THE EFFICACY OF SMALL-SCALE REMOVAL OF AN INVASIVE SPECIES (REDBREAST SUNFISH, *LEPOMIS AURITUS*) BY ELECTROFISHING

A thesis presented to the faculty of the graduate school of Western Carolina University in partial fulfillment of the requirements for the degree of Masters of Science in Biology.

By

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ABSTRACT

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Redbreast sunfish (*Lepomis auritus)* in Richland Creek, Haywood County, NC, were studied to determine if back-pack electrofishing was an effective method of removal of this invasive species. Three study sites were established, with 100 m removal reaches and paired 100 m control reaches which were resampled at intervals over a nine month period to test for population depletion by electrofishing. All redbreast sunfish were sacrificed from removal reaches while those captured in control reaches were returned to the stream after marking by fin clipping. While electrofishing, any rock bass (*Ambloplites rupestris*) captured were measured noted to look for re-establishment in areas where redbreast sunfish had been removed.

The results indicate that the population was significantly smaller in removal reaches; averaged over the period of the study, population estimates from removal reaches were approximately 50% lower than those from control reaches. However, there was not a significant difference of the interaction of month by treatment. While the local populations of redbreast sunfish in removal reaches in Richland Creek may have been reduced by electrofishing, fin-clipped fish from control reaches were occasionally captured in removal reaches suggesting upstream-downstream movement. Given that the small reservoir downstream (Lake Junaluska) may serve as a source population, I suspect that a continuing program of

removal would be needed in order to control the population by electrofishing. I was unable to detect a response of rock bass to the removal of redbreast sunfish due to their extremely low capture rate.

INTRODUCTION

Invasive Species

Freshwater biodiversity is affected by many different factors including invasive species introductions, river impoundments, climate change, over exploitation, and pollution. One main threat to freshwater biodiversity is the introduction of non-native species. Where as not all introduced species become invasive or cause negative ecological effects (Leprieur et al. 2009), there is a long history of human-assisted invasions in freshwater habitats (Almeida and Grossman 2012). There are several ways non-native fishes have been introduced: intentional introduction by state or federal agencies, through canal systems, "bait-bucket" introductions by anglers, intentional stocking by anglers, and “hitchhiking” on boats (Kohler and Hubert 1999). Globalization has also led to aquatic organism introductions through ballast-dumping, aquarium fish releases, and escape of aquaculture fish (Kohler and Hubert 1999).

Invasions in freshwater systems are sometimes difficult to detect or are only detected after the population has become established. The process of invasion of a non-native species can be divided into four stages, introduction, establishment, spread, and the integration or impact (Leprieur et al. 2009). A successful invasion, establishment, and spread of an invasive species can be affected by several factors (Hoddle 2014). One factor is propagule pressure, the number and frequency of introduction into a new area. It is more likely for a species to establish with 10 separate introductions of 100 individuals each than with the introduction of 1000 individuals at one time. Numerous small introductions over time are more likely to coincide with proper conditions for the organism to establish, such as food and temperature. Another factor affecting the phases of an invasion is the minimum viable population size of an organism. A minimum number of individuals are needed to sustain an organism’s population, if an introduction is to result in the establishment of a population. Having a minimum number of individuals in a population ensures a small population doesn’t go extinct because of random environmental events, such as storms. A minimum population size also has an importance for locating mates to ensure that reproduction can replace members of the population lost through mortality. The lag period is another factor of the invasion; a population can persist for quite some time before the observation of exponential growth is observed. Explanations for this lag period are that genetic adaptation is occurring which may take several generations, or time for the organism to disperse from a less desirable introduction area to more desirable areas (Hoddle 2014). Lag periods can also be explained by environmental causes, the inherent lag effect, and by not detecting an already expanding species. The final factor of an invasion is the climate and environment of the invaded or introduced area. The area of introduction must have a suitable climate in order for the introduced species to survive, and must provide adequate resources for growth and reproduction (Hoddle 2014).

The impact stage of an invasion is rarely examined and very little documentation on the impact of many introductions may be found (Leprieur et al. 2009). For example, in the DIAS (United Nations Food and Agriculture Organization's Database of Invasive Aquatic Species) database, for 13.9% of 3141 records of introductions, it is unknown whether the establishment of the species was successful and in 80% of cases it is unknown if the species caused ecological effects (Leprieur et al. 2009). A successful species introduction is an ecological perturbation that alters the biotic community (Kohler and Hubert 1999).

Non-native fishes may have a variety of effects on native fishes including habitat alteration, predation, hybridization, vectoring diseases, food web alteration, and interspecific competition (Almeida and Grossman 2012). Interspecific competition can occur as exploitative competition or interference competition. The introduced species could prey upon or compete for food with native fishes and this could lead to a decline or loss of population of the native fish (Leprieur et al. 2009). Introduced species often have a wide range of physiological tolerances giving them a competitive edge in disturbed habitats. Thus, more tolerant invasive species often replace native species (Almeida and Grossman 2012).

Although competition occurs in many cases, it is difficult to quantify the effect of competition and sometimes difficult to detect.

Freshwater invaders can cause changes in an individual's behavior, to the community, or the population. Predation or competition by invaders has altered native species behavior and this altered behavior can influence habitat use and foraging, in addition diet shifts can occur (Simon and Townsend 2003). At the population level, changes in abundance or distribution of other species can be changed by the invaders. Community level effects include alterations to direct and indirect interactions among populations and can cause trophic cascade. Invaders can cause energy and nutrient movement changes to their pathways and magnitude at the ecosystem level and the impacts are related to energy and nutrient flux in the system (Simon and Townsend 2003). Fish communities and assemblages have been altered through extirpations and distribution changes of other species. If the impact is sufficiently large, the regional distribution of the native species can change or populations can be extirpated (Simon and Townsend 2003). Size selection is another area that can be impacted by invasions if predation is size selective, causing prey size class structure to change (Simon and Townsend 2003). A reduction in fitness and the ability to adapt to condition changes by the population can increase the risk of extinction through the process of introgression (Simon and Townsend 2003). If an invader is able to build up high densities and biomasses and replace a native species that is an ecological equivalent and develop a higher biomass than the native, then they are able to exert a stronger ecosystem impact (Simon and Townsend 2003). Direct ecosystem impacts can be caused by the invaders mode of resource acquisition by generating or enhancing energy and nutrient pathways (Simon and Townsend 2003). Invaders in freshwater systems can have severe consequences on the native species on many levels, and much of the time those impacts are unknown.

A fish species is deemed undesirable if it has adverse effects on threatened or endangered native fishes, serves as a source of pathogenic organisms, does not contribute to commercial or sport fishing, inhibits the development or maintenance through predation, direct competition on desirable fishes, or interferes with other wildlife management practices (Kohler and Hubert 1999). Fish introductions in the past were usually not viewed as a negative occurrence; it was believed that fishes introduced were compatible with other vertebrates that use the same aquatic habitat (Kohler and Hubert 1999). There is a lack of data on the long-term trends of introductions of non-native fish and fish extinctions in freshwater ecosystems. Interactions among species leading to extinctions may take decades to complete leading to time-lags in community dynamics (Leprieur et al. 2009). The potential of possible extinction debts of native species due to the establishment of an invasive species is an important factor in researching and studying invasive species in aquatic systems a priority and increases the importance of the removal of these invasive organisms.

Redbreast Sunfish And Rock Bass

Lepomis auritus (Linnaeus, 1758), redbreast sunfish, are native to creeks and small to medium rivers of the Atlantic Coastal drainages from the Gulf of Mexico to central New Brunswick. Non-native occurrences have been seen in 13 states including North Carolina (Fuller et al. 1999). In North Carolina, they were widely stocked as a popular sport fish and now are found throughout the state including the upper Tennessee and New River drainages. It's not known when they were introduced to Richland Creek, a tributary of the Pigeon River located in Haywood County, North Carolina. Messer (1964) reported that he sampled two individuals from the Pigeon River, downstream of the mouth of Richland Creek (one adult at each of two stations), but found none in his Richland Creek sample, suggesting that the current population probably dates from around that time or later.

They are often reported to feed on or near the bottom (Etnier and Starnes 1993), and aquatic and terrestrial invertebrates are typically reported as dominant food items (Davis 1972, Etnier and Starnes 1993). They are occasionally reported to eat small fish (Davis 1972, Gautreau and Curry 2012). Under favorable conditions, they live in excess of 6 years and routinely grow to lengths greater than 220 mm total length (Carlander 1977, Davis 1972). At the northern extreme of their native range in southwestern New Brunswick, their maximum length was recorded as approximately 195 mm for a 6+ aged individual (Gautreau and Curry 2012).

Introduction of the *L. auritus* outside their native range could have consequences on native fish assemblages and fish-fish interactions (Etnier and Starnes 1993). Circumstantial evidence has indicated that *L. auritus* are displacing the native longear sunfish (*L. megalotis*) through direct competition in eastern Tennessee (Fuller et al. 1999). They are also reported as an ecological threat in Texas and have helped placed several fish on the endangered species list (Bonner et al. 2005). What effects, if any, that *L. auritus* have on Richland Creek species is still unknown. The possibility of competition between *L. auritus* and native rock bass (*Ambloplites rupestris*) has been suggested as a possibility, (B.H. Tracy, N.C. Department of Environment and Natural Resources, personal communication).

Ambloplites rupestris (Rafinesque, 1817), the rock bass, is native to the St. Lawerence River through the Great Lakes, Hudson Bay, and Mississippi River drainages, from Quebec to Georgia (Page and Burr 2011). *Lepomis auritus* and *Ambloplites rupestris* have similar habitat requirements, a similar diet, similar reproductive behaviors, and are similar in size when adults. Both species are found in rocky or vegetated pools. The *A. rupestris* is known to feed on insects, small fish, mollusks, and crayfish (Werner 2004). Both species spawn in early summer, and males build and guard nests in shallow water. Females carry a similar number of eggs, rock bass carry 5,000 or more and *L. auritus* carry between 1,000 and 8,000 eggs (Werner 2004). These overlapping life-history characteristics could potentially lead to competition or at least daily interactions between these two species.

Reproductive ecology of fishes is an important life history attribute that can have consequences on population growth and community composition. Redbreast sunfish is found in headwater streams to coastal rivers, lakes, and reservoirs, but its reproductive ecology is not well known (Lukas and Orth 1993). In Virginia streams, the nesting sites were selected where there was low to negligible water velocity, typically in pools, sheltered backwaters, side channels, and stream margins (Helfrich et al. 1991). They typically began spawning when the water temperature increased to 20° C which normally occurs in late May (Lukas and Orth 1993). High or variable water discharges and thermal regime fluctuations can have a negative impact on stream centrarchids, by disrupting spawning (Helfrich et al. 1991). Centrarchids are freshwater ray-finned fish that include sunfish, rock bass, and largemouth bass. The reproductive success of fishes is partially dependent upon having suitable nest substrate available (Helfrich et al. 1991).

During spawning, females approach the nests from downstream and are actively courted by the males swimming in circular patterns above their nests (Lukas and Orth 1993). The spawning act takes around four minutes and while the female is in the center of the nest the male leaves the nest and chases away other redbreast sunfish (Lukas and Orth 1993). The eggs are mixed into the gravel by the male to form a stable gelatinous matrix (Lukas and Orth 1993). In *L. auritus*, after the parental male's nest has been visited by females and is full of incubated eggs, he keeps them aerated and free of silt by fanning them (DeWoody et al. 1998). The male redbreast sunfish spends a considerable amount of time defending his nest from perceived predators, and in the North Anna River in Virginia this included smallmouth and largemouth bass, and other redbreast sunfish (Lukas and Orth 1993). Even though the redbreast sunfish uses these tactics to protect its nest and brood, there is still mortality and nest failure for a number of reasons. An important source of egg and larvae mortality may be predation (Lukas and Orth 1993). High flow is a cause of early nesting failure and departure of the male may suggest that predation is a source of nest failure (Lukas and Orth 1993). The spawning behaviors of redbreast sunfish possibly create incubation conditions that are better suited for stream environments, suggesting adaptation for a lotic existence (Lukas and Orth 1993).

Although rock bass have similar reproductive behaviors, their population is not thought to be currently sustaining itself in Richland Creek (B.H. Tracy, N.C. Department of Environment and Natural

Resources, personal communication). Spawning occurs for the rock bass during late spring to early summer and males excavate nests in the gravel of shallow areas in lakes and streams (Noltie and Keenleyside 1986), and can occur on different dates in different years (Gross and Nowell 1980). After the females deposit their eggs they depart and the males stay and guard their nests and broods. The reproductive behavior of the rock bass is similar to the redbreast sunfish. During the nesting cycle of centrarchids, the quality of parental care and thus brood vulnerability may vary (Noltie and Keenleyside 1986). The ability of a male to complete more than one nesting cycle in a season influences his reproductive success and can be enhanced by larger male size, low flow conditions, early start date, and warm water (Noltie and Keenleyside 1986). The males carry the burden of having the high energy cost behavior of building and maintaining their nests and guarding their brood (Noltie and Keenleyside 1986). Rock bass that are larger have the ability to defend the nest against predation attempts and fast for longer periods of time with their stored reserves (Noltie and Keenleyside 1986). Once they have hatched, the young remain in the nest for around 10 days until the free-swimming larvae disperse (Noltie and Keenleyside 1986). The brood can be lost by predation, flooding, and fouling by algae. High water flow has the ability to wash the young from the nests, or can increase deposition of silt or algae in the nest (Noltie and Keenleyside 1986). Once the fry leaves, some males linger, but most males quickly become free-swimming and others find new nests and spawn for a second time (Gross and Nowell 1980). Unlike *Lepomis*, rock bass use anal and pectoral fins in the construction of the nest and the male color darkens and during spawning they perform irregular circling behavior (Gross and Nowell 1980).

Fish Movement

There are many purposes of fish movement in streams that include spawning, migrations, and colonization of vacated areas of the stream. The mobility of an animal could influence interactions within the community, population responses to disturbance, and the scale at which organisms can respond to variation in the environment. The ability of stream fishes to travel long distances is further demonstrated by seasonal migrations and the re-colonization of disturbed streams (Freeman 1995). It has been known that stream-dwelling species restrict movements to limited areas, and documented limited movements have included centrarchids (Gatz and Adams 1994). Resource availability may reflect a given species pattern of movement and this movement may differ between streams (Gatz and Adams 1994). A small percentage of a population seems to be able to repopulate devastated areas in a stream where recolonization can be rapid for sunfish with small home ranges (Gatz and Adams 1994).

Case studies show that movement is often limited, but longer migrations are possible and necessary. Gatz and Adams (1994) found that two-thirds of all documented movements were less than 100 meters and rock bass and redbreast sunfish are highly sedentary species. Their estimated home range length is 50 to 99 meters for both species. Even though they have the capability to travel greater distances small fish frequently occupy limited areas and are considered habitat specialists. Freeman (1995) found that juvenile redbreast sunfish moved considerable distances both within and between mesohabitats, and they displayed a tendency to move upstream rather than downstream. She further suggested that longer distance movements may be routine. She interpreted rapid re-colonization of stream sections after being defaunated as evidence of routine exploratory movement (Freeman 1995). Gatz and Adams (1994) found redbreast sunfish to be highly sedentary during winter months, but highly mobile in the spring and have intermediate movement in the summer and fall. In Midwestern streams, it was found that longear sunfish, green sunfish, and rock bass are species with restricted home ranges (Gerking 1953). The majority of transplanted longear sunfish moved to their original home ranges downstream, and this movement indicated that riffles are a behavioral barrier rather than a physical one (Gerking 1953). The majority of longear sunfish and rock bass were found to stay in a section of 100 to 200 feet (Gerking 1953). The occasional significant upstream or downstream movement patterns of redbreast sunfish are representative of site resource responses and season rather than behavior preferences (Gatz and Adams 1994). Gerking (1953) found that the natural intraspecific and interspecific changes to a fish population in one part of the stream may not have any relationship to events in another part. The mobility of redbreast sunfish will play a large role in determining if their removal will affect their population or if they have the ability to re-colonize as fast as they are removed.

Fish Community Structure

The Southeastern United States is a biological hotspot of biodiversity and this includes fish communities. There are estimated to be over 600 fishes to occur in Southeast drainages and approximately 28% of this fish fauna is deemed in need of conservation (Butler 2002). There are a number of jeopardized fishes that are unlisted in this region that need to be managed and conserved before listing them becomes necessary (Butler 2002). Causes of these conservation concerns include introduced species, river impoundments and reservoirs, and environmental changes.

Biodiversity loss and fish assemblage structure are often affected by impoundments and stream flow. Biodiversity loss in freshwater ecosystems is often driven by impoundments (Rypel 2011). Physical, physiochemical, and ecological conditions are often suboptimal for many lotic specialists, and they are unable to emigrate from these areas (Rypel 2011). Rypel (2011) found that rock bass and redbreast sunfish had significantly higher growth rates in riverine systems. Fish have different abilities to deal with environmental variability and show a wide range of life history patterns. Stream flow can have strong effects on the structure of stream fish assemblages and can vary among groups (Schlosser 1985). Sunfish breed later in the year, which appears to make juvenile abundance more sensitive to high flow conditions than species breeding earlier (Schlosser 1985). Stream fish assemblage structure is likely regulated by both stochastic and deterministic processes. Younger age class abundance, species richness, and species composition is likely influenced by stochastic or physical factors (Schlosser 1985). These factors can include temperature, water level, and storms. Deterministic or biotic interactions may strongly regulate the older age classes (Schlosser 1985). Growth rates, biodiversity, and assemblage structure can all be affected by different environmental conditions and impoundments (natural and manmade) that change composition of species.

Reservoirs are a common occurrence on rivers throughout the world, and the fish communities within them can change over time. High reproductive rates, short generation times, and wide tolerances facilitate invasion of new environments, such as reservoirs, by early colonizing species (Paller et al. 1992). Some of these early colonizers are not native to the system and can establish and become invasive. Studies of fish communities in newly formed reservoirs focus on sport fish management instead of ecological mechanisms responsible for community development (Paller et al. 1992). The redbreast sunfish is one species that is characterized as a successful colonist with parental care, low fecundity, large adult body size, and is an insectivore. In one South Carolina reservoir, redbreast sunfish was the only species out of the eight that initially dominated the fish community of the reservoir with increasing numbers (Paller et al. 1992). The decline of early colonists could have been caused by several types of biological interactions. Fish stocking can cause the rate of community change to accelerate and contribute to an abrupt species turnover.

Fisheries Management

Chemical control of unwanted species is often used as a quick and efficient method in eliminating an invader, but unlike other means of removal it is non-discriminatory. In the early 1900s, chemicals were first used to control fish when a Vermont lake was treated with copper sulfate, and by the 1930s to 1950s chemical toxicants were widely used to control undesirable species (Kohler and Hubert 1999). Non-target species are killed when toxicants (e.g. rotenone and antimycin) are used, so in many cases it is necessary for biologists to find an alternative method of removal or control (Meyer et al. 2006). Native piscivorous fish can be used to control an invasive species. One recent attempt at controlling common carp focused on controlling juvenile abundance by targeting younger age fish as a source of prey of a native predator (Weber and Brown 2012). A shift of predator preference was expected when the complexity of habitats was altered, due to the availability of prey (Weber and Brown 2012). Prey that was easier to catch was selected by predators (Weber and Brown 2012).

Suppression using electrofishing is a feasible option due to its ability to significantly reduce nonnative densities (Peterson et al. 2008). This removal method is most likely to be successful in small streams because of the lower capture efficiencies in larger systems (Carmona-Catot et al. 2010). The shortcomings of this method includes the difficulty of complete removal of the target species, and fish are mobile species and able to colonize rapidly unless there is a barrier between treated and untreated areas (Meyer et al. 2006). Additionally, there is the potential for harmful physiological effects to the fish that receive electroshock. The level of these effects is dependent upon variables such as current type (e.g. AC, continuous DC, or pulsed DC), voltage, duration, wave form, size of fish, fish species, and conductivity of water (Gatz and Linder 2008). Larger fish are shocked more severely than smaller fish and are more likely to be injured which makes them more likely to be captured (Gatz and Linder 2008). Physiological stress responses such as elevated blood cortisol, glucose, and lactate can result in acidosis and can be caused by electroshock (Gatz and Linder 2008). Impaired swimming is a consequence of these effects that can be seen a day after the shocking event and for wild fish this can reduce the ability to maintain position and access food which can lead to growth effects (Gatz and Linder 2008). In addition to the electroshock, the handling or tagging of the fish can be as or more detrimental than the shock (Gatz and Linder 2008). Even with the consequences of electroshocking, it remains a suitable way to remove target species or evaluate species composition. Lemly (1985) found that when invasive green sunfish (*Lepomis cyanellus*) were selectively removed, previously suppressed native species increased in number in three first-order piedmont North Carolina streams.

Location Of Study

The study was conducted in Richland Creek, in Haywood County, North Carolina (Figure 1). Richland Creek flows in a generally northeastern direction from its headwaters near the boundary between Haywood and Jackson Counties, NC, through Waynesville, NC to its mouth on the Pigeon River (35.549799^0N, 82.946653^0W) approximately 10 km downstream of Canton, NC. Richland Creek was dammed in 1913 approximately 4 km upstream from its mouth, creating a small (81 ha) reservoir, Lake Junaluska. The Richland Creek watershed encompasses an area of approximately 17,700 ha and while the headwaters drain mostly protected forestland, the main stem flows through the most heavily developed portion of Haywood County. Lake Junaluska is a popular recreation destination and provides revenue to the local economy (EPA 2012). This reservoir isolates most of the stream from the rest of the Pigeon River drainage.

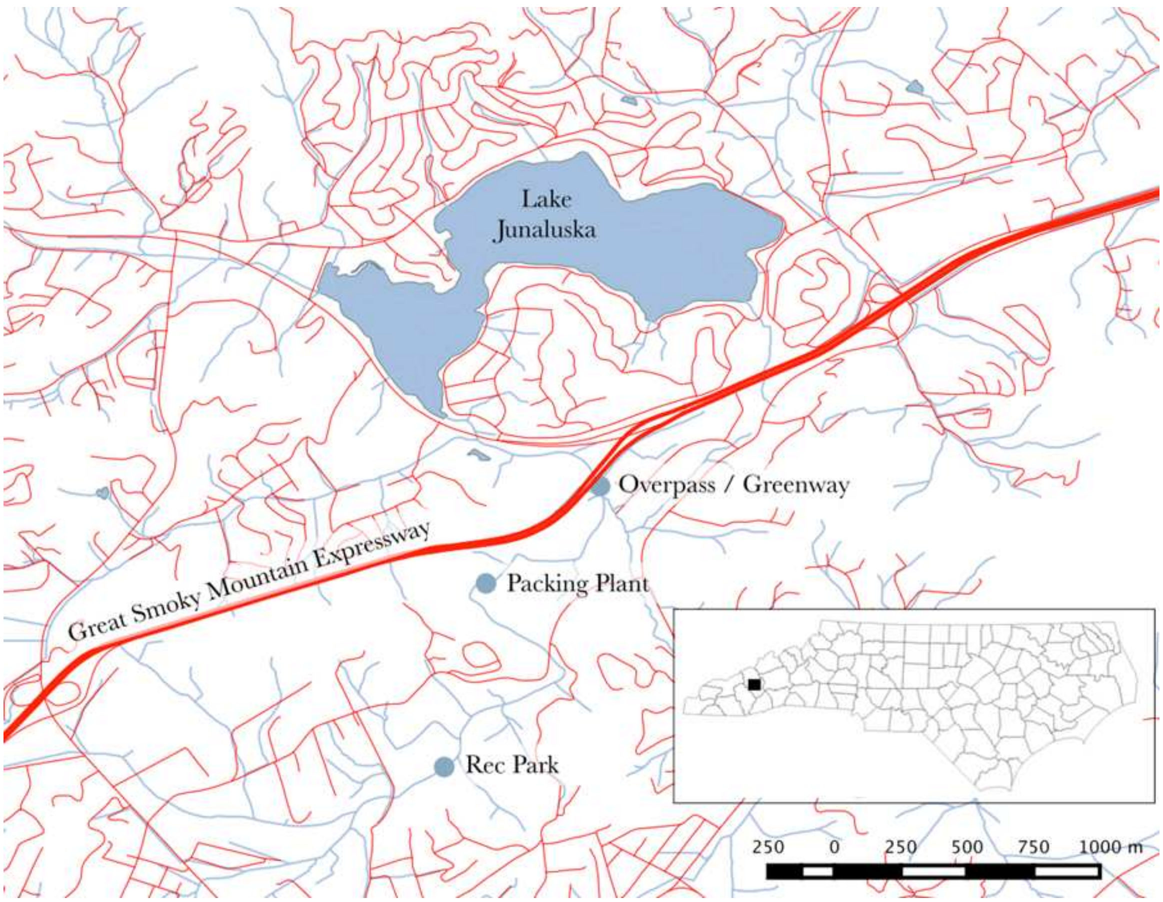

Figure 1: Map of Richland Creek. The study locations of the experiment are in the city of Waynesville in Haywood County, NC.

In recent years, much of Richland Creek has been placed on the CWA *303(d)* list of impaired streams, due to low biological integrity and/or water quality. Collaborative efforts led by NCDENR personnel to improve the quality of the stream and reintroduce native species to the stream are ongoing. Through 2011, best management practices have been implemented to include checking dams, planting in critical areas, diversions, adding exclusion fencing, adding riparian herbaceous cover, and stabilizing the stream channel (Bornholm 2014). In addition to these best management practices, native fish introductions of warpaint shiner, river chub, saffron shiner, mirror shiner, rock bass, mottled sculpin, greenfin darter, Tuckasegee darter, and fantail darter have been taking place twice a year with over 14,000 fish having been released thus far (B.H. Tracy, N.C. Department of Environment and Natural Resources, personal communication).

Purpose Of Study

The objectives of this study were as follows: (1) to test if back-pack electrofishing methods are effective in removing *L. auritus* from Richland Creek, (2) to test if removing *L. auritus* has any effect on the population size of *A. rupestris*, and (3) to determine if *L. auritus* quickly re-establish the voids that are made by their removal.

METHODS

Study Sites

The study sites (Figure 1) were selected based on ease of electrofishing accessibility, and known *L. auritus* and *A. rupestris* distribution. Each removal and control reach was 100 meters long. In past years, NCDENR fish community sampling on Richland Creek has found *L.* auritus as far upstream as their E44 (Boyd Avenue) sample site, but there have been more consistent catches farther downstream at their E47 (SR 1184) site (NCDENR 2013).

The upstream-most site was located alongside the Waynesville Recreation Park (35.503932, -82.976297; Figure 1). The removal reach started just downstream of the Vance Street bridge and the control reach was located immediately downstream of the removal reach. Both reaches contained riffles, pools, and runs. There was some woody debris and rocky substrate. There was a walking path on one side of the stream and railroad tracks on the other. There was minimal riparian zone on either side of the stream with few trees and shrubs and approximately 75% to 80% canopy cover. The wet width of the stream was 12 meters and had a depth range of 0.3 to 1 meter. Both control and removal reaches were located within the city limits of Waynesville, NC.

The second location was located near the Evergreen Paper Company packaging plant (35.511579, -82.974559; Figure 1). The removal reach ran parallel to the side of the building and the control reach was located alongside the Waynesville walking trail behind the building. There was a break between the reaches of approximately 50 meters due to the depth and swift current of the stream that made wading and electrofishing difficult and dangerous. Both reaches contained riffles, pools, and runs. There was some woody debris, a rocky substrate, and some sandy areas associated with the larger pools. One side of the stream had forest habitat and one side was mowed lawn with a small riparian zone less than 10 meters.

Canopy cover was approximately 40%, wetted width was 12 meters, and the stream depth range was approximately 0.5 to 1 meter.

The third removal site was located along the Waynesville Greenway near an underpass of highway 23/74 (35.515627, -82.969819; Figure 1). This was the most downstream site and was located approximately 1 km upstream from Lake Junaluska. The control reach was directly downstream of the experimental reach with no break in between reaches. There were riffles, pools, and runs. There was a significant riparian zone on both sides of the stream, but there were cutout trails to the stream from the walking trail. Beyond the riparian zone was a golf course as well. Highway 23/74 ran directly over the stream allowing for runoff from the road. Canopy cover was approximately 60%, wet width was 13.4 meters, and stream depth was up to 1 meter. There were riffles, pools, and runs. In the removal reach there was a small tributary, Raccoon Creek.

Fish Collection, Measurement, And Euthanization

The collection of *L. auritus* was done using a Halltech Aquatic Research Incorporated, model HT 2000/05 electrofisher. The frequency was set at 80 Hz, and the output voltage was determined based on conductivity of the water. The voltage used for this stream, ranged from 550 to 650 volts depending on the conductivity and the effect the voltage had on the fish in the stream. Shocking was initiated at the downstream area of the reach and progressed upstream moving in a zigzag pattern moving the anode back and forth. Only *L. auritus* and *A. rupestris* were collected, all other species were quickly viewed to identify and returned to the stream downstream of the shock zone. Any *L. auritus* and *A. rupestris* caught were either euthanized or measured and released. The fish collected for each pass were processed separately.

All *L. auritus* were euthanized by over-dosing with MS-222, (American Fisheries Society 2002). Barbiturates, benzocaine, 2-phenoxyethanol or MS-222 are the agents recommended in the United States

(Ross and Ross 2008). The fish being euthanized remained immersed in the solution for 10 minutes after their last observed opercular movements had occurred (Cornell University institutional animal use and care committee 2013).

After the fish were euthanized, their length and weight were measured. Maximum total length was measured to the nearest millimeter. Wet weight was measured to the nearest gram. If the fish weighed less than one gram it was marked as less than one gram. Whether the fish had a fin clip or not was recorded, as well as any other characteristics that had importance. Once the measurements had been taken, the fish were put into a sealed bag and stored for travel on ice. They were transported to Western Carolina University, where they were stored until they were used for additional analysis of their diet and age characteristics (Woods 2014).

Any *A. rupestris* captured were released as quickly as possible after their length and weights were recorded, using minimal handling. *L. auritus* sampled from control reaches and all *A. rupestris* were marked using a small pelvic fin clip that allowed recognition of previously sampled individuals. The cut fin regenerated, but evidence of the cut allowed for easy identification. All fish not sacrificed (including non-target species encountered) were immediately returned to the river downstream (out of the electrical field). Non-target species are noted on the data sheet. Identification of species that were unknown or questionable was done using *Peterson Field Guide to Freshwater Fishes of North America North of Mexico*, second edition (Page and Burr 2011). After the initial collection occurred, subsequent collections occurred during monthly monitoring trips to the study sites through June 2014. At each sampling, a portable DO meter (YSI 85, Yellow Springs, OH), was used to take readings of temperature, dissolved oxygen, and conductivity.

Statistical Methods

Stream fish abundance was estimated using depletion or removal methods, for redbreast sunfish in Richland Creek. The statistical software R (v.3.1.0, R Core Team 2014) was used to estimate the population using the K-Pass removal algorithm from the FSA package (v. 0.4.22) for R (Ogle 2014). The collection was performed using the same amount of effort on a closed population that was sampled repeatedly k times (Ogle 2013). The number of fish that were removed was recorded for each sampling pass. Estimation of the population can be done from the number of animals that are removed from the successively removed organisms, using certain assumptions. These assumptions include the population was closed and the capture probability for an individual was constant for all animals from sample to sample. Zippin (1956, 1958) showed the method of iteratively solving for q and N_0. A slight modification of Zippin's method was shown by Carle and Strub (1978) and noted that if X $\leq \frac{T(k-1)}{2}$ the general k-pass method will fail to give an appropriate estimate of the population size. The criterion for failure is equal to X≤T (i.e. $C_1 \leq C_3$ when k=3). Where k= is the total number of removal periods, C_i= is the number of animals captured in the ith removal period, T= is the total number of individuals captured, and X= (k-i)C_i. This means, the general k-pass method will fail if the number of fish removed on the last pass is greater than or equal to the first pass number of fish removed (Ogle 2013). For this study, The Zippin's K-Pass Removal Method was used when the number of fish removed was lower for each sequential pass. If a greater amount of fish were removed in a later pass, then The Carle & Strub's K-Pass Removal Method was used.

The difference in population estimates between removal and control sites was tested using mixed effects model for repeated-measures ANOVA via R package lme4 (v. 1.1-6, Bates et al. 2014). The degrees of freedom for the F-test were estimated using the Satterthwaite approximation (lmerTest package v. 2.0-6, Kuznetsova et al. 2014). The population estimates were log(X+1) transformed to meet the assumption of homogeneity of variances.

RESULTS

The direct counts of redbreast sunfish showed a drop in the numbers that were caught in the areas of the stream that were electrofished. Fewer fish were being caught as sampling continued over the course of the study. This drop was seen in all reaches including control and removal reaches. Some reaches dropped more than others and some months had larger catches than others. Over the length of the study the total number of fish caught in the control areas of the stream dropped from 91 fish caught in September to 27 fish caught in June and in the removal reaches dropped from 102 fish caught in September to 20 fish caught in June.

At site one, 72 redbreast sunfish were euthanized during the length of the study. In the control reach of this site a total of 50 fish were caught and 11 fish had evidence of fin clips (Table 1), indicating that they were caught previously in this reach. The fish with clipped pelvic fins remained within the reach or returned to the section of stream before sampling. In the removal reach, five fish were caught with fin clips. This indicates that they had traveled from the control reach and into the removal reach between the sampling times of the reaches. The recapture rate for the control reach was 22% and for the removal reach was 6.9%.

At site two, 66 redbreast sunfish were euthanized over the course of the study. In the control reach of this site 162 fish were caught with 15 recaptures (Table 1). In the removal reach, two fish were caught with fin clips. This was evidence that they traveled from the control reach to the removal reach within the time between sampling one section to the next. The rate at which fish were recaptured was 9.3% for the control reach and 3% for the removal reach.

At site three, 133 redbreast sunfish were euthanized during the study. In the control reach of this site a total of 186 fish were caught with 23 recaptures (Table 1). No fish in the removal reach had evidence of fin clips; this indicates that no fish from the control reach caught previously traveled upstream to the adjacent section. The fish were recaptured at a rate of 12.4% for the control reach and 0% in the removal reach.

Table 1: The total number of redbreast sunfish caught, euthanized, recaptured, and percentage recaptured (C= control, R= removal).

Site	**Treatment**	**Total**	**Euthanized**	**Recapture**	**Recapture Rate (%)**
1	C	50	0	11	22
1	R	72	72	5	6.9
2	C	162	0	15	9.3
2	R	66	66	2	3
3	C	186	0	23	12.4
3	R	133	133	0	0

<u>Population Estimates</u>

The population estimates showed a significant decrease in population size similar to the direct counts over the duration of the study (Figure 2, Table 2). There was also an overall difference between control and removal sites (P=0.0521). There was a significant difference between sampling months (p=0.0001).

Table 2: Summary of the results of the mixed-model repeated-measures ANOVA.

	SS	MS	NumDF	DenDF	F value	Pr(>F)
Treatment	2.49	2.49	1	24.03	4.18	0.0521
Month	25.50	3.64	7	24.15	7.84	0.0001
Treatment X Month	1.22	0.17	7	24.03	0.37	0.9080

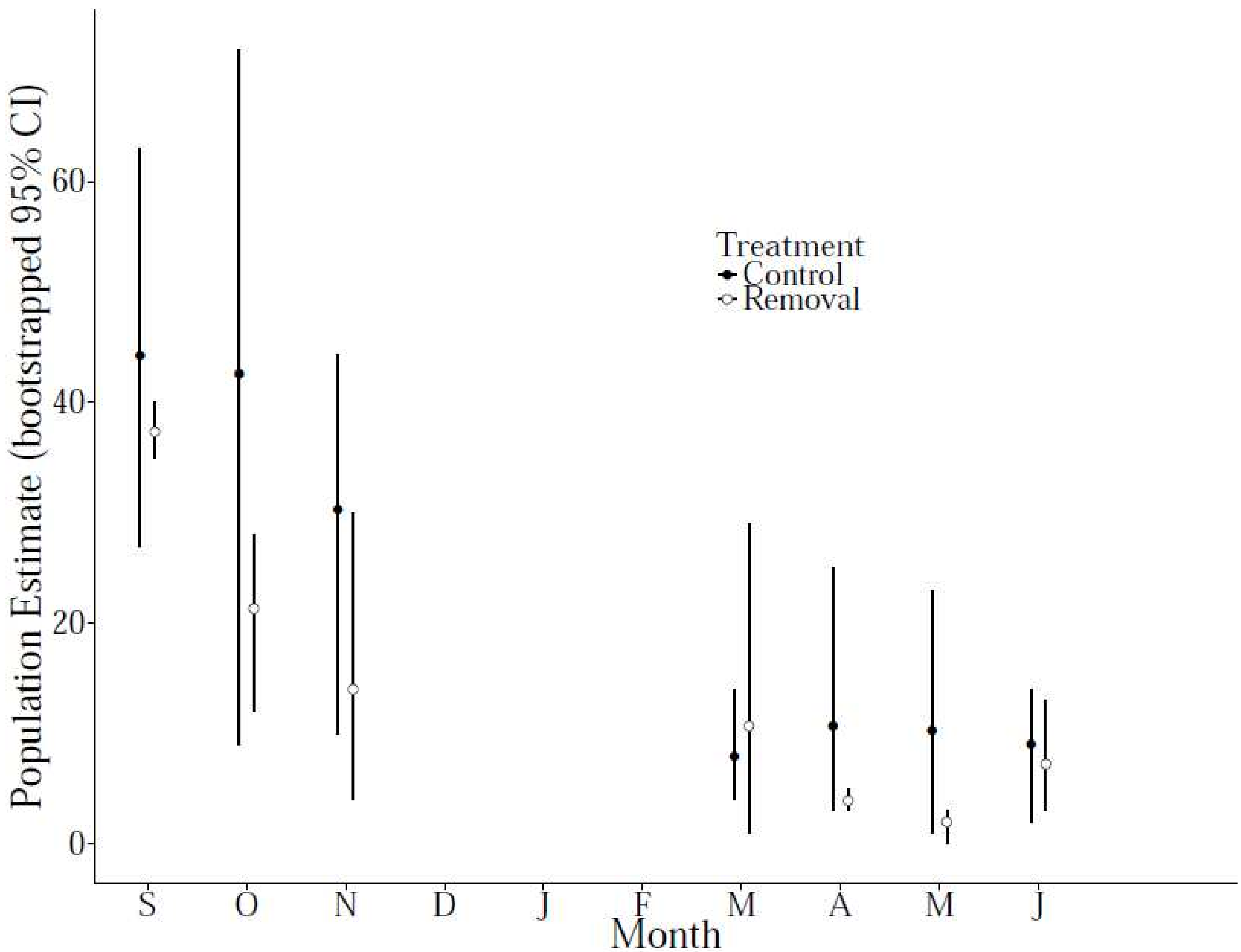

Figure 2: Population estimates of redbreast sunfish for each sample month of the study.

Length-Weight Relationship

Overall, redbreast sunfish caught in this stream were relatively small to mid-size fish (Table 3, Figure 3). The majority, approximately 70%, of the sampled population was between 50-100 mm in length (Figure 3) and had a mean length of 81.12 mm (Table 3).

Table 3: Average length and weight of all sampled redbreast sunfish in Richland creek.

	Mean (mm)	Median (mm)	Standard Deviation
Length	81.12	80.00	24.92
Weight	12.32	9.00	13.07

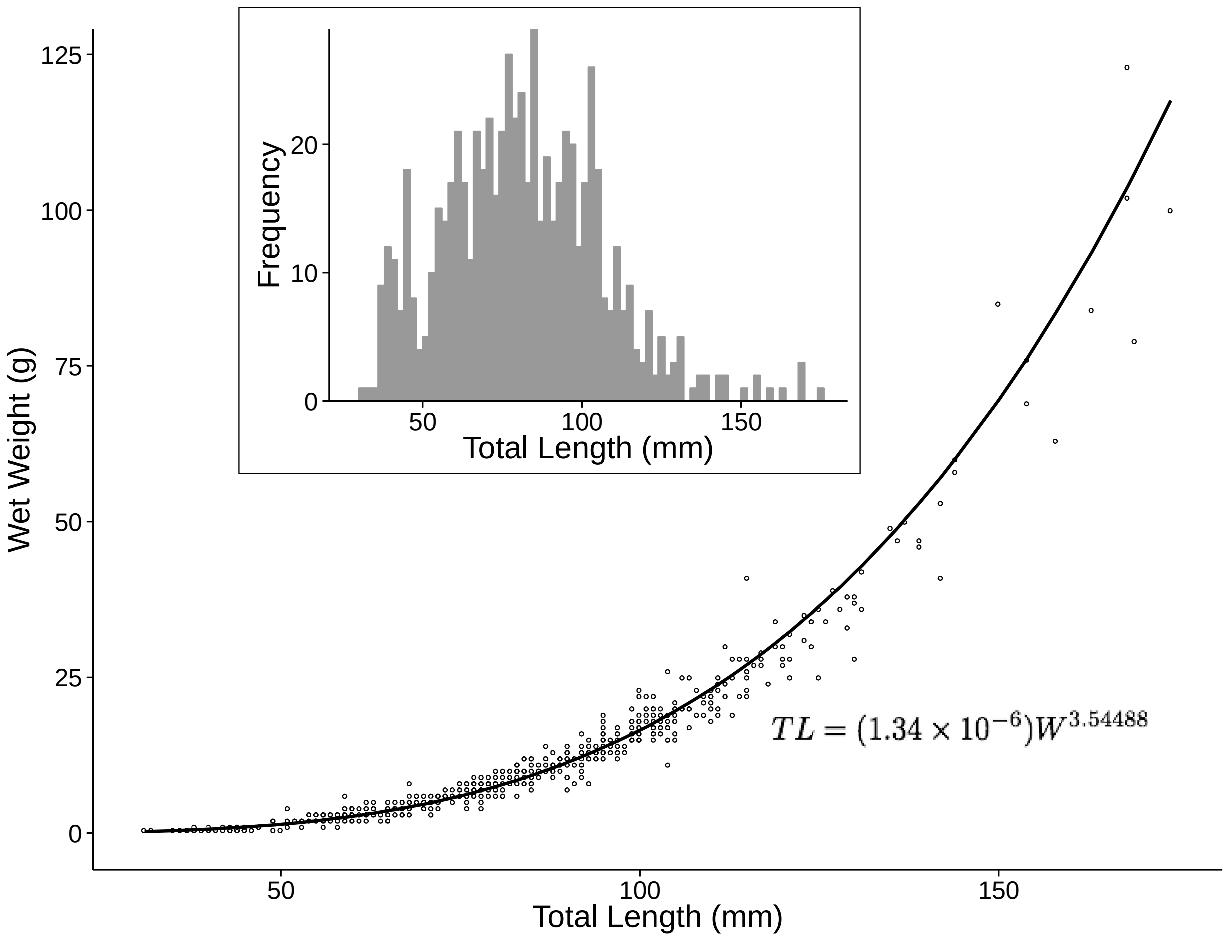

Figure 3: Length - weight relationship and length distribution (inset) for redbreast sunfish sampled from Richland Creek. Data from all redbreast sunfish collected over all reaches and all months.

Temperature, Dissolved Oxygen, And Conductivity

As expected, dissolved oxygen (DO) was negatively correlated with temperature (Table 4, Figure 4). The conductivity varied widely during the spring samples, with April's specific conductivity peaking at 78.6 µS/cm, this is approximately 30µS/cm higher than the preceding or following month's sample (Table 4).

Table 4: Average monthly readings of environmental variables in Richland Creek.

Month	**DO (mg/L)**	**Conductivity (µS/cm)**	**Temperature (˚C)**
Sep	7.95	66.27	16.83
Oct	10.33	63.90	11.35
Nov	11.02	53.90	8.68
Mar	13.18	47.78	9.10
Apr	12.70	78.60	9.20
May	11.00	46.67	14.67
Jun	9.57	55.30	18.10

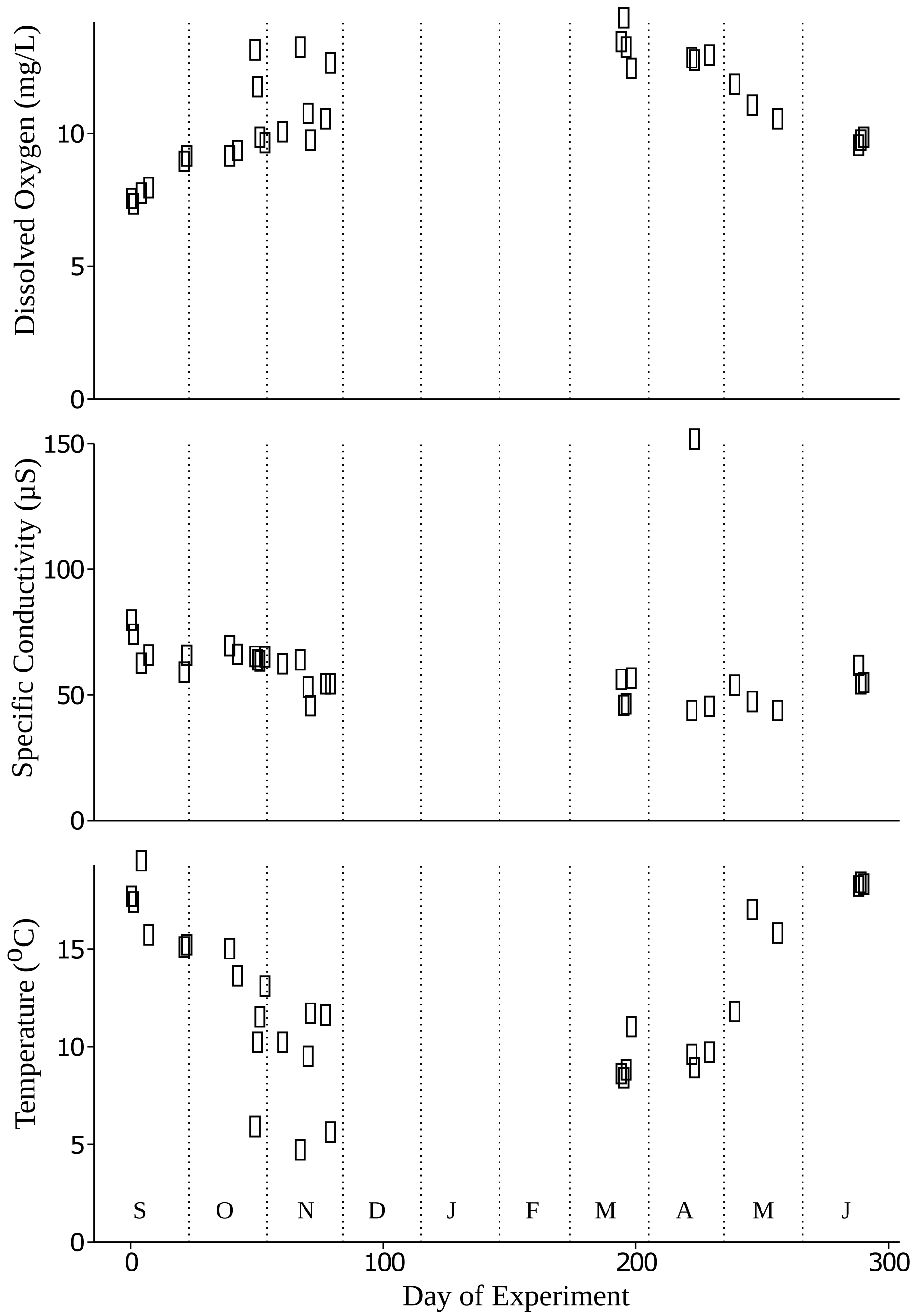

Figure 4: Dissolved oxygen, temperature, and conductivity data for each sample day in Richland Creek.

Rock Bass

During the study, only three rock bass were captured (Table 5). They were caught at site one, one in the control reach and two in the experimental reach. They were all caught within two days of each other during the first trip to site one. Their fins were clipped in case they were seen on future dates, but they were not seen again. All three had a length greater than 100 mm (Table 5). No other rock bass were seen in any section throughout the rest of the study.

Table 5: Length and weight of rock bass sampled in Richland Creek.

Site	**Length (mm)**	**Weight (g)**
1	103	18
1	135	48
1	142	50

DISCUSSION

During this study, redbreast sunfish were removed from Richland Creek, and their population within the study reaches was monitored to evaluate the population's response to the removal. A significant drop in the number of redbreast sunfish was seen throughout all of the study sites. Because the population within both control and removal sites declined, it is my belief that the population of redbreast sunfish in Richland Creek may be a transient population that comes and goes with the seasons from the nearby reservoir, and given the size distribution of captured fish the majority of the population may be juvenile fish. However, there was also a significance difference in population estimates of the redbreast sunfish between control and removal reaches. Thus, the selected removal may have affected this population, and the removal via electrofishing may be a potential management tool for this population with intensive and repeated removal. This is an interesting population of redbreast sunfish that appears to be successful in colonizing Richland Creek, but after heavy removal of individuals they do not appear to be quickly returning within the immediate area.

The movement of the marked fish suggests that this population is mobile and moves frequently among stream areas, side-streams, and potentially the reservoir. The fish that had been fin clipped to monitor the control reaches showed movement out of their area, as they were found in the experimental reaches. I assumed this would occur, because there was evidence in previous studies that fish often move around in river systems, but the distance is often unknown and depends on the species. Redbreast sunfish, according to Gatz and Adams (1994), are generally a sedentary animal and have limited movement with a normal home range of 50 to 99 meters in Eastern Tennessee. If this was the case in Richland Creek, then in the control reaches there should be a heavy reoccurrence of fish showing evidence of fin clips and the population size should have remained relatively stable. However, what I found was some fish stayed within the 100 meter control reach sampled, some moved out of the reach and into the neighboring 100 meter removal reach, and still others have moved out of the sampling range and new individuals moved in. This means that instead of being a sedentary animal in this stream, this population of the species may be more mobile than those studied by Gatz and Adams (1994). Evidence of stream fish's capability of long-distance movements is demonstrated through seasonal migration and the recolonization of intermittent and disturbed streams (Freeman 1995). The sizes of the fish that I found in this stream were smaller, juvenile fish, mostly in their first couple of years of life (Woods, 2014). The abundance of juvenile fish can dramatically fluctuate annually and based on season (Weber and Brown 2012). Freeman's (1995) recapture data showed considerable distances moved by juvenile redbreast sunfish, within and between mesohabitats. Relatively long movements (maximum of 200 m) were exhibited in this study, including three fish less than 40mm SL (Freeman 1995). This proved that small fish moved along the stream bank unrestricted (Freeman 1995). Freeman (1995) suggested that the juvenile redbreast sunfish's routine movements are representative of longer distance movements and movements between mesohabitats.

In Richland Creek, stream conditions often appeared to change depending on the weather conditions. Areas within the reaches that had calm or slow moving current became swift moving with the rising water level. This may have changed the amount of potential sunfish habitat within the reaches during the study. The redbreast sunfish's movement could be associated with the location's proximity to the reservoir.

The role that the reservoir plays for this population is unknown. Redbreast sunfish are generally a riverine species; however they also inhabit reservoirs, ponds, and lakes (Lukas and Orth 1993). In February 2014, the reservoir was allowed to drain to allow sediment removal. The effect of the radical water level change on resident fish is unknown. If they stayed in the remaining stream channel, they would have been severely crowded. They may have moved downstream to below the dam with the draining water, or they could have travelled upstream into Richland Creek. I did not see an increase of redbreast sunfish in the study area during the time of the draining or after. Once downstream of the dam, there is no way back upstream without transplantation by humans. During the month the lake was drained, no sampling occurred due to unsafe sampling conditions, but sampling did take place the following month in March while the lake remained empty.

Variables such as temperature, dissolved oxygen, and conductivity are known to have strong effects on aquatic organisms. If the temperature goes above or below the preferred range of an organism for too long, then the number of individuals in a population could decrease to extinction (Perlman 2014). In Richland Creek, the water did not reach extreme temperatures that would have negatively affected the population of redbreast sunfish. Dissolved oxygen levels can fluctuate seasonally and daily, and can vary with water temperature and altitude (APHA 1992). Cold water holds more oxygen than warm water, this was evident in the results I had from the data I collected. The temperature and DO data was negatively correlated with one another, which was expected. Redbreast sunfish inhabit slow moving water areas even though there is less DO, and they can withstand these levels associated with these areas. The presence of inorganic dissolved solids can affect the conductivity in water (APHA 1992). There was a spike in the conductivity during the month of April and this may reflect an influx of inorganic solids into Richland Creek. The source is unknown, but the stream flows through the city of Waynesville, NC and could have come from any number of sources. Overall, the environmental factors associated with Richland Creek remained at standard levels and did not reach levels that suggested an unhealthy system for fish growth.

Only three rock bass were caught over the course of the study, this leads me to believe that there is not a sustaining population in Richland Creek. NCDENR has stocked rock bass into Richland Creek in the past, but have not found any growth in their population. The fish that I caught were most likely some of these stocked fish. All three fish were similar in size, so there was no variation of size classes found among them. There’s a possibility that these fish were fished out by anglers, because of their size and proximity to a highly traveled recreational fishing spot. Due to the lack of rock bass found in the stream I am unable to make any estimation on their population size. It is still a likely possibility that there is competition occurring among rock bass and redbreast sunfish in this stream system. Frequently, competition is the major reason cited for replacement of native fish by the introduced species, however most of the evidence is anecdotal or inferential and a limited resource is not conclusively demonstrated in these cases (Kohler and Hubert 1999). If competition was a factor in this case, then rock bass were unable to survive until spawning season, or competition for nesting space between the two species were occurring limiting reproductive success of rock bass. In Tennessee, there is anecdotal evidence that redbreast sunfish have displaced the native longear sunfish (Etnier and Starnes 1993). Documentation of the negative effects on native species by the ecologically similar pumpkinseed sunfish exists, but there is a lack of direct observation field studies (Almeida and Grossman 2012). Pumpkinseed sunfish exhibit interspecific aggression toward fish that use similar microhabitats and food sources (Almeida and Grossman 2012). This aggression was higher when low water velocities occurred, close to river banks, and when abundances of prey were high (Almeida and Grossman 2012). Almeida and Grossman (2012) showed that pumpkinseed sunfish shared food and habitat resources with the native Southern Iberian chub, and impacted the native fish by disturbing foraging and movement. Besides competition, introgression with native species is another outcome of fish invasions (Simon and Townsend 2003). The ecosystem effects are less profound with the hybrids than with the pure-bred invaders (Simon and Townsend 2003). Predation and competition are clear causes of direct impacts that introduced species can cause, however they can also have indirect impacts that alter community interactions and can potentially result in trophic cascade (Simon and Townsend 2003).

The experience I had electrofishing to reduce the population of redbreast sunfish provided insight into the habitats where these fish are most likely to be found and how the population was responding to the treatment. Using electrofishing to eradicate or control this population could be complicated, but would be feasible for decreasing the population size of this invasive species using intensive and repeated removals. Redbreast sunfish was a suitable species to work with on this project, due to their relativity easy catchability and they were fairly predictable in their habitat choice. This allowed me to have an idea ahead of time which locations had a higher probability of seeing larger numbers of sunfish. Other studies that used electrofishing removals of invasive trout species showed mixed results. Meyer et al. (2006) found in one Rocky Mountain stream that after three years of intensive removals via electrofishing there were no long-term effects on brook trout abundance. They reported the short comings of the study as difficulty completely removing the target species and rapid recolonization of this mobile species, unless a barrier was established between the treated and untreated reaches (Meyer et al. 2006). Similar to this study, complete removal of my target species was difficult. A barrier was not established between my treated and untreated reaches. The fish were allowed to travel in and out of the reaches after sampling occurred. The sampling within the reach occurred in a short time span, considering the population to be a closed population. However in another study, a three year intensive removal by electrofishing reduced the brook trout population in the study creek (Carmona-Catot et al. 2010). In that study, the population reduction indicated a potential for those methods to improve the rearing conditions of the native rainbow trout. In my study, the reduction of redbreast sunfish likely led to additional resources and freed up habitat for native fish species, especially rock bass, if they were in the stream. Carmona-Catot et al. (2010) showed that projects that provided considerable effort of one to eight years removing by electrofishing in small streams, succeeded in eliminating the nonnative trout. A model, constructed for a cutthroat trout and invasive brook trout, predicted that suppression by electrofishing gave native cutthroat trout a demographic boost and assisted in their persistence in sharing resources with nonnative brook trout (Peterson et al. 2008). It also supported the conclusion that in order to interrupt the recruitment cycle of the nonnative trout species, repeated and consistent suppression was needed.

Richland Creek will need continued evaluation of its water quality and of its fish composition. The fish composition will need to be watched in order to evaluate if the missing native fish species are increasing in population size. The continued evaluation of fish assemblage will monitor how the population of redbreast sunfish has responded long term to its removal during this study. In addition to monitoring and evaluating, it’s my recommendation that Richland Creek be electrofished annually to keep the redbreast sunfish population low. I believe eradication of redbreast sunfish in this stream is improbable, but population control is possible with continued effort. If the population level of redbreast sunfish can remain low enough, than there is a possibility for native fish species to have the opportunity to increase their population size to healthy and stable numbers. At healthy and stable numbers, native fish species are less likely to be outcompeted by an invader. Since redbreast sunfish numbers were much lower than at the start of this study, it is an ideal time to transplant rock bass into the stream. If competition is occurring between the two species, rock bass have a better chance of survival and resource acquisition with less redbreast sunfish to compete with. Rock bass have a greater chance of survival if a sufficient number can be transplanted into the stream. The general public is a resource that can be useful in quality control of streams as well. Public education, outreach, and training programs on invasive species and competition that an invasive species may have with native species can increase awareness, understanding, and acceptance. These programs can motivate people to take action in their community on ecological issues. The support of invasive species removal by anglers is another option that can reduce invasive numbers quickly, when they are allowed to remove the unwanted species without limits or licensing restrictions. When all members of a community work together toward a goal, then greater ecological breakthroughs can be made, possibly eliminating a pest species and bringing back a native fish to one local stream.

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APPENDIX A

Fish Species Identified

Table 6: Fish species viewed in the sample areas during the study. Some have been identified to species and others to genus or family. These fish were not collected and only handled for a minimal amount of time to identify; no counts were taken on these species.

Common Name	**Scientific Name**
Bluegill sunfish	*Lepomis macrochirus*
Brown Trout	*Salmo trutta*
Channel Catfish	*Ictalurus punctatus*
Creek Chub	*Semotilus atromaculatus*
Dace sp.	*Cyprinidae*
Fry unknown sp.	*unknown*
Darter sp.	*Etheostoma sp.*
Largemouth Bass	*Micropterus salmoides*
Largescale Stoneroller	*Campostoma oligolepis*
Minnow sp.	*Cyprinidae*
Molted Sculpin	*Cottus bairdii*
Northern Hog Sucker	*Hypentelium nigricans*
Rainbow Trout	*Oncorhynchus mykiss*
River Chub	*Nocomis micropogon*
Shiner sp.	*Cyprinidae*

Smallmouth Bass	*Micropterus dolomieu*
Warpaint Shiner	*Luxilus coccogenis*