial model for each variable (depth, temperature,velocity, and substrate, for example) using the OpenBUGS code framework provided by Hightower et al. (2012) and available at: http://dx.doi.org/10.3996/082011-JFWM-047.S7

The development of a Habitat Suitability Index for Alabama Shad in this reach would be useful for identification of additional suitable spawning locations in the lower Flint River and within other river systems, especially for those located within the Gulf Coastal Plain physiographic region of the southeastern United States. Collection data over the past 30 years show that populations of Alabama Shad still persist within the Pea-Choctawhatchee and Conecuh river systems in southern Alabama and northern Florida, the Pascagoula-Leaf-Chicasawhay river system in eastern Mississippi, and the lower portions of the Little Missouri and Ouchita river systems in southwestern Arkansas (Smith et al. 2011). While collections within these systems have been sporadic and typically consisted of a few individuals, these rivers may represent the last remaining habitats where Alabama Shad are reproducing successfully outside of the ACF basin. Most importantly, all of these river systems, including the lower Flint River, lie completely or mostly within the Inner Coastal Plain physiographic region of the larger Gulf Coast Coastal Plain (Berkowitz et al. 2012). It stands to reason that a HSI created for Alabama Shad in the lower Flint River would be generally applicable to other rivers within the same physiographic region where recent collections have occurred, and may prove to be a useful tool to aid in conservation of Alabama Shad stocks outside of the ACF basin.

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Table 1. Substrate class percent composition and associated definitions developed for the Lower Flint River substrate map (Kaeser et al 2013) and adapted for this study.

Substrate class	Composition	Definition
Sandy	46%	>75% of area of composed of particles < 2mm in diameter (sand, silt, clay or fine organic detritus) and Unsure Sandy
Limerock Fine	17%	≥75% of the area composed of limestone as bedrock or an outcropping with relatively smooth texture (not fractured in blocks >500mm in diameter)
Rocky Boulder	11%	An area ≥ 314 m^2 that includes three or more boulders, each >500mm across longest axis, and each boulder within 1.5 m of the next adjacent boulder, regardless of underlying substrate
Rocky Fine	6%	>25% of area composed of rocks >2 mm but <500mm diameter across the longest axis
Missing Data	6%	A combination of No Data (out of range but within river channel) and Sonar Shadow (within range but behind reflective objects)
Mixed Rocky	9%	An area comprising two or more substrates (at least one being rocky) arranged such that not one single unit is >314 m^2 and Unsure Rocky
Limerock Boulder	4%	≥75% of area composed of limestone fractured into blocks >500mm diameter across longest axis and otherwise meeting the same criteria as Rocky Boulder
Island	1%	Any area of land wholly contained within the river channel that is surrounded by water during typical winter or spring discharge

Table 2. Average distance (m) to nearest polygon of each substrate type from fish locations and regular grid of points placed over study area. Also includes average perimeter of substrates contained within 15-m buffers around fish locations and regular grid of points placed over study area.

Substrate Type	Distance From Fish	Distance From Grid
Sandy	29.1	24.5
Limerock Fine	255.4	171.1
Rocky Boulder	339.0	208.0
Rocky Fine	5,810.0	1,204.3
Mixed Rocky	161.9	101.6
Limerock Boulder	150.2	367.8
Island	17,258.5	6,641.1

Perimeter of 15-meter Circle	Perimeter at Fish Locations	Perimeter at Grid Points
94.13	169.0	162.0

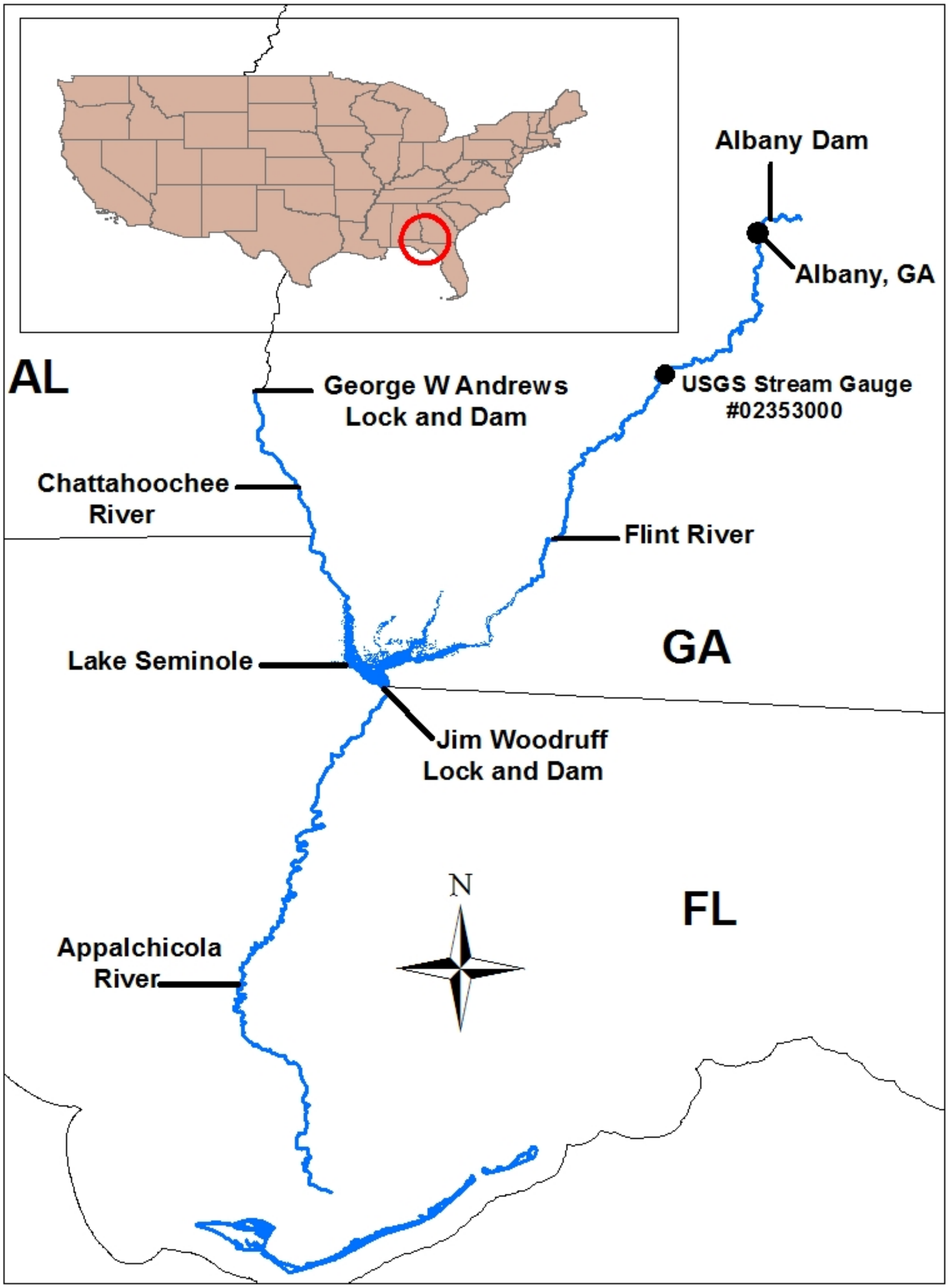

Figure 1. The lower Apalachicola-Chattahoochee-Flint River Basin, including the Lower Flint River from Jim Woodruff Lock and Dam to Albany, Georgia.

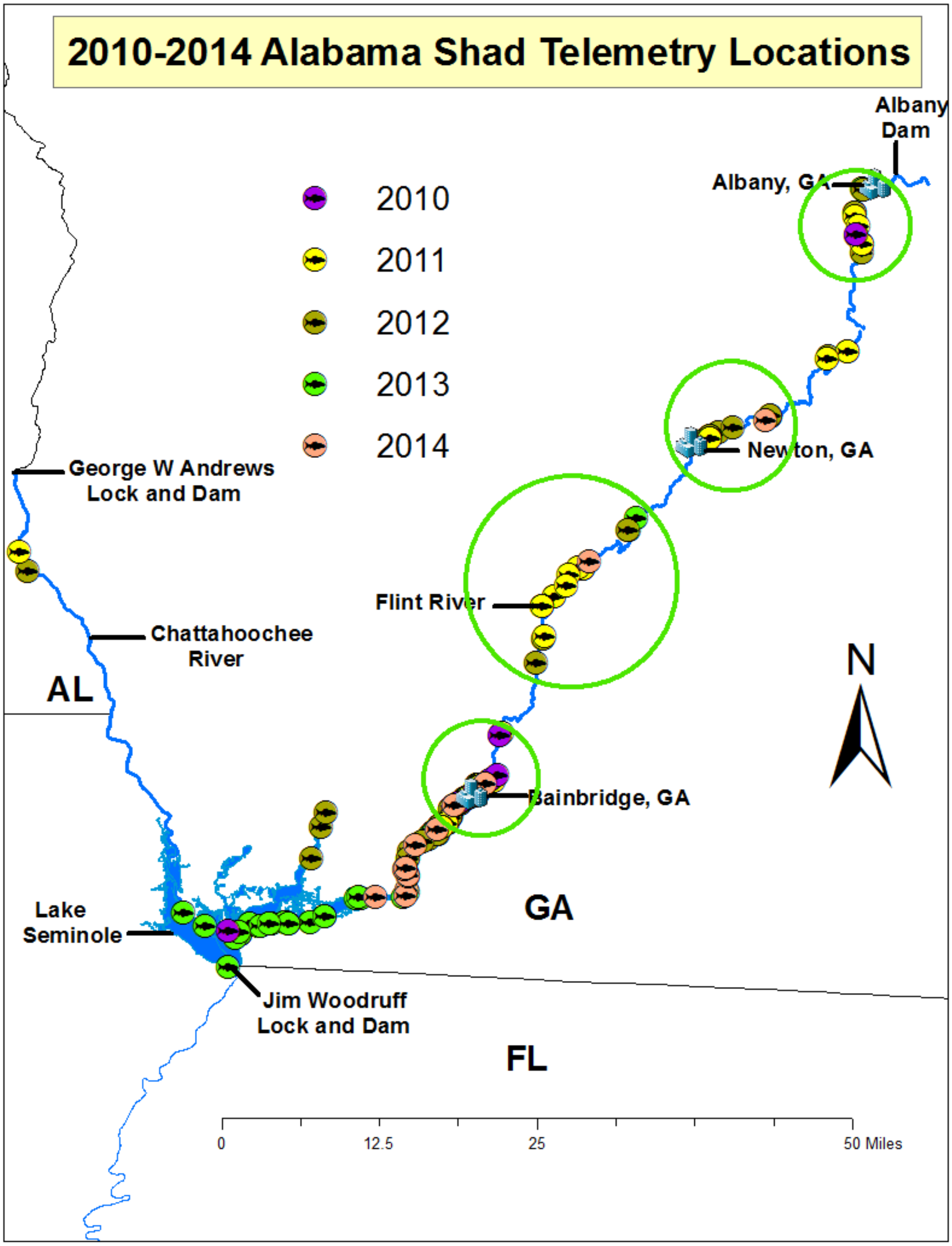

Figure 2. Locations of telemetered Alabama Shad in the lower Flint River, Georgia, showing river reaches that attracted fish over multiple years.

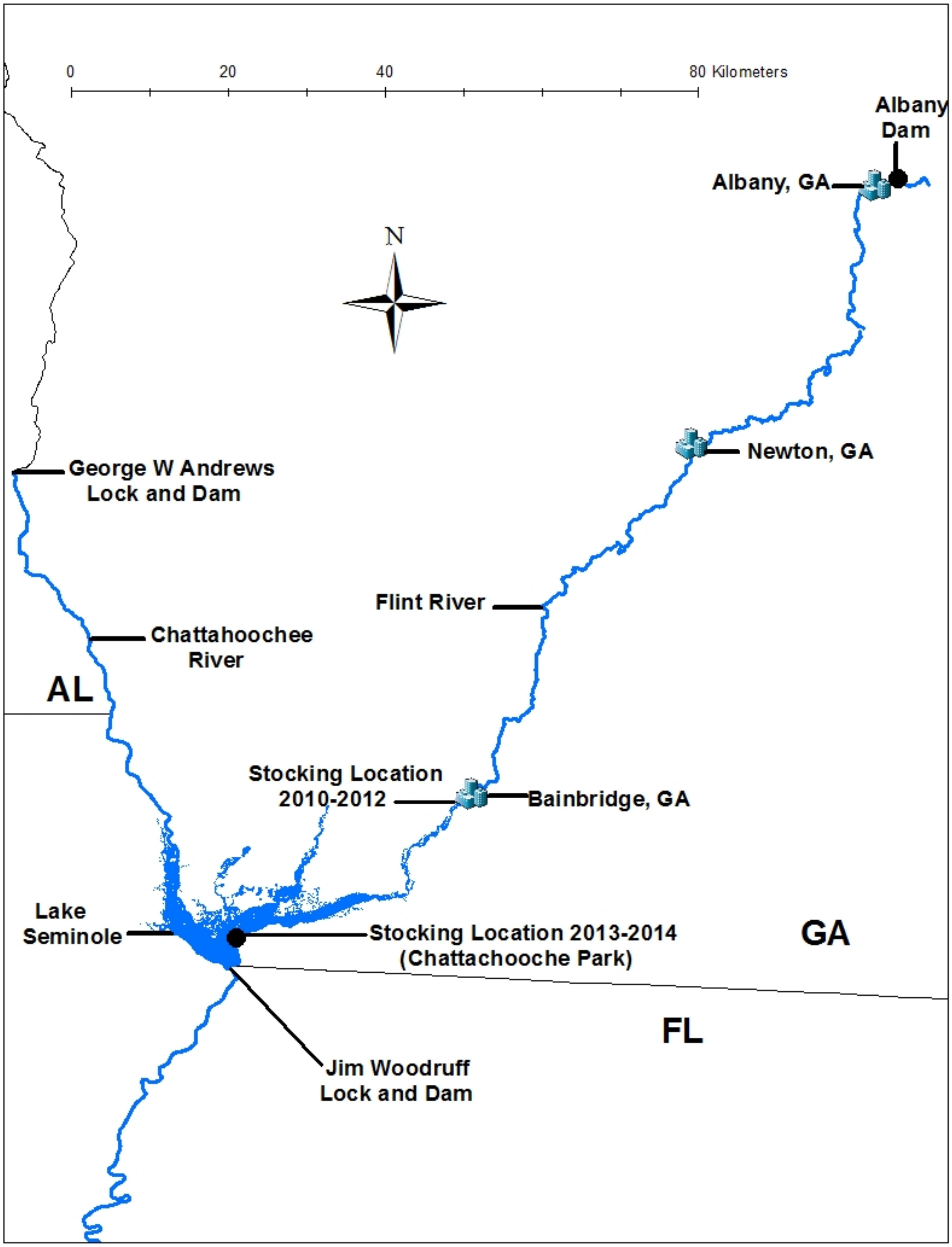

Figure 3. Locations of stocking sites upstream of Jim Woodruff Lock and Dam for transmitted Alabama Shad in the lower Flint River, Georgia.

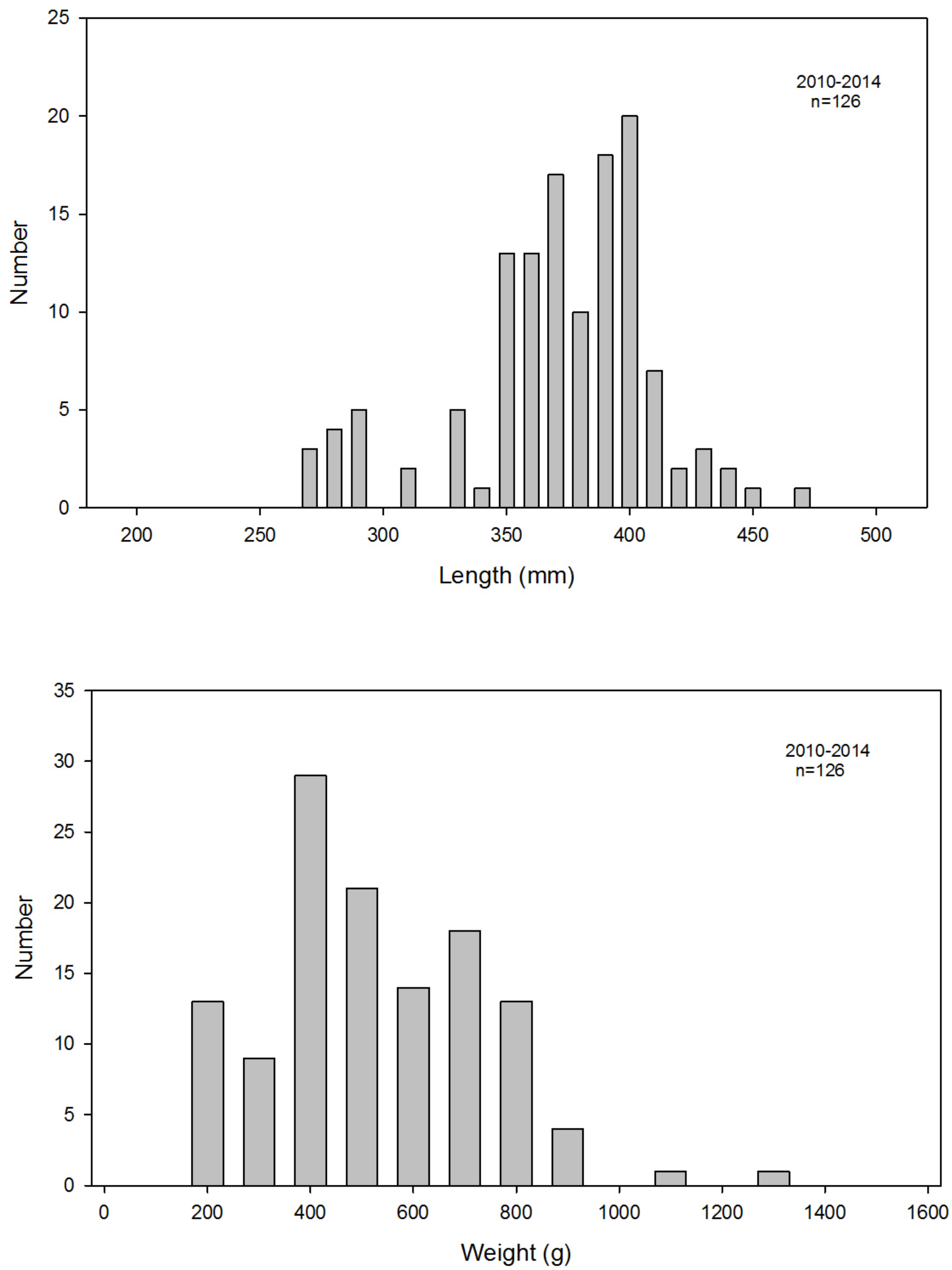

Figure 4. Length (10-mm groups) and weight (100-mm groups) frequencies of transmitted Alabama Shad relocated in the lower Flint River, Chattahoochee Rivers, and Lake Seminole, Georgia over 2010-2014.

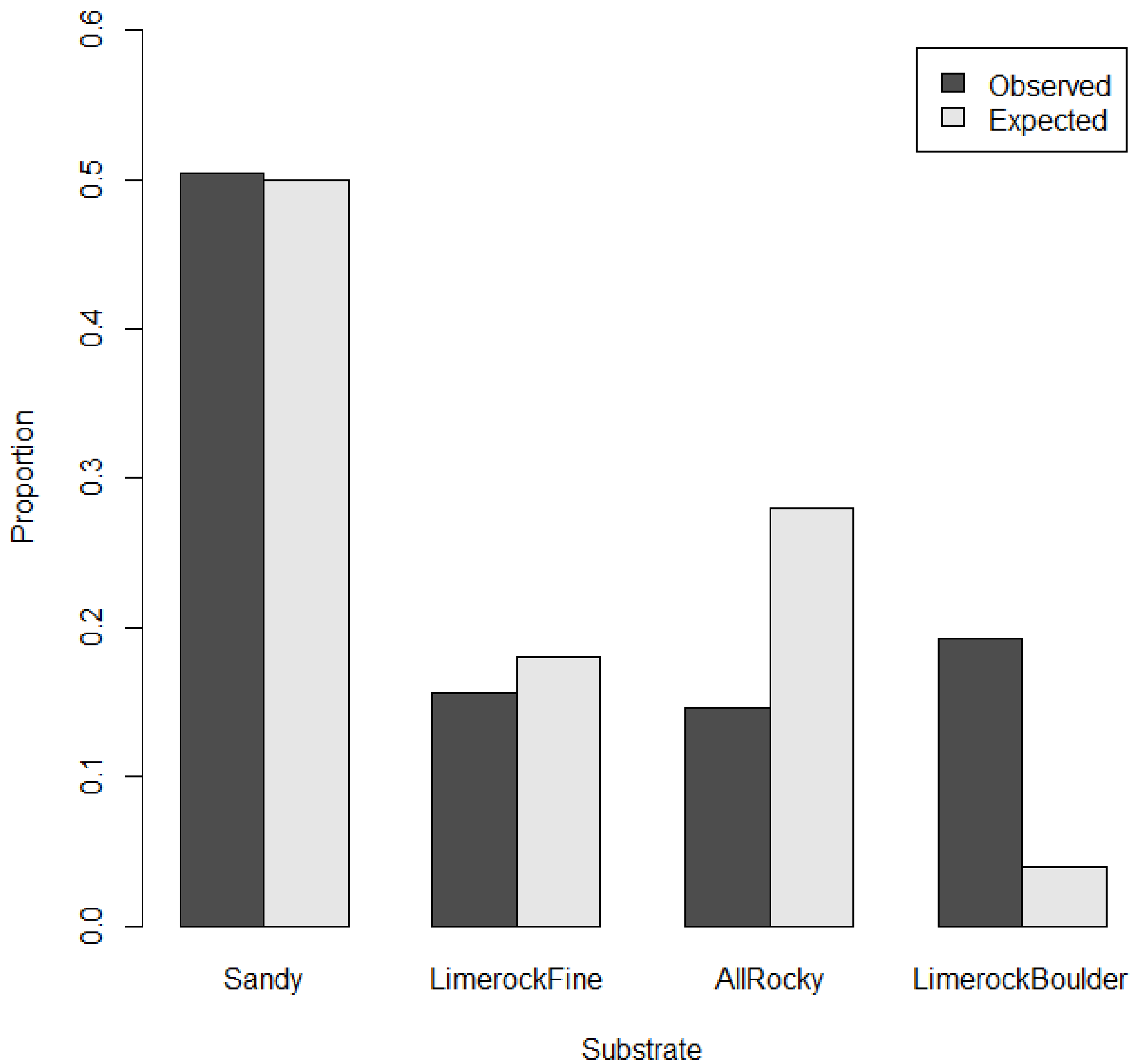

Figure 5. Observed and Expected frequencies of substrate use by Alabama Shad in the Lower Flint River, Georgia 2010-2014.

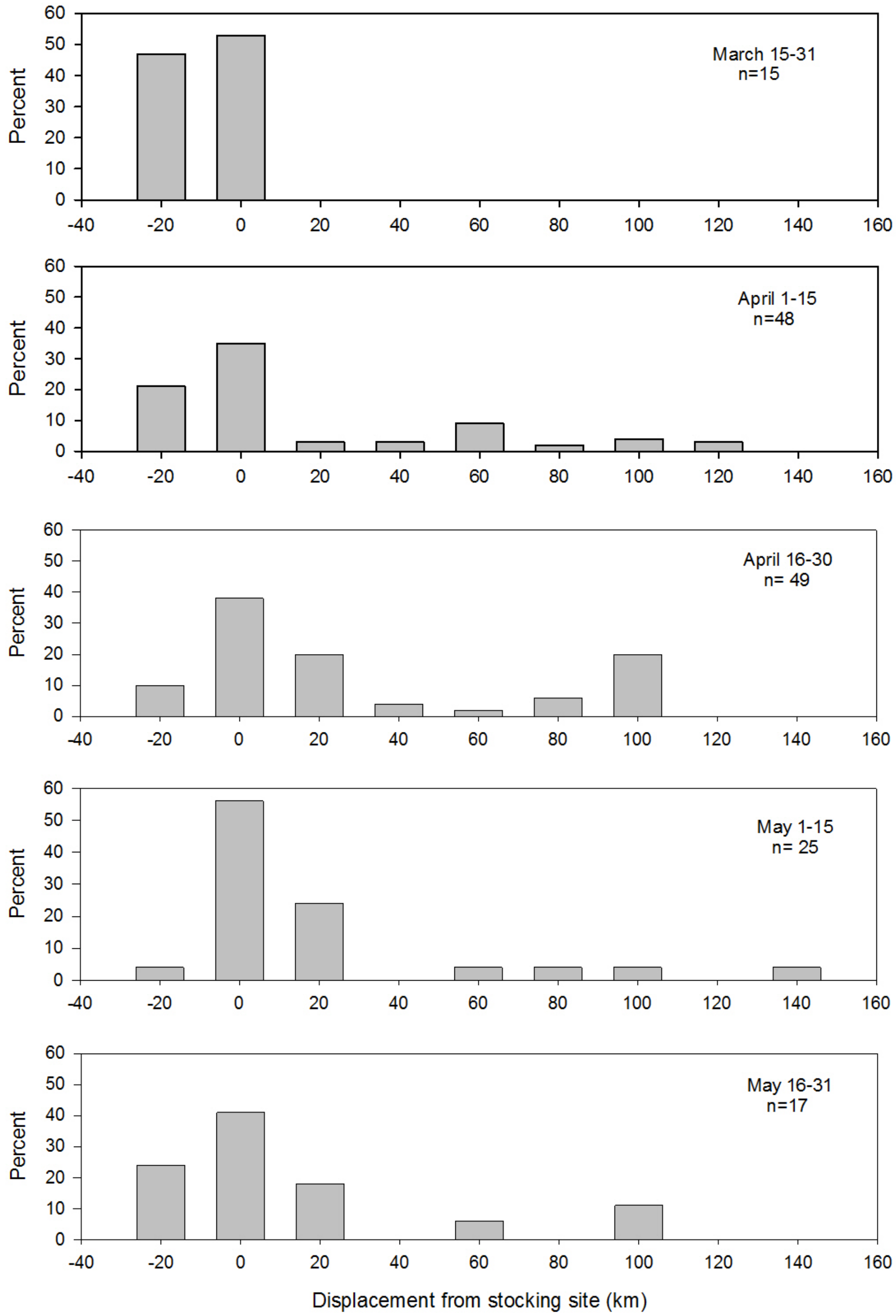

Figure 6. Temporal displacement from stocking sites for Alabama Shad in the lower Flint River, Georgia 2010-2014.

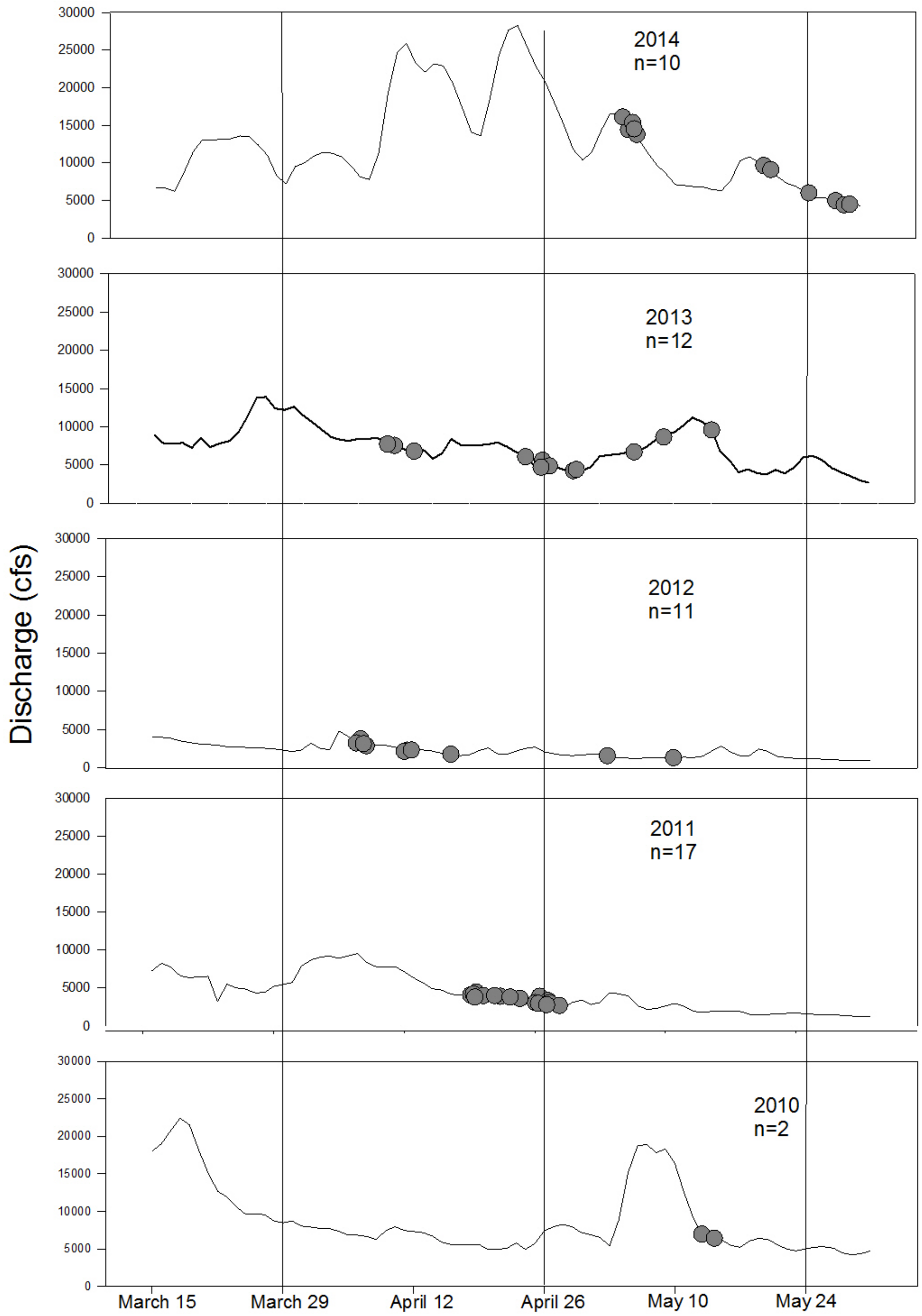

Figure 7. Alabama Shad movements ≥ 20-km in relation to daily discharge (cfs) in the lower Flint River 2010-2014.

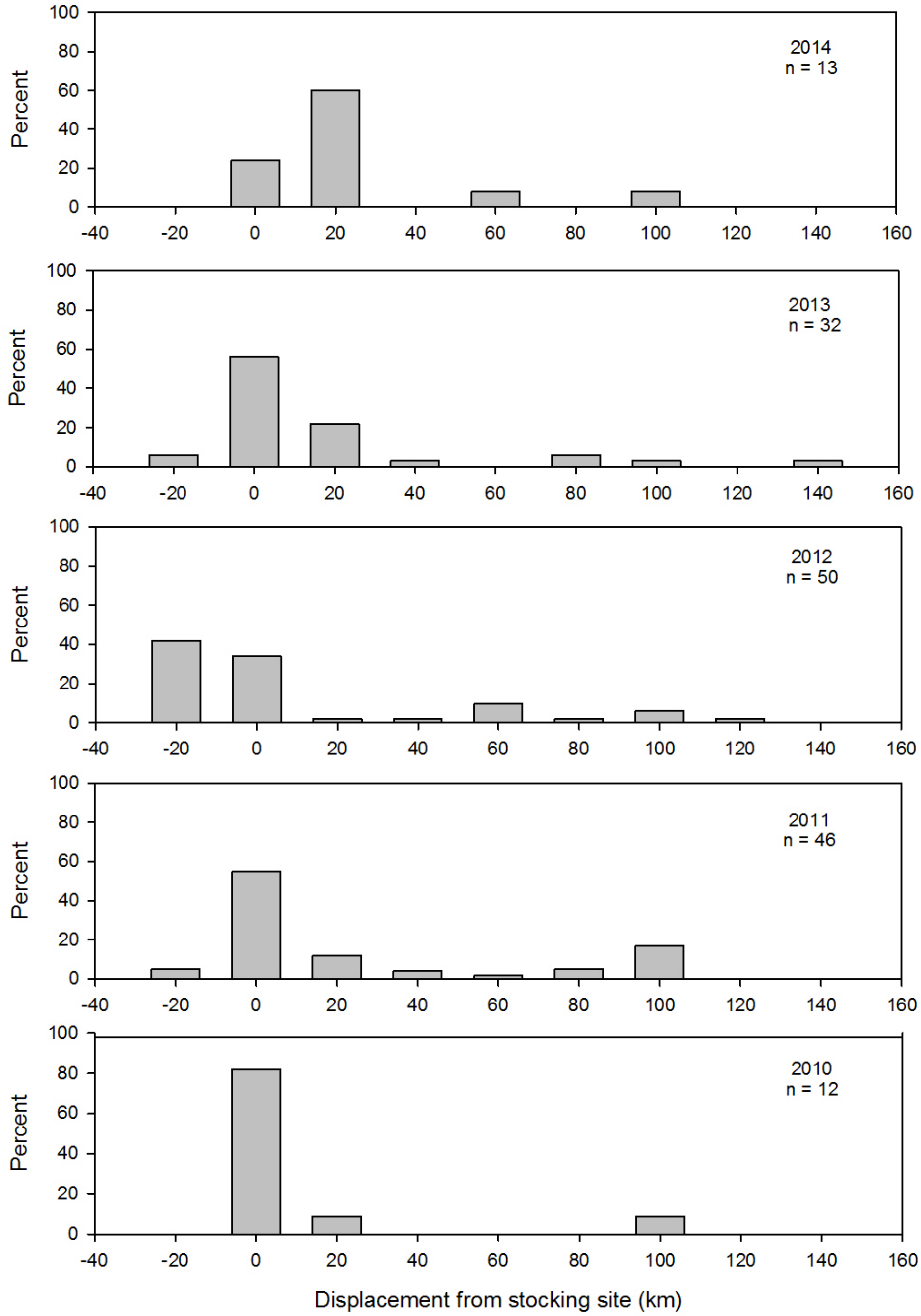

Figure 8. Total relocations and displacement, by year, from stocking sites for Alabama Shad in the Lower Flint River, Georgia 2010-2014.

Appendix 1. Tag #, total length, weight, locations and dates of relocation for transmitted Alabama Shad in the lower Flint River, Lake Seminole, and Chattahoochee River.

Tag #	Sex	TL (mm)	Weight (g)	Location(s)	Date
8.054	M	292	264	Mortality at stocking site	4/17/2014
8.221	F	339	716	Mortality at stocking site	5/27/2014
8.312	M	335	336	Mortality at stocking site	4/17/2014
8.361	F	291	241	Mortality at stocking site	5/27/2014
8.021	M	282	190	Mortality near Bainbridge, GA	5/28/2014
8.241	F	310	284	Lake Seminole	4/17/2014
8.463	M	351	456	Lake Seminole	4/17/2014
8.473	F	289	199	Mortality in Lake Seminole	6/2/2014
8.574	F	298	269	Lake Seminole	5/5/2014
8.832	M	405	772	Lake Seminole	5/5/2014
8.894	?	331	328	Flint River near Newton, GA	5/21/2014
8.982	F	354	440	Mortality near Bainbridge, GA	5/20/2014
9.022	M	415	802	Flint River above Bainbridge, GA	5/6/2014
9.052	F	319	272	Flint River near Hopeful, AL	5/31/2014
9.171	F	330	359	Lake Seminole	4/17/2014
9.263	M	270	183	Lake Seminole	5/5/2014
8.832	M	359	375	Flint River n Bainbridge, GA	5/5/2014
9.412	F	342	408	Lake Seminole	5/5/2014
213	F	447	815	Mortality near stocking site	4/5/2013
				Apalachicola River	4/22/2013
262	F	440	?	Lake Seminole	4/5/2013
302	M	357	351	Mortality in Lake Seminole	5/16/2013
340	M	357	405	Lake Seminole	4/5/2013
				Lake Seminole	4/4/2013
362	M	396	635	Mortality in Lake Seminole	5/05/13
373	F	405	692	Flint River near Bainbridge, GA	4/26/2013
				Flint River near Bainbridge, GA	4/29/2013
382	F	401	524	Lake Seminole	5/6/2013
423	F	379	545	Mortality near Release Site	5/05/13
432	F	418	803	Mortality near Release Site	5/13/2013
443	F	393	584	Mortality near Release Site	5/5/2013
453	M	370	383	Lake Seminole	4/8/2013
				Lake Seminole	4/5/2013
464	M	369	442	Flint River near Bainbridge, GA	4/10/2013
				Near Stocking Site	4/5/2013

Appendix 1 continued

Tag #	Sex	TL (mm)	Weight(g)	Location(s)	Date
513	F	386	502	Mortality near Bainbridge, GA	4/29/2013
523	M	360	420	Near Stocking Site	4/5/2013
				Flint River near Newton, GA	5/16/2013
543	F	382	562	Flint River near Bainbridge, GA	4/26/2013
				Lake Seminole	4/5/2013
564	F	408	665	Near Stocking Site	4/5/2013
				Flint River near Norman's Ferry	4/12/2013
				Flint River near Norman’s Ferry	4/24/2013
584	M	392	464	Near Stocking Site	4/4/2013
				Lake Seminole	4/5/2013
604	M	372	468	Mortality in Lake Seminole	5/5/2013
632	F	372	507	Mortality in Lake Seminole	5/16/13
654	F	387	576	Flint River near Bainbridge, GA	4/9/2013
664	M	385	481	Flint River below Albany Dam	5/8/2013
				Near Stocking Site	4/5/2013
694	F	389	568	Lake Seminole backwater	5/13/2013
				Lake Seminole	4/9/2013
722	F	358	460	Near Stocking Site	4/5/2013
				Lake Seminole	4/4/2013
813	F	410	672	Mortality near Stocking Site	4/4/2013
833	F	381	548	Apalachicola River below JWLD	5/7/2013
843	F	399	580	Mortality in Lake Seminole	5/5/2013
853	F	390	702	Mortality near Bainbridge, GA	5/6/2013
863	F	392	454	Mortality near Bainbridge, GA	5/6/2013
872	F	402	560	Flint River near Bainbridge, GA	4/26/2013
				Lake Seminole	4/29/2013
8.061	F	475	1342	Flint River near Bainbridge, GA	4/15/2012
8.241	M	363	444	Flint River near Baconton, GA	4/6/2012
8.624	F	437	812	Flint River near Bainbridge, GA	3/30/2012
				Flint River near Bainbridge, GA	4/15/2012
				Flint River near Bainbridge, GA	4/17/2012
				Flint River near Bainbridge, GA	5/18/2012
8.684	M	370	415	Flint River near Bainbridge, GA	3/6/2012
8.712	F	393	680	Flint River near Baconton, GA	5/10/2012
8.743	M	375	536	Flint River near stocking site	3/21/2012
				Flint River near stocking site	4/15/2012

Appendix 1 continued.

Tag #	Sex	TL (mm)	Weight(g)	Location(s)	Date
8.802	F	408	742	Flint River below Albany Dam	4/12/2012
				Flint River near Bainbridge, GA	4/15/2012
8.833	M	390	618	Flint River near Bainbridge, GA	3/21/2012
				Flint River near Bainbridge, GA	3/30/2012
				Flint River near Bainbridge, GA	4/15/2012
8.923	M	368	495	Flint River near Bainbridge, GA	3/30/2012
8.952	M	375	530	Flint River below Albany Dam	4/12/2012
				Flint river near Bainbridge, GA	5/8/2012
8.983	F	402	763	Flint River near Bainbridge, GA	4/15/2012
				Flint River below Albany Dam	4/17/2012
				Flint River near Bainbridge, GA	5/8/2012
9.014	M	375	550	Flint River near Bainbridge, GA	3/14/2012
9.022	M	363	390	Flint River near Bainbridge, GA	4/15/2012
				Flint River near Bainbridge, GA	5/18/2012
9.044	F	400	760	Flint River near Bainbridge, GA	4/27/2012
9.052	M	352	410	Flint River Near Baconton, GA	4/6/2012
9.112	M	368	460	Flint River near Bainbridge, GA	4/24/2012
				Flint River near Bainbridge, GA	5/18/2012
9.123	F	425	724	Flint River near Bainbridge, GA	3/14/2012
				Flint river near Bainbridge, GA	4/15/2012
9.142	M	354	410	Flint river near Bainbridge, GA	3/21/2012
				Flint river near Bainbridge, GA	4/15/2021
9.152	F	411	868	Flint River near Newton, GA	4/5/2012
9.163	M	368	410	Flint River near Baconton, GA	4/6/2012
				Flint River near Baconton, GA	5/10/2012
9.172	F	402	662	Mortality near Bainbridge, GA	3/14/2012
9.192	F	417	827	Flint River near Bainbridge, GA	4/15/2012
9.203	F	452	1156	Mortality near Albany, GA	3/14/2012
				Mortality near Albany, GA	3/30/2012
9.212	F	408	775	Flint River near Hopeful, GA	4/2/2012
9.221	M	380	490	Flint River near Bainbridge, GA	4/15/2012
9.234	M	357	408	Flint River near Bainbridge, GA	4/15/2012
9.264	F	400	799	Flint River below Albany Dam	4/12/2012
9.273	F	420	992	Flint River near Baconton, GA	4/5/2012
				Flint River near Baconton, GA	5/2/2012
9.322	F	436	880	Flint River near Bainbridge, GA	4/15/2012

Appendix 1 continued.

Tag #	Sex	TL (mm)	Weight(g)	Location(s)	Date
9.343	F	406	707	Flint River near Bainbridge, GA	5/18/2012
9.352	M	367	470	Mortality near Bainbridge, GA	3/14/2012
				Mortality near Bainbridge, GA	3/20/2012
				Mortality near Bainbridge, GA	3/21/2012
				Mortality near Bainbridge, GA	4/1/2012
8.743	M	375	536	Chattahoochee River Andrews L&D	4/26/2012
?	?	?	?	Chattahoochee River Andrews L&D	4/26/2012
9.061	M	387	579	Spring Creek	5/4/2012
9.052	M	380	490	Spring Creek	5/4/2012
9.001	M	374	524	Spring Creek	5/4/2012
8.012	M	373	672	Flint River near Bainbridge, Georgia	4/14/2011
				Flint River near Bainbridge, Georgia	4/17/2011
8.024	M	332	398	Flint River near Albany, Georgia	4/27/2011
8.032	F	403	900	Flint River near Newton, GA	4/26/2011
8.041	M	361	509	Flint River near Bainbridge, GA	4/14/2011
8.053	F	395	784	Flint River near Bainbridge, GA	4/14/2011
8.061	F	391	725	Flint River near Bainbridge, GA	4/15/2011
8.092	F	373	491	Flint River near Bainbridge, GA	4/14/2011
				Flint River below Albany Dam	4/19/2011
8.101	M	297	287	Flint River near Bainbridge, GA	4/17/2011
8.133	M	372	542	Flint River near Bainbridge, GA	4/14/2011
8.141	F	363	488	Flint River near Bainbridge, GA	4/17/2011
				Flint River below Albany Dam	4/19/2011
8.152	F	392	746	Flint River near Newton, GA	4/26/2011
8.162	M	392	682	Flint River near Bainbridge, GA	4/14/2011
				Flint River near Bainbridge, GA	4/17/2011
				Flint River above Newton, GA	4/20/2011
8.192	M	358	486	Flint River near Newton, GA	4/26/2011
8.203	M	365	540	Flint River near Newton, GA	4/26/2011
8.211	F	414	930	Flint River near Bainbridge, GA	4/14/2011
				Flint River near Bainbridge, GA	4/17/2011
				Flint River below Albany Dam	4/19/2011
8.223	F	395	808	Mortality near Bainbridge, GA	4/24/2011
8.252	F	373	616	Mortality near Bainbridge, GA	4/17/2011

Appendix 1 continued.

Tag #	Sex	TL (mm)	Weight(g)	Location(s)	Date
8.262	M	370	541	Flint River near Bainbridge, GA	4/14/2011
				Flint River below Albany Dam	4/19/2011
8.282	F	382	763	Below JWLD (emigrated)	4/3/2011
8.291	M	282	234	Mortality at stocking site	3/21/2011
8.313	M	357	491	Mortality at stocking site	3/21/2011
8.331	F	389	694	Flint River near Bainbridge, GA	4/17/2011
8.36	F	430	982	Flint River near Bainbridge, GA	4/17/2011
				Flint River above Newton, GA	4/20/2011
8.37	F	398	880	Flint River below Albany Dam	4/19/2011
8.402	F	370	571	Flint River below Albany Dam	4/19/2011
8.433	F	400	860	Flint River near Bainbridge, GA	4/17/2011
8.463	F	383	741	Flint River near Vada, GA	4/22/2011
				Flint River below Albany Dam	4/27/2011
8.472	F	392	659	Flint River near Bainbridge, GA	4/14/2011
				Flint River above Newton, GA	4/20/2011
8.481	F	392	762	Flint River near Newton, GA	4/26/2011
8.49	F	419	803	Flint River near Bainbridge, GA	4/14/2011
8.502	F	396	720	Flint River near Bainbridge, GA	4/17/2011
8.51	F	400	818	Flint River near Bainbridge, GA	4/19/2011
				Flint River below Albany, GA	4/24/2011
8.523	F	403	803	Flint River near Bainbridge, GA	4/14/2011
				Flint River near Bainbridge, GA	4/17/2011
8.531	M	358	479	Flint River near Bainbridge, GA	4/14/2011
				Flint River near Bainbridge, GA	4/17/2011
				Flint River near Newton, GA	4/26/2011
8.535	M	275	215	Flint River near Bainbridge, GA	4/14/2011
36590	F	401	685	Mortality near Bainbridge, GA	5/13/2010
36639	F	374	546	Flint River near Bainbridge, GA	5/28/2010
36590	F	401	685	Flint River above Bainbridge, GA	5/13/2010
36632	M	357	333	Flint River above Bainbridge, GA	5/13/2010
36613	M	361	410	Flint River above Bainbridge, GA	5/13/2010
36607	?	?	?	?	5/13/2010

Appendix 1 Continued.

Tag #	Sex	TL (mm)	Weight (g)	Location(s)	Date
36598	M	278	211	Flint River near Bainbridge, GA	5/28/2010
36639	F	374	546	Flint River near Bainbridge, GA	5/28/2010
36607	F	396	735	Flint River near Bainbridge, GA	5/28/2010
36590	F	401	685	Flint River near Bainbridge, GA	5/28/2010
36613	M	361	410	Flint River near Bainbridge, GA	5/28/2010

Appendix 2. Tag #, length, weight, # of relocations, substrate(s) at relocation (s), and year of collection/sex for Alabama Shad located within the boundaries of the substrate map between Bainbridge, Georgia and Albany Dam.

Tag #	TL (mm)	Weight (g)	Relocations	Substrate(s)	Year/Sex
8.011	403	656	2	Limerock Fine, Rocky Boulder	2012/F
8.241	363	444	1	Sandy	2012/M
8.684	370	415	1	Limerock Boulder	2012/M
8.712	393	680	1	Rocky Fine	2012/F
8.743	373	511	2	Sandy	2011/F
8.802	408	742	1	Rocky Boulder	2012/F
8.833	390	618	2	Sandy	2012/M
8.894	331	328	1	Sandy	2014/?
8.952	375	530	2	Sandy, Rocky Fine	2012/M
8.983	402	763	2	Sandy, Limerock Fine	2012/F
9.022	363	390	2	Sandy	2012/M
9.052	352	410	2	Sandy, Mixed Rocky	2012/M
9.123	425	724	1	Sandy	2012/F
9.142	354	410	2	Sandy	2012/M
9.152	411	868	1	Sandy	2012/F
9.163	368	410	1	Sandy	2012/M
9.192	417	827	1	Sandy	2012/F
9.212	408	775	1	Sandy	2012/F
9.264	400	799	1	Rocky Fine	2012/F
9.273	420	992	2	Sandy, Limerock Fine	2012/F
9.283	359	375	1	Sandy	2014/M
9.352	367	470	4	Sandy(2), Limerock Boulder(2)	2012/M
8.012	373	672	1	Limerock Boulder	2011/M
8.024	332	398	1	Sandy	2011/M
8.032	403	900	1	Mixed Rocky	2011/F
8.041	361	509	1	Sandy	2011/M
8.053	395	784	1	Limerock Fine	2011/F
8.092	373	491	2	Sandy, Limerock Fine	2011/F
8.133	372	542	1	Sandy	2011/M
8.141	363	488	1	Limerock Boulder	2011/F
8.152	392	746	1	Sandy	2011/F
8.162	392	682	3	Sandy(2), Rocky Boulder	2011/M
8.192	358	486	1	Sandy	2011/M
8.203	365	540	1	Sandy	2011/M
8.211	414	930	3	Sandy, LR Fine, LR Boulder	2011/F
8.223	395	808	3	Sandy, Limerock Boulder (2)	2011/F
8.252	373	616	3	Sandy, Limerock Boulder(2)	2011/F
8.262	370	541	2	Sandy, Limerock Fine	2011/F
8.291	282	234	2	Sandy, Limerock Boulder	2011/M
8.332	?	?	1	Mixed Rocky	?
8.291	282	234	2	Sandy, Limerock Boulder	2011/M

Appendix 2 continued.

Tag #	TL (mm)	Weight (g)	Relocations	Substrate(s)	Year/Sex
8.402	370	571	1	Sandy	2011/F
8.360	430	982	2	Limerock Fine, LR Boulder	2011/F
8.360	430	982	2	Limerock Fine, LR Boulder	2011/F
8.433	400	860	1	Sandy	2011/F
8.463	383	741	1	Sandy	2011/F
8.472	392	659	2	Limerock Fine	2011/F
8.481	392	762	1	Sandy	2011/F
8.490	419	803	1	Sandy	2011/F
8.502	396	720	1	Sandy	2011/F
8.510	400	818	1	Limerock Boulder	2011/F
8.523	403	803	2	Limerock Fine, Limerock Boulder	2011/F
8.531	358	479	3	Sandy	2011/M
8.545	275	215	1	Sandy	2011/M
8.573	408	904	1	Sandy	2011/F
8.583	404	802	4	Sandy, Limerock Fine(3)	2011/F
8.921	385	522	4	Sandy(2), Limerock Fine(1), Rocky Boulder(1)	2011/F
9.150	396	769	1	Mixed Rocky	2011/F
9.182	285	212	1	Mixed Rocky	2011/M
9.370	?	?	1	Sandy	?
464	369	442	1	Sandy	2013/M
523	360	420	1	Mixed Rocky	2013/M
564	408	665	2	Island, Rocky Boulder	2013/F
664	385	481	2	Rocky Boulder	2013/M
36590	401	685	2	Limerock Boulder	2010/F
36598	278	211	1	Limerock Boulder	2010/M
36607	396	735	2	Sandy, Limerock Boulder	2010/F
36613	361	410	2	Sandy, Limerock Boulder	2010/M
36618	289	256	1	Limerock Fine	2010/M
36632	357	333	1	Sandy	2010/M
36639	374	546	1	Limerock Boulder	2010/F

Appendix 3. Area and X-Y coordinates for Limerock Boulder substrate polygons in the Lower Flint River. Individual substrate polygons are listed in an upstream-to-downstream fashion, beginning near Albany Dam near Albany, Georgia. *** denotes those polygons that contained a fish location or were within 500-m of a fish location.

FID	Substrate	Shape Area(m2)	Latitude	Longitude
71	Limerock boulder***	831.81	31.60170	-84.13840
64	Limerock boulder***	685.34	31.60030	-84.13950
63	Limerock boulder	365.30	31.59840	-84.14210
62	Limerock boulder	392.93	31.59490	-84.14410
61	Limerock boulder	682.66	31.59380	-84.14450
60	Limerock boulder	484.68	31.59120	-84.14460
44	Limerock boulder	3952.57	31.58860	-84.14570
45	Limerock boulder	1569.47	31.58590	-84.14510
59	Limerock boulder***	523.96	31.57380	-84.820
58	Limerock boulder***	419.16	31.56910	-84.14630
65	Limerock boulder***	464.62	31.56750	-84.14560
56	Limerock boulder***	306.06	31.56440	-84.14440
57	Limerock boulder***	1150.75	31.56430	-84.14520
55	Limerock boulder***	689.51	31.56040	-84.14380
54	Limerock boulder***	1571.18	31.55880	-84.14310
53	Limerock boulder***	1717.56	31.55650	-84.14490
42	Limerock boulder***	2427.38	31.55490	-84.14770
43	Limerock boulder***	901.35	31.55380	-84.14630
38	Limerock boulder***	257.73	31.55230	-84.14590
52	Limerock boulder***	540.35	31.55190	-84.14650
51	Limerock boulder***	538.48	31.54980	-84.14610
50	Limerock boulder***	550.85	31.54130	-84.14030
37	Limerock boulder***	112.79	31.54060	-84.14000
82	Limerock boulder***	374.36	31.53710	-84.13980
152	Limerock boulder***	1017.52	31.53590	-84.13940
151	Limerock boulder***	312.53	31.53100	-84.13850
150	Limerock boulder***	303.78	31.53040	-84.13870
83	Limerock boulder***	757.44	31.52620	-84.14100
84	Limerock boulder	220.04	31.52520	-84.14120
85	Limerock boulder	1102.98	31.52230	-84.14130
86	Limerock boulder	1988.22	31.51910	-84.14230
149	Limerock boulder	635.43	31.51790	-84.14500
146	Limerock boulder	3122.08	31.50990	-84.15250
72	Limerock boulder	2043.82	31.50030	-84.14730
49	Limerock boulder	3944.89	31.48650	-84.14950
47	Limerock boulder	1956.55	31.48340	-84.15330
46	Limerock boulder	1418.75	31.46690	-84.840
41	Limerock boulder	4406.80	31.45440	-84.15570
73	Limerock boulder	928.64	31.42630	-84.800
74	Limerock boulder	379.89	31.42560	-84.14750

Appendix 3 continued.

FID	Substrate	Shape Area(m2)	Latitude	Longitude
75	Limerock boulder	569.41	31.42340	-84.14990
35	Limerock boulder***	783.57	31.41730	-84.15880
34	Limerock boulder	682.42	31.41310	-84.16840
145	Limerock boulder***	1890.57	31.41120	-84.17720
144	Limerock boulder***	1357.82	31.40830	-84.17960
143	Limerock boulder***	1354.08	31.40560	-84.17970
141	Limerock boulder	9481.55	31.39940	-84.18250
92	Limerock boulder	759.20	31.39830	-84.18530
140	Limerock boulder	427.64	31.39770	-84.18060
139	Limerock boulder	545.19	31.39520	-84.17650
131	Limerock boulder	250.88	31.39470	-84.18200
137	Limerock boulder	338.42	31.39420	-84.18220
138	Limerock boulder	593.75	31.39420	-84.17700
136	Limerock boulder	281.68	31.39370	-84.17730
130	Limerock boulder	722.91	31.39340	-84.18250
135	Limerock boulder	346.20	31.39300	-84.19440
134	Limerock boulder	3816.91	31.39170	-84.18230
142	Limerock boulder	1552.12	31.39090	-84.19640
129	Limerock boulder	5375.78	31.39080	-84.18050
133	Limerock boulder	452.58	31.38800	-84.19690
132	Limerock boulder	1415.61	31.37930	-84.20020
33	Limerock boulder	459.51	31.35680	-84.20100
32	Limerock boulder	844.03	31.35610	-84.22440
31	Limerock boulder	616.97	31.35480	-84.22640
30	Limerock boulder	452.48	31.35370	-84.22650
29	Limerock boulder	826.65	31.34640	-84.23020
28	Limerock boulder	883.01	31.34540	-84.23220
27	Limerock boulder	814.61	31.34450	-84.23280
77	Limerock boulder***	3146.14	31.34280	-84.24340
70	Limerock boulder***	650.69	31.34260	-84.24490
26	Limerock boulder***	730.98	31.34210	-84.24520
25	Limerock boulder	1469.00	31.34180	-84.26840
69	Limerock boulder	1970.74	31.34040	-84.23790
24	Limerock boulder	2345.36	31.34020	-84.23500
78	Limerock boulder	1438.86	31.33980	-84.25790
67	Limerock boulder***	325.85	31.33970	-84.24170
79	Limerock boulder	1398.29	31.33940	-84.26020
23	Limerock boulder	711.96	31.33930	-84.25630
22	Limerock boulder***	2350.72	31.33840	-84.24110
76	Limerock boulder***	3183.09	31.33820	-84.24750
18	Limerock boulder	1766.31	31.33670	-84.27240
17	Limerock boulder***	423.51	31.33640	-84.25350

Appendix 3 continued.

FID	Substrate	Shape Area(m2)	Latitude	Longitude
21	Limerock boulder***	3888.42	31.33620	-84.25530
16	Limerock boulder	4397.71	31.33280	-84.27040
194	Limerock Boulder	1087.08	31.33220	-84.27100
68	Limerock boulder	362.11	31.33190	-84.27290
14	Limerock boulder	1389.89	31.33050	-84.28070
13	Limerock boulder	3103.27	31.32970	-84.27830
48	Limerock boulder***	3816.48	31.32950	-84.28700
12	Limerock boulder***	316.05	31.32900	-84.28570
11	Limerock boulder***	482.52	31.32830	-84.28530
10	Limerock boulder***	645.47	31.32740	-84.28390
9	Limerock boulder	916.40	31.32670	-84.29380
8	Limerock boulder	1992.01	31.32660	-84.29590
7	Limerock boulder***	2502.34	31.32590	-84.29980
15	Limerock boulder***	3890.83	31.32590	-84.28960
6	Limerock boulder***	523.50	31.32510	-84.30890
5	Limerock boulder	788.18	31.32460	-84.31080
4	Limerock boulder***	851.38	31.32390	-84.30440
3	Limerock boulder***	675.01	31.32370	-84.30300
2	Limerock boulder***	933.86	31.32290	-84.31350
1	Limerock boulder***	2873.33	31.32190	-84.31400
0	Limerock boulder***	785.73	31.32000	-84.31380
147	Limerock boulder***	4567.61	31.31420	-84.32430
8	Limerock boulder***	954.79	31.31300	-84.32670
66	Limerock boulder***	761.10	31.31290	-84.31450
89	Limerock boulder	669.00	31.31230	-84.33190
90	Limerock boulder***	2972.04	31.31220	-84.32220
159	Limerock boulder	498.98	31.30900	-84.33640
91	Limerock boulder	686.85	31.30420	-84.34250
87	Limerock boulder	4239.88	31.30000	-84.33740
160	Limerock boulder	867.12	31.29060	-84.33620
88	Limerock boulder	761.74	31.28880	-84.33750
158	Limerock boulder	2082.98	31.28740	-84.33770
161	Limerock boulder	1630.82	31.28500	-84.34170
157	Limerock boulder	1857.87	31.28220	-84.34210
153	Limerock boulder	390.08	31.28030	-84.34180
155	Limerock boulder	470.77	31.27740	-84.34110
154	Limerock boulder	3472.00	31.27620	-84.34240
162	Limerock boulder	1848.36	31.26960	-84.34960
156	Limerock boulder	1226.80	31.26610	-84.35520
94	Limerock boulder	1177.22	31.23770	-84.38020
93	Limerock boulder	2317.61	31.22980	-84.39510
172	Limerock boulder	1206.41	31.18150	-84.44480
171	Limerock boulder***	512.31	31.17030	-84.45620

Appendix 3 continued.

FID	Substrate	Shape Area(m2)	Latitude	Longitude
170	Limerock boulder	17785.89	31.16540	-84.47000
169	Limerock boulder***	311.00	31.16000	-84.47490
168	Limerock boulder***	831.11	31.15910	-84.47840
167	Limerock boulder***	966.78	31.15880	-84.47690
166	Limerock boulder	7730.22	31.15680	-84.48300
165	Limerock boulder	5550.24	31.15270	-84.48270
164	Limerock boulder***	3367.80	31.13270	-84.49630
163	Limerock boulder	1689.56	31.12900	-84.49870
122	Limerock boulder***	1543.92	31.12630	-84.50360
121	Limerock boulder***	1892.94	31.12540	-84.50650
120	Limerock boulder***	486.67	31.12420	-84.50750
119	Limerock boulder***	5793.70	31.12270	-84.50730
124	Limerock boulder***	589.61	31.12110	-84.50490
118	Limerock boulder	347.28	31.12030	-84.50320
117	Limerock boulder	2043.99	31.11940	-84.50070
116	Limerock boulder	2241.92	31.11750	-84.50030
123	Limerock boulder	1759.79	31.11200	-84.50230
115	Limerock boulder	6215.43	31.10970	-84.50660
114	Limerock boulder	1574.59	31.10390	-84.50770
113	Limerock boulder	3089.33	31.10010	-84.50680
112	Limerock boulder	3150.06	31.09480	-84.50450
110	Limerock boulder***	2141.98	31.09220	-84.50530
126	Limerock boulder***	3099.43	31.08930	-84.50360
109	Limerock boulder***	1762.34	31.08700	-84.50470
108	Limerock boulder***	838.20	31.08470	-84.50710
96	Limerock boulder	3727.87	31.07400	-84.50950
107	Limerock boulder	3027.13	31.06940	-84.51210
106	Limerock boulder	763.66	31.06920	-84.51330
105	Limerock boulder	1063.04	31.06780	-84.51400
104	Limerock boulder	2531.07	31.06650	-84.51370
111	Limerock boulder	6136.23	31.06530	-84.51440
103	Limerock boulder***	3539.01	31.06170	-84.51270
97	Limerock boulder	283.29	31.05140	-84.51440
102	Limerock boulder	1734.71	31.04430	-84.51400
101	Limerock boulder	1641.31	31.03860	-84.51260
100	Limerock boulder	3734.92	31.03550	-84.51540
99	Limerock boulder	909.00	31.03330	-84.51500
127	Limerock boulder	982.55	31.03000	-84.51750
98	Limerock boulder	2108.07	31.02810	-84.51710
125	Limerock boulder	7898.24	31.01690	-84.51720
128	Limerock boulder	11123.44	31.01400	-84.51980
192	Limerock boulder	4090.10	30.99610	-84.53930
190	Limerock boulder	7948.54	30.99410	-84.54460
189	Limerock boulder	7162.22	30.99210	-84.55180

Appendix 3 continued.

FID	Substrate	Shape Area(m2)	Latitude	Longitude
188	Limerock boulder	1933.61	30.98900	-84.54940
187	Limerock boulder	5241.89	30.98690	-84.55020
186	Limerock boulder	952.89	30.98510	-84.55130
185	Limerock boulder***	1178.37	30.97990	-84.55370
184	Limerock boulder***	6100.04	30.97750	-84.55360
183	Limerock boulder***	2481.69	30.97400	-84.55550
182	Limerock boulder***	2832.03	30.97270	-84.55640
181	Limerock boulder***	8985.78	30.97090	-84.55730
180	Limerock boulder	6713.15	30.96740	-84.55620
179	Limerock boulder***	1030.88	30.92370	-84.55970
177	Limerock boulder***	822.74	30.92300	-84.56470
178	Limerock boulder***	10966.26	30.92300	-84.56180
193	Limerock boulder***	4921.39	30.91730	-84.58150
176	Limerock boulder***	17100.56	30.91610	-84.57660
175	Limerock boulder***	10729.31	30.91200	-84.58130
174	Limerock boulder***	41816.79	30.90710	-84.58330
173	Limerock boulder***	18322.10	30.90260	-84.59290

Appendix 4. Programming code used for statistical analysis in R software.

Observed/Expected Barplot

```
observed=c(55,17,16,21)
expected=c(0.50,0.18,0.28,0.04)
total=sum(observed)
observed.prop=observed/total
observed.prop
Input=(
"Value      Sandy       LimerockFine     AllRocky      LimerockBoulder
Observed   0.5045872   0.1559633       0.1467890       0.1926606

Expected   0.50         0.18         0.28        0.04")

Data=as.matrix(read.table(textConnection(Input),
header=TRUE,
row.names=1))
Data
barplot(Data,
beside=TRUE,
legend=TRUE,
ylim=c(0,0.6),
xlab="Substrate",
ylab="Proportion")
```

**Exact Multinomial GOF Test **

```
library(EMT)
observed=c(50,17,16,21)
prob=c(0.50,0.18,0.28,0.04)
output=multinomial.test(observed,prob)
```

Exact Binomial Post-Hoc Testing for EMT

```
**Sandy**
binom.test(55,109,0.50)

**Limerock Fine**
binom.test(17,109,0.18)

**AllRocky**
binom.test(16,109,0.28)
```

```
**Limerock Boulder**
binom.test(21,109,0.04)
```

Bonferroni Correction for EMT Post-Hoc Tests

```
Input=(
"Factor              Raw.p
Sandy               0.9999
Limerock Fine        0.6178
All Rocky            0.0013
Limerock Boulder     2.209e-09
")
Data=read.table(textConnection(Input),header=TRUE)
Data$Bonferroni=p.adjust(Data$Raw.p,method="bonferroni")
Data
```

**XNomial Exact GOF Test **

```
library(XNomial)
observed=(55,17,16,21)
prob=c(0.50, 0.18, 0.28,0.04)
xmulti(observed,prob,detail=3)
```

** Manley Selection Ratios **

```
library(adehabitatHS)
Sandy<-
c(0,0,1,0,0,2,0,2,1,1,1,2,1,1,2,1,1,1,1,0,1,1,2,0,1,0,1,0,1,1,0,1,2,1,1,1,1,1,1,1,0,0,0,1,1,1,0
,1,1,1,0,0,3,1,1,1,2,0,0,1,1,0,0,0,0,0,1,0,1,0)
length(Sandy)

LimerockFine<-
c(1,0,0,0,0,0,0,0,0,1,0,0,0,0,0,1,0,0,0,1,0,0,0,0,0,0,1,1,0,0,0,0,0,0,1,0,0,1,0,0,0,1,0,0,0,2,0
,0,0,0,1,0,0,0,3,1,0,0,0,0,0,0,0,0,0,0,0,1,0,0)
length(LimerockFine)

AllRocky<-
c(1,0,0,2,0,1,0,0,1,0,0,1,0,0,0,0,0,0,1,0,0,0,0,0,1,0,0,0,0,0,0,1,0,0,0,0,0,0,0,0,1,0,0,0,0,0,0
,0,0,0,0,0,0,0,0,1,1,1,0,0,1,2,0,0,0,0,0,0,0,0)
length(AllRocky)

LimerockBoulder<-
c(0,0,1,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,2,1,0,0,0,0,0,0,1,0,0,0,0,1,2,2,0,1,1,0,1,0,0,0,0,0
,0,0,1,1,0,0,0,0,0,0,0,0,0,0,0,0,2,1,1,1,0,0,1)
```

Appendix 4 continued

```
length(LimerockBoulder)
SubUsed<-data.frame(Sandy,LimerockFine,AllRocky,LimerockBoulder)
SubAvail<-c(0.50,0.18,0.28,0.04)
names(SubAvail)<-c("Sandy","LimerockFine","AllRocky","LimerockBoulder")
widesII(SubUsed,SubAvail,avknown=TRUE,alpha=0.05)
```