OCCURRENCE OF LEAST KILLIFISH AT THE NORTHERN LIMIT OF ITS RANGE IN SOUTH AND NORTH CAROLINA

Travis J. Nelson and Erin J. Burge

tjnelson@coastal.edu and eburge@coastal.edu

Coastal Carolina University, Marine Science, PO Box 261954, Conway, SC 29528-6054

INTRODUCTION

The Least Killifish (*Heterandria formosa*), is a small, live-bearing poeciliid native to the southeastern United States. *Heterandria* is the smallest freshwater fish in North America and one of the smallest in the world, growing to an average adult size of approximately 2.0 cm (0.8 in). Common habitat includes shallow, vegetated ditches and ponds, with some populations occasionally venturing into brackish water (Rohde et al., 2009). Females give birth to up to 8 young at intervals of about 10 days. Least Killifish are diurnal feeders on small arthropods, snails, algae, plants, and detritus and have a lifespan of about 2 years (Menhinick and Braswell, 1997). They are found on the coastal plain from Louisiana to Wilmington, North Carolina, including Florida, and have been found as far as 161 km (100 miles) inland (Chaney and Bechler, 2006). The Least Killifish is an easily overlooked species because of its small size, occurrence in heavily vegetated habitat, and similarity to more widespread species such as the Eastern Mosquitofish (*Gambusia holbrooki*).

Spotty records of *Heterandria* exist in Horry County, South Carolina, but no records of which we are aware exist between this region and collections of *H. formosa* near Wilmington, North Carolina, a distance of approximately 64 km (40 miles) between the closest verified occurrences (Menhinick and Braswell, 1997; Hogue and Raine, 2006; Rohde et al. 2009). Genetic evidence indicates that a historically recent range expansion of *H. formosa* into the Carolinas has not achieved Hardy-Weinberg equilibrium. Populations of *Heterandria* are in flux, and there is evidence that populations in the Carolinas have been established more recently than populations further south (Baer, 1998). Anecdotal observations suggest that Least Killifish are more common now in Horry County than they were in previous decades (R.H. Moore, pers. comm.). Stream capture under low flow conditions may function as a conduit for establishing new natural populations over time (Chaney and Bechler, 2006), but an isolated population indicates the likelihood that specimens caught in the lower Cape Fear River drainage around Wilmington are introduced, possibly from the stocking of larger game fish or through bait-bucket transfer.

Bait-bucket transfer can occur when bait sold by a retailer is released. *H. formosa* is unlikely to be used for bait due to its diminutive size, however store-sold bait often contains non-bait species, or bait being sold may not be the species advertised. Bait may also be transported between basins by stores receiving shipments of bait stock containing *H. formosa* or from fishermen transporting bait personally. There is the potential for non-native species to be introduced to a new environment when fishermen release their bait or when bait escapes. The combination of these factors creates a high likelihood that species are frequently introduced to new environments through bait-bucket transfer (Ludwig and Leitch, 1996).

Least Killifish (*Heterandria formosa*), Waccamaw River tributary, SC. (Photo by Fritz Rohde)

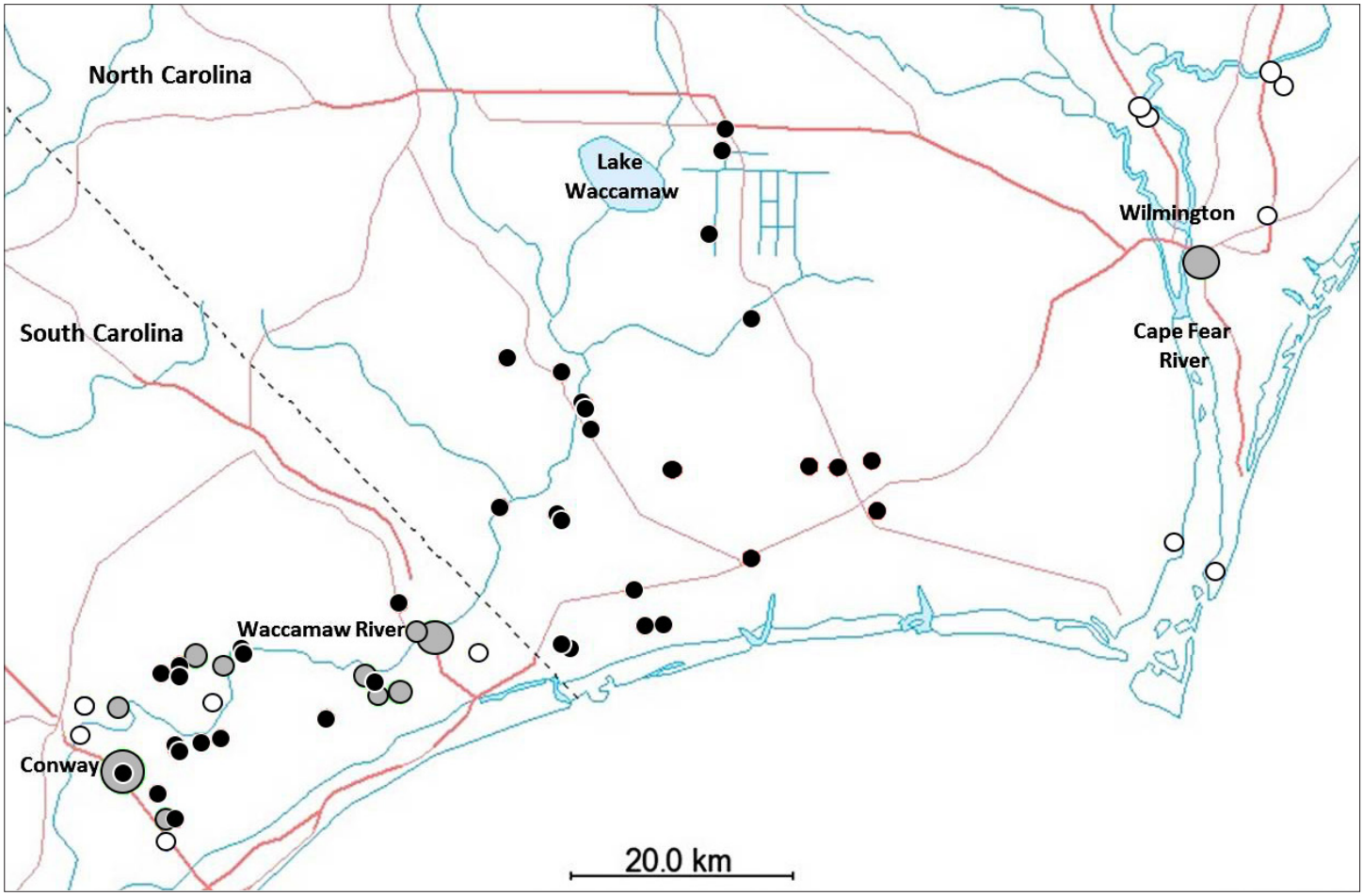

Figure 1. Map of localities sampled for the presence of *Heterandria formosa* in 2012 (n = 61). Gray circles indicate the presence of *Heterandria* (n = 14) and the size indicates the population density (smallest = 1, largest = 37). Black circles indicate sites where fish were caught, but *Heterandria* were not. White circles mark past catches of *H. formosa* (see Rohde et al. 2009, http://people.clemson.edu/~jwfoltz/scfish/search.htm, http://collections.naturalsciences.org/searchFishes.aspx). See also an interactive Google Map (http://goo.gl/maps/9MDZM) containing collection information, representative photographs of habitat, and locations.

Such a small species of fish is understandably highly affected by the environment in which it lives. Other small poeciliids known to prey on *Heterandria*, such as *G. holbrooki*, may influence the size and sex distribution of populations of *Heterandria* by skewing the population towards large-bodied females (Belk and Lydeard, 1994; Schaefer et al., 1994). The density of *Heterandria* in communities containing few predators seems to fluctuate significantly between monthly censuses while there is greater stability of populations of *H. formosa* in communities with strong predator presence (Richardson et al. 2006). The type of environment, whether creek, river, or lake, can affect the average size of *Heterandria* due to differences in vegetation and depth (Leips and Travis, 1999). Average size may also correlate positively with brood size and negatively with life expectancy of mothers (Henrich, 1988).

The purpose of this study was to resolve the uncertainty regarding the occurrences of *H. formosa* at the northern limit of its range. A lack of continuity in the occurrence of *H. formosa* between South Carolina collections and those around Wilmington, North Carolina, would strengthen the contention that disjunct populations are the result of introduction events in the Wilmington area. Confirmation of a disjunct distribution presents opportunities for research on ecological, behavioral, and genetic variation within the species.

FIELD SAMPLING

To investigate the northern limit of the range of *H. formosa*, sampling of 61 sites was done in Horry County, South Carolina, and Brunswick, Columbus, and New Hanover counties, North Carolina. Sampling sites were preselected using Google Maps based on apparent accessibility and proximity to visible water sources. Several sites that historically had populations of *H. formosa* were also sampled (Hogue and Raine, 2006; Rohde et al., 2009). Sites were also visited opportunistically dur-

Figure 2. Typical *Heterandria formosa* habitat.

ing sampling trips if they were visible from the road and had apparent characteristics of *H. formosa* habitat (e.g., shallow, vegetated water). Sampling took place between March and November, 2012, and focused on the region east of Conway and north of Myrtle Beach, South Carolina in the Waccamaw and Carolina Coastal-Sampit watersheds. Routes that were sampled included South Carolina highways 90, 905, and 9, as well as North Carolina highways 904, 130, 179, and 211. Several localities in Wilmington, North Carolina, part of the lower Cape Fear watershed, were also sampled to confirm the persistence of *H. formosa* in North Carolina.

At each site, a digital photograph and GPS coordinates were recorded and the water was sampled using a long sweep of a fully submerged 40 x 24 cm dip net with a 109 cm handle. Sampling occurred in water with moderate vegetation, if any was present. If fish were present in initial sweeps, four additional sweeps of the net were performed to obtain data on population density. If Least Killifish were caught, a solution of tricaine methanesulfonate was used to sedate the fish at an optimal dosage between 60 and 100 mg/L (Carter et al. 2010). Length and sex of the first 20 specimens were recorded to the nearest 0.1 mm using calipers. After measurements, fish were revived and released. *Heterandria* caught in Wilmington were preserved in 100% ethanol for use in future population genetic studies. To obtain data on associated fish species, individuals were field identified and released for easily recognizable types, while unfamiliar specimens were preserved in 10% neutral buffered formalin, and identified using a dissecting scope and dichotomous keys (Rohde et al., 2009). Sampling was conducted under South Carolina Department of Natural Resources permit F-12-05 and North Carolina Wildlife Resources Commission permit 12-SFC00086 issued to Erin Burge.

DATA ANALYSIS

Google Maps (https://maps.google.com) and GPS Visualizer (http://www.gpsvisualizer.com) were used to map all sample sites and display relative density data for *Heterandria* (Figure 1). Previous occurrences of *H. formosa* in South Carolina and North Carolina were also obtained from publicly available databases and included on maps (South Carolina, JW Foltz: http://people.clemson.edu/~jwfoltz/scfish/search.htm; North Carolina, North Carolina Museum of Natural Sciences http://collections.naturalsciences.org/searchFishes.aspx). The map was used to infer the distribution of *H. formosa* at the northern reported limit of its range and to determine how the range correlated with specific drainage basins.

RESULTS

Occurrences of *H. formosa* were found primarily in South Carolina, with the northeasternmost population found along Highway 9 at the Waccamaw River. No *H. formosa* were found in North Carolina except for isolated occurrences in Wilmington (Figure 1, Table 1). Distances between the northeasternmost South Carolina site containing *Heterandria* (33° 54' 30.70" N, 78° 41' 50.22" W) and the closest historical locations in Wilmington were 67.3 km (33° 59' 4.92" N, 77° 58' 22.44" W), 77.2 km (34° 12' 42.80" N, 77° 56' 42.40" W), and 79.7 km (34° 19' 50.16" N, 77° 59' 53.88" W). Localities with *Heterandria* were usually shallow roadside ditches with heavy vegetation, and rarely lakes, ponds, or natural streams (Figure 2). All but one site containing *H. formosa* also contained *G. holbrooki*. See Table 1 for additional species associated with collections of *H. formosa*, and an interactive Google Map containing collection information, representative photographs of habitat, and locations (http://goo.gl/maps/9MDZM).

DISCUSSION

Field sampling data (n = 61 sites) indicated that the population of *H. formosa* in Wilmington, North Carolina is geographically disjunct from South Carolina localities. To our knowledge, no *H. formosa* have been collected in Brunswick or Columbus counties, North Carolina, prior to and including this study, but isolated occurrences reported in Menhinick and Braswell (1997) in Wilmington, part of New Hanover County, were confirmed by these collections. In South Carolina, the northeasternmost easily accessible point of the normal range of Least Killifish is along Highway 9 in the Waccamaw River drainage. Wilmington, North Carolina,

lies within a different drainage basin—the Cape Fear River—and the absence of *H. formosa* in collections between South Carolina localities and established populations in North Carolina strengthen the likelihood that the Wilmington population was introduced and is separate from the species' natural range.

It is possible that the Wilmington area was once within the species' natural range. At some point in recent geological history the population may have been cut off by some barrier to migration, resulting in a relict population of *H. formosa*. Similar relict, rare, or endemic fishes, such as the Bluefin Killifish (*Lucania goodei*), and the endemic Waccamaw Killifish (*Fundulus waccamensis*), are also present in southeastern North Carolina (Menhinick and Braswell, 1997). This division, possibly by shifting watersheds or development, would have created vicariance, isolating the population. As a result, a population of Least Killifish within the main range may have a similar genetic fingerprint to the isolated population in Wilmington. Similar processes modifying the distribution and isolating populations of Central American species, including other poecilids, has been studied previously (Costa and Schlupp, 2010; Lee and Johnson, 2009; Smith and Bermingham, 2005).

Acknowledgements

The authors thank Coastal Carolina University's Honors Program for funding portions of this project, and the Department of Marine Science for providing collecting materials and supplies. Keira Williams provided constructive criticism and review on early revisions of this manuscript. Amanda Kau and Dylan Johnson assisted with sampling efforts.

Literature Cited

Baer, C. F. 1998. Species-wide population structure in a southeastern U.S. freshwater fish, *Heterandria formosa*: Gene flow and biogeography. Evolution 52(1): 183–193.

Belk, M., and C. Lydeard. 1994. Effect of *Gambusia holbrooki* on a similar-sized, syntopic poeciliid, *Heterandria formosa*: competitor or predator? Copeia 1994(2): 296–302.

Carter, K. M., C. M. Woodley, and R. S. Brown. 2010. A review of tricaine methanesulfonate for anesthesia of fish. Reviews in Fish Biology & Fisheries 21(1): 51–59.

Chaney, J., and D. Bechler. 2006. The occurrence and distribution of *Heterandria formosa* (Teleostei, Poeciliidae) in Lowndes County, Georgia. Georgia Journal of Science 64(2): 67–75.

Costa, G. C., and I. Schlupp. 2010. Biogeography of the Amazon molly: ecological niche and range limits of an asexual hybrid species. Global Ecology & Biogeography 19(4): 442–451.

Henrich, S. 1988. Variation in offspring sizes of the poeciliid fish *Heterandria formosa* in relation to fitness. Oikos 51(1): 13–18.

Hogue, G. M. and J. A. Raine. 2006. From the ledger to the web: setting 21st Century documentation standards for the collections of the North Carolina State Museum of Natural Sciences. Collection Forum 21(1–2): 175–191.

Lee, J. B., and J. B. Johnson. 2009. Biogeography of the livebearing fish *Poecilia gillii* in Costa Rica: are phylogeographical breaks congruent with fish community boundaries? Molecular Ecology 18(19): 4088–4101.

Leips, J., and J. Travis. 1999. The comparative expression of life history traits and its relationship to the numerical dynamics of four populations of the least killifish. Journal of Animal Ecology 68: 595–616.

Ludwig Jr., H., and J. Leitch. 1996. Interbasin transfer of aquatic biota via anglers' bait buckets. Fisheries 21(7): 14–18.

Menhinick, E. F., and A. L. Braswell. 1997. Endangered, threatened, and rare fauna of North Carolina Part IV. A reevaluation of the freshwater fishes. Occasional Papers of the North Carolina Museum of Natural Sciences and the North Carolina Biological Survey 11: 106.

Rohde, F. C., R. G. Arndt, J. W. Foltz, and J. M. Quattro. 2009. Freshwater fishes of South Carolina. University of South Carolina Press. Columbia, SC.

Richardson, J. M. L., M. S. Gunzburger, and J. Travis. 2006. Variation in predation pressure as a mechanism underlying differences in numerical abundance between populations of the poeciliid fish *Heterandria formosa*. Oecologia 147(4): 596–605.

Schaefer, J. F., S. T. Heulett, and T. M. Farrell. 1994. Interactions between two poeciliid fishes (*Gambusia holbrooki* and *Heterandria formosa*) and their prey in a Florida marsh. Copeia 1994(2): 516–520.

Smith, S. A., and E. Bermingham. 2005. The biogeography of lower Mesoamerican freshwater fishes. Journal of Biogeography 32(10): 1835–1854.

The following table was inadvertenly omitted from "Occurrence of Least Killifish at the northern limit of its range in South and North Carolina" by Travis J. Nelson and Erin J. Burge, *American Currents* 39.3 (Spring, 2014) pp. 25–28.

Table 1. Details of sample sites, occurences of *Heterandria formosa* and associated fish species. See also an interactive Google Map (http://goo.gl/maps/9MDZM) containing collection information, representative photographs of habitat, and locations.

Site description					Number of *Heterandria formosa*	
State	**County**	**Location**	**Habitat**	**Latitude N, Longitude W**		**Other species**
North Carolina	Brunswick	Ash Little River Rd and Ashlong Trail	creek	34° 00' 17.65", -78° 34' 21.54"	0	*Gambusia holbrooki*
		Ash Little River Rd and Ashlong Trail	creek	34° 00' 18.10", -78° 34' 21.31"	0	*G. holbrooki*
		Beckman Cir	stormwater pond	33° 55' 04.12", -78° 28' 19.52"	0	*G. holbrooki*
		Big Neck Rd and 4 Seasons Way	roadside ditch	34° 02' 38.49", -78° 27' 47.22"	0	*G. holbrooki*
		Horseshoe Rd and Ash Little River Rd	roadside ditch	34° 00' 27.50", -78° 34' 36.28"	0	*G. holbrooki*
		Little Macedonia Rd	creek	34° 02' 44.90", -78° 18' 08.49"	0	*G. holbrooki*
		Makatoka Rd and Little Macedonia Rd	stormwater pond	34° 02' 48.90", -78° 19' 45.79"	0	*G. holbrooki, Lepomis gulosus*
		Middle River Rd and Skyview Ln	creek	34° 03' 04.75", -78° 16' 06.50"	0	*G. holbrooki*
		NC 130	creek	34° 04' 39.42", -78° 32' 35.56"	0	*G. holbrooki, Lepomis* sp.
		NC 130 and Mulberry St	river	33° 58' 20.36", -78° 23' 11.53"	0	*G. holbrooki*
		NC 211	river	34° 00' 37.79", -78° 15' 47.97"	0	*G. holbrooki*
		NC 904 and NC 179	stormwater pond	33° 55' 01.22", -78° 29' 26.73"	0	*G. holbrooki*
		NC 904 and NC 179	stormwater pond	33° 55' 01.43", -78° 29' 25.15"	0	*G. holbrooki*
		NC 904 and US 17	roadside ditch	33° 56' 46.80", -78° 30' 05.65"	0	*G. holbrooki*
		Rivergate Dr and NC 904	stormwater pond	34° 00' 49.92", -78° 37' 56.11"	0	*G. holbrooki, Enneacanthus gloriosus*
		Saltaire Dr and Persimmon Rd	stormwater pond	33° 54' 08.50", -78° 34' 22.26"	0	*G. holbrooki*
		Thicketwood Dr and Pinewood Dr	roadside ditch	33° 53' 59.86", -78° 33' 52.42"	0	*G. holbrooki*
		Tram Rd	roadside ditch	34° 10' 01.80", -78° 23' 07.75"	0	*G. holbrooki*
	Columbus	Tram Rd	roadside ditch	34° 18' 14.45", -78° 24' 52.59"	0	*G. holbrooki*
		13th St and NC 214	roadside ditch	34° 19' 15.78", -78° 24' 43.15"	0	*G. holbrooki*
		Happy Home Rd and Hoover Ray Rd	creek	34° 08' 5.03", -78° 37' 33.93"	0	*G. holbrooki, Fundulus chrysotus, L. gulosus*
		NC 130	swamp	34° 05' 51.18", -78° 33' 01.43"	0	*G. holbrooki*
		NC 130	swamp	34° 07' 24.64", -78° 34' 21.33"	0	*G. holbrooki*
		NC 130 and Old King Rd	Waccamaw River	34° 05' 43.95", -78° 32' 53.19"	0	*G. holbrooki, Lepomis* sp.
		Tram Rd and Harlem Rd	roadside ditch	34° 14' 09.65", -78° 25' 39.56"	0	*G. holbrooki*
	New Hanover (Wilmington)	US 421 N	pond	34° 19' 50.06", -77° 59' 55.87"	0	*G. holbrooki*
		US 421 N and S Front St	Greenfield Lake	34° 12' 42.80", -77° 56' 42.40"	19	*G. holbrooki, En. gloriosus*
South Carolina	Horry	Berley Mc Rd and SC 905	roadside ditch	33° 52' 42.36", -78° 57' 51.35"	0	*G. holbrooki*
		Cain Wilson Rd	roadside ditch	33° 53' 29.79", -78° 55' 49.34"	7	*G. holbrooki, F. chrysotus*

South Carolina	Horry (continued)	Caines Landing Rd and Black Harbor Dr	stormwater pond	33° 53' 05.19", -78° 54' 11.04"	3	*G. holbrooki*
		Chelsey Lake Dr and SC 90	stormwater pond	33° 49' 20.39", -78° 55' 30.30"	0	*G. holbrooki*
		Doodle Hill Dr	swamp	33° 53' 47.64", -78° 53' 05.63"	0	*Aphredoderus sayanus, En. gloriosus*
		Doodle Hill Dr	stormwater pond	33° 53' 55.80", -78° 53' 05.02"	0	*G. holbrooki*
		Grey Oaks Dr and Old Reaves Ferry Rd	Waccamaw River	33° 51' 02.69", -78° 53' 52.00"	0	*G. holbrooki*
		Hidden Creek Dr and Carolina Forest Blvd	roadside ditch	33° 45' 35.16", -78° 57' 04.70"	0	*G. holbrooki*
		Husted Rd and US 501 N	stormwater pond	33° 47' 54.72", -79° 00' 07.30"	0	*G. holbrooki*
		Husted Rd and US 501 N	railroad ditch	33° 47' 54.91", -79° 00' 06.49"	37	*G. holbrooki, F. chrysotus, Elassoma zonatum, L. auritus*
		Jones Rd and SC 90	creek	33° 51' 47.25", -78° 43' 52.15"	8	*G. holbrooki*
		Lindrem Lake Dr and SC 90	partially dry pond	33° 49' 14.12", -78° 57' 01.72"	0	*Umbra pygmaea*
		Lindrem Lake Dr and SC 90	stormwater pond	33° 48' 55.33", -78° 56' 56.80"	0	*G. holbrooki*
		Living Stones Ln and Star Bluff Rd	stormwater pond	33° 52' 20.61", -78° 45' 17.19"	0	*G. holbrooki*
		Monaca Dr	stormwater pond	33° 50' 31.80", -78° 48' 10.29"	0	*G. holbrooki, El. zonatum*
		Old Pireway Rd and Hardees Ferry Rd	roadside ditch	33° 51' 02.93", -79° 00' 25.14"	4	*G. holbrooki*
		Old Reaves Ferry Rd	natural pond	33° 51' 11.34", -78° 54' 36.72"	7	none
		Old State Hwy 90 and SC 90	roadside ditch	33° 51' 02.54", -78° 50' 08.94"	0	*G. holbrooki, En. gloriosus*
		Old State Hwy 90 and SC 90	roadside ditch	33° 51' 03.51", -78° 50' 03.85"	5	*G. holbrooki*
		Pireway Rd and SC 9 S	stormwater pond	33° 56' 06.36", -78° 43' 52.78"	0	*G. holbrooki*
		Postal Way and Carolina Forest Blvd	roadside ditch	33° 45' 38.08", -78° 57' 35.64"	5	*G. holbrooki, El. zonatum*
		Rivers Edge Dr and SC 90	Tilly Lake	33° 49' 32.39", -78° 54' 22.79"	0	*G. holbrooki, L. marginatus*
		Rodney Rd	creek	33° 52' 40.12", -78° 56' 52.22"	0	*G. holbrooki*
		Rodney Rd	roadside ditch	33° 53' 07.07", -78° 56' 45.77"	0	*G. holbrooki*
		SC 9 N	roadside ditch	33° 54' 47.11", -78° 42' 56.82"	1	*G. holbrooki, F. chrysotus, Lepomis* sp.
		Shad Row and Goggle Eye Dr	lake	33° 54' 30.70", -78° 41' 50.22"	21	*G. holbrooki, F. chrysotus, El. boehlkei, Lepomis* sp.
		Southgate Pkwy and Carolina Forest Blvd	stormwater pond	33° 45' 30.04", -78° 56' 32.70"	0	*G. holbrooki*
		Star Bluff Rd and Old Star Bluff Rd	roadside ditch	33° 51' 37.11", -78° 45' 06.90"	3	*G. holbrooki*
		Thomas Rd and Old Reaves Ferry Rd	roadside ditch	33° 51' 03.33", -78° 51' 58.40"	0	*G. holbrooki, A. sayanus*
		Tibwin Dr and Gardner Lacy Rd	stormwater pond	33° 46' 52.41", -78° 58' 05.27"	0	*G. holbrooki*
		Tibwin Dr and Gardner Lacy Rd	stormwater pond	33° 46' 49.52", -78° 58' 05.74"	0	*G. holbrooki*
		Tibwin Dr and Gardner Lacy Rd	roadside ditch	33° 46' 52.47", -78° 58' 05.92"	0	*G. holbrooki*
		Todd Rd and SC 90	roadside ditch	33° 50' 00.31", -78° 53' 06.98"	10	*G. holbrooki*
		Vera Rd	roadside ditch	33° 52' 34.26", -78° 45' 45.70"	9	*G. holbrooki*