THE INFLUENCE OF PHYSICOCHEMCIAL CHARACTERISTICS AND INTERSPECIES INTERACTIONS ON THE INVASION SUCCESS OF A NON-NATIVE CRAYFISH

by

LINDSEY WINEMAN SARGENT

(Under the Direction of Alan P. Covich and Stephen W. Golladay)

ABSTRACT

Species invasions have the potential to reduce native biodiversity and alter ecosystem processes. Examining the conditions that allow non-native species to be successful allows for better identification of ecosystems that are vulnerable to invasion and those species that are likely to be invaders. We examined two crayfish species in the lower Flint River basin, GA, USA, a native, *Procambarus spiculifer*, and a non-native, *Orconectes palmeri*. We correlated abundance of both species with physicochemical habitat variables, examined selective fish predation, and assessed temperature selection of both species in the laboratory. Results suggest that *P. spiculifer* is superior at avoiding fish predation, and *O. palmeri* may be less successful in locations with intact *P. spiculifer* populations. *P. spiculifer* abundance is reduced in the upstream portion of the lower Flint River compared to the downstream portion likely due to warmer water temperatures. It remains unclear whether temperatures have increased in recent years due to more human water use.

INDEX WORDS: *Orconectes palmeri*, *Procambarus spiculifer*, invasion, crayfish, water abstraction, temperature, fish, predator avoidance

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Project Overview

Species invasions are established as a primary threat to global biodiversity (Elton 1958, Mooney et al. 2005) and to ecosystem function (Vitousek 1990, Spencer et al. 1991, Lodge 1993, Dukes and Mooney 2004, Peters et al. 2008). Freshwater ecosystems are particularly vulnerable to impacts from invasive species (Ricciardi and Rasmussen 1999, Sala et al. 2000). Invasive crayfish threaten native crayfish biodiversity and have caused changes to stream and lake ecosystems (Lodge et al. 2000). This study examines the invasion of a lotic crayfish, *Orconectes palmeri*, in the lower Flint River basin in southwest Georgia, USA. The purpose of this study was to document the distribution of native and invasive crayfish in the lower Flint River and adjacent tributaries. In addition, we examined how interspecies interactions and physicochemical habitat characteristics interact to determine the distributions of *O. palmeri* and a native crayfish, *Procambarus spiculifer*. The following literature review provides a background on the causes and effects of crayfish invasions and the factors that control crayfish abundance and distribution. Pertinent *O. palmeri* and *P. spiculifer* life history characteristics were also reviewed.

Literature Review

Impacts of Invasive Crayfish

North America supports 75% of the world's crayfish species, and the southeastern United States has the greatest species diversity of crayfish in North America (Hobbs 1981, Lodge et al. 2000). Hobbs (1981) recorded 45 native species of crayfish in Georgia. Crayfish act as keystone species in many aquatic ecosystems, feeding largely on detritus, algae, macrophytes and invertebrates (Creed 1994, Lodge et al. 1994, Nystrom et al. 1996, Dorn and Wojdak 2004). Crayfish also act as an important food source for some species of fish and may reduce recruitment of larval fish through predation on fish eggs (Rabeni 1992, Nystrom et al. 1996, Dorn and Mittelbach 1999). The impact crayfish will have on an ecosystem is difficult to predict as they are selective omnivores, and differential feeding behavior between species can dramatically change their functional role (Nystrom and Strand 1996, Covich et al. 1999, Usio et al. 2006)

Currently, one of the most important threats to native crayfish is displacement by non-native crayfish (Lodge et al. 2000). Invasive crayfish have reduced native crayfish populations as well as impacting algae, macrophytes, macroinvertebrates, fish, and amphibians (Charlebois and Lamberti 1996, Wilson et al. 2004, Riley et al. 2005, McCarthy et al. 2006, Correia and Anastacio 2008). The high crayfish species diversity in the southeastern United States combined with small home ranges of many native species makes crayfish biodiversity in this area particularly susceptible to the impacts of invaders.

Physicochemical Factors

Previous research has found specific physical and chemical habitat requirements to be important in influencing crayfish distributions. Of particular importance are temperature (Taylor 1984, Paglianti and Gherardi 2004), flow regime (Flinders and Magoulick 2003, 2005, Dorn and Trexler 2007), and substratum size and availability (Nystrom et al. 2006, Jones and Bergey 2007). Human induced habitat alteration often favors invaders by creating conditions to which native species are not well adapted and invasive species are well adapted by chance (Moyle and Light 1996b, Gido and Brown 1999, Light 2003). Determining which physicochemical factors are important in this *O. palmeri* invasion may help us to predict the future invasion potential of this species.

Predation

A number of well-documented cases in which invasive crayfish displace natives have been attributed to the superior ability to avoid fish predation. Predator avoidance may be accomplished through competitive displacement of native crayfish from shelters (Capelli and Munjal 1982, Soderback 1994, Nakata and Goshima 2003, Chucholl et al. 2008), or differential behavioral responses to predator encounters (Garvey et al. 1994, Gherardi and Daniels 2004, Fortino and Creed 2007). In addition, species may respond differently to chemosensory cues from predators (Willman et al. 1994, Gherardi et al. 2002, Hazlett et al. 2002, Acquistapace et al. 2004).

Invasive crayfish may also avoid predation if they have faster growth rates or larger chelae than native crayfish as smaller crayfish are more vulnerable to fish predation (Stein and

Magnuson 1976, Didonato and Lodge 1993, Garvey and Stein 1993, Kuhlmann et al. 2008). Fish often select small crayfish due to reductions in handling time (Didonato and Lodge 1993). In addition, larger crayfish or crayfish with larger chelae are often more successful in shelter competition. Due to selective fish predation on small crayfish, young of the year crayfish may be absent from a site even if adults are present (Fortino and Creed 2007). This age structure occurs when adults migrate from a source area with lower predation pressure. Larger crayfish are often located in deeper water than small crayfish. This strategy is important in predator avoidance because it is beneficial for small crayfish to avoid predatory fish by remaining in very shallow water and for large crayfish to avoid terrestrial predators by remaining in deeper water. Unlike terrestrial predators, fish are often gape limited, so they may be unable to consume large crayfish unless the crayfish have recently moulted (Power 1987, Schlosser and Ebel 1989). Large crayfish are also less likely than small crayfish to change their behavior when predatory fish are present (Stein et al. 1977, Keller and Moore 2000).

Crayfish in the lower Flint River Basin

Orconectes palmeri was first found in the Flint River in 2001 (C Skelton, personal communication, 2006). The historic range of *O. palmeri* includes the lower Mississippi valley in Kentucky, Tennessee, Louisiana, and Mississippi, and eastern Missouri and Arkansas (Hobbs 1989). The crayfish found in the Flint River is the subspecies *O. palmeri creolanus*, which has historically been located in the Pearl and Pascagoula Rivers and the Lake Pontchartrain drainage basin in Mississippi and Louisiana (Penn 1957). Previous to this study, this species had only been reported at one location in the Flint River, at the bridge crossing below the confluence of the Flint River and Coolewahee Creek. Currently, the vector of its introduction is unknown. *O.*

palmeri is a tertiary burrower, spending most of its time in open water habitat (Holdich, 2002). This species has only been recorded in lotic systems (Penn 1952).

Procambarus spiculifer has historically been common throughout the lower Flint River and its tributaries. Its range extends from eastern Alabama through Georgia and northern Florida, and it is native to the Flint River basin (Hobbs 1981). This species is also a tertiary burrower that is restricted to lotic systems (Hobbs 1981). During our preliminary sampling we found both species utilizing cobble and boulder particles for shelter. *P. spiculifer* was also associated with coarse wood.

Objectives

This study seeks to answer the following questions:

1. What are the distributions of the native and invasive crayfish species in the lower Flint River basin and do these species co-occur?
2. Are physicochemical variables such as temperature and/or particle size important in structuring the distribution of crayfish species in the lower Flint River basin?
3. Are predatory fish disproportionately feeding on either native or invasive crayfish species and does fish predation affect crayfish distribution?

Answers to these questions provide information about the extent of the *O. palmeri* invasion, the future invasion potential of this species, and the interspecies interactions occurring between *O. palmeri* and species native to the Flint River. In addition, examining the traits and conditions that contribute to invasion success allows for better forecasting of impacts on ecological systems that are vulnerable to invasion and those species that are likely to be invaders. Examining invasions can provide insight into ecological and evolutionary processes because invasions provide examples of species succeeding in environments in which they did not evolve.

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CHAPTER 2

PHYSICOCHEMICAL HABITAT ASSOCIATION IN A NATIVE AND A NON-NATIVE CRAYFISH: IMPLICATIONS FOR INVASION SUCCESS[1]

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Abstract

Native species are sometimes competitively inferior to invasive species despite their history of adaptation to the local environment. Here we examine how the physicochemical environment contributes to and limits the invasion success of *Orconectes palmeri*, a non-native crayfish in the lower Flint River basin, Georgia, USA. We examined the distribution of a native crayfish, *Procambarus spiculifer*, along with the distribution of *O. palmeri* within the lower Flint River basin, and correlated relative abundance of both species with physicochemical variables. Crayfish abundance was estimated by surveys of 26 sites in the Flint River and its adjacent tributaries. Within the Flint River, the relative abundance of the two species varied from upstream to downstream. *O. palmeri* was found almost exclusively at upstream sites, while *P. spiculifer* was more abundant downstream. *O. palmeri* collected downstream were larger than those collected at upstream sites, suggesting little recruitment of *O. palmeri* downstream. Within tributaries, only native crayfish were observed. The abundance of *O. palmeri* was correlated with warmer water temperatures and with the availability of large substrata which act as refugia from predation. The abundance of *P. spiculifer* was correlated with cooler water temperatures, which were maintained by inputs from ground water, and with coarse wood. Laboratory studies demonstrated that *O. palmeri* selected significantly warmer temperatures than *P. spiculifer*, suggesting that temperatures in the upstream area favor *O. palmeri* fitness. It is possible that *O. palmeri* is by chance better suited than *P. spiculifer* to the temperature regime in this portion of the river, despite *P. spiculifer's* advantage of adaptation to the local environment. Alternatively, evidence suggests that temperatures have increased in recent years due to human withdrawals from the aquifer and the installation of upstream dams, creating a thermal regime that *P. spiculifer* has not historically encountered. Our findings indicate that the maintenance of

groundwater inputs to the river and tributaries is crucial to protect populations of native *P. spiculifer* and prevent further invasion by *O. palmeri*. In addition, the contribution of natural riparian buffers in providing in-stream wood and shading may be important in maintaining *P. spiculifer* habitat.

Introduction

Invasive species are a primary threat to global biodiversity and ecosystem processes (Elton 1958; Coblentz 1990; Mooney et al. 2005). While some species naturally disperse and successfully colonize new habitats, the impacts of invasive species are currently more prevalent due to increased rates of transport of non-native species and habitat alterations by human activities (Meyerson and Mooney 2007; Rahel 2007). It is important to understand the traits and conditions that allow a species to be successful outside of its native range in order to predict and inhibit future invasions. The ability of some non-native species to outcompete natives suggests that native species are not necessarily optimally adapted to the conditions within their historical ranges due to the imperfect nature of natural selection (Jacob 1977; Mack 2003; Sax et al. 2007). Long-term data are needed to determine if native species can sustain their populations and even dominate during the highly variable conditions that characterize their habitat. In well-documented, short-term studies, human-induced habitat alteration has been attributed to the success of many non-native species (Moyle and Light 1996; Gido and Brown 1999; Light 2003). In these cases, native species may be the superior competitors in their natural environment, but habitat alteration has created conditions that mimic other environments and therefore favor non-native species. These explanations for the success of invasive species are not necessarily mutually exclusive; however, whether invasive species are typically the drivers or passengers of ecological change has recently been debated (MacDougall and Turkington 2005; Light and Marchetti 2007). We examined the distribution of a native and a non-native crayfish in the lower Flint River basin of southwest Georgia, and determined how habitat characteristics contribute to this invasion.

Orconectes palmeri is native to the lower Mississippi River valley, and was recently found in the Flint River. The subspecies of *O. palmeri* found in the Flint River basin, *Orconectes palmeri creolanus*, is native to MS and LA, USA, and has historically been found in the Pearl and Pascagoula Rivers and the Lake Ponchartrain drainage basin (Penn 1957). This species was first reported in the Flint River in 2001 at one location below the confluence of the Flint River and Coolewahee Creek (C. Skelton, personal communication, 2006). It was also collected in the Flint River below the confluence with Kinchafoonee and Muckalee Creeks in 2003 (G. Stanton, personal communication, 2008). Aside from these two records, the extent of this invasion was unknown. *O. palmeri* was not observed during historical surveys of crayfish in the lower Flint River basin (Hobbs 1981); however, the vector of its introduction is unclear. *O. palmeri* is classified as a tertiary burrower, meaning that it spends most of its time in open water, and has been recorded only in lotic systems (Penn 1952; Holdich 2002).

Procambarus spiculifer is native to GA, USA and has historically been found in the lower Flint River and adjacent tributaries (Hobbs 1981). This species is widespread throughout GA and its range extends from eastern AL through GA and northern FL. *P. spiculifer* is also a tertiary burrower which is restricted to lotic systems (Hobbs 1981). In addition to *P. spiculifer*, a number of other crayfish species have been historically found in the tributaries to the lower Flint River. Among them are *Cambarus diogenes, Procambarus gibbus*, and *Procambarus paeninsulanus* (Hobbs 1981).

In this study, we assessed the distributions of native and non-native crayfishes in the lower Flint River and adjacent tributaries. We correlated the relative abundance of each species with biologically relevant physicochemical factors such as temperature and sizes of substrata. The installation of a series of upstream dams, increased urbanization in the upstream area of the Flint

River, and increased groundwater withdrawals in recent years may have altered some of the physicochemical characteristics of these streams. Primarily, we expect that water temperatures in this region are warmer than they were historically. To establish how increased temperatures may affect *O. palmeri* and *P. spiculifer* populations, we assessed temperature preference in a laboratory experiment. By determining which physicochemical factors control the distributions of these species, we can determine the impact of human induced habitat alteration on the success of this invasion.

Materials and Methods

Field sampling was conducted within the lower Flint River basin to determine the distribution of crayfish species and the correlation between distribution and physicochemical variables. Sixteen sites were located within the mainstem of the lower Flint River, and ten sites were located within adjacent tributaries (Figure 2.1). Sites were distributed throughout the Dougherty Plain physiographic district, a region in the Coastal Plain characterized by karst topography and extensive subsurface aquifers. Limestone geology and substantial spring water inputs make the physicochemical characteristics of streams in this region distinct. Baseflows are supported by discharge from the upper Floridan Aquifer through a combination of spring discharge and diffuse groundwater inputs. Each site was sampled along a 50 m transect on one side of the stream. Crayfish are not evenly distributed the lower Flint River basin because they appear to prefer specific microhabitats that serve as refugia from fish predators. Sampling 50 m of channel allowed us to find crayfish at most sites and helped ensure similar sampling effort among sites. Sites were located in areas containing loose boulders, cobbles or pebbles which could be sampled by snorkeling. Preliminary sampling indicated that crayfish were abundant in this type of substrata and rare in other types of habitat including macrophytes, coarse wood, sand, silt and bedrock.

Sites were surveyed from June to September of 2007 to determine the presence and relative abundance of crayfish species. At each site, two experienced observers each sampled 25 m of the 50 m transect for 1 h. We detected crayfish by turning over boulders, cobbles and pebbles while snorkeling and wading within 2 m of the transect line. Observed crayfish were collected in a hand net. We were unable to capture roughly 35% of crayfish observed, but able to visually identify the species. In our assessments of relative abundance we used counts of crayfish species that were identified but not captured along with counts of crayfish species that were captured. Captured crayfish were transferred to a bucket or mesh bag for the duration of the sampling and subsequently identified to species, and measured for carapace length, chelae length and chelae width using vernier calipers as in Stein *et al.* (1977). Sex of each captured crayfish was also recorded.

For a number of analyses, we grouped the sites upstream in the Flint River, the sites downstream in the Flint River, and the sites in the adjacent tributaries. These groups were established based on observed shifts in the relative abundance of crayfish species. The upstream location included all sites in the mainstem above Coolewahee Creek, and the downstream location included all sites in the mainstem below Coolewahee Creek (Figure 2.1). Tributary sites included all sites located in Kinchafoonee Creek, Muckalee Creek, Coolewahee Creek, Chickasawhatchee Creek, Ichawaynochaway Creek, and Spring Creek. T-tests were used to compare carapace length between *O. palmeri* and *P. spiculifer* and between conspecifics found in different locations. Length and width of chelae were compared between species using ANCOVA to control for differences in carapace length. In addition, we used chi-square tests to examine differences in the proportion of males to females between *O. palmeri* and *P. spiculifer* and also between conspecifics found in different locations.

To characterize physicochemical variables at each site we used a stratified pebble count method. Type of substrata (clay, silt, sand, gravel, pebble, cobble, or boulder) and the presence of macrophytes or coarse wood (wood that was at least 1 m in length and 10 cm in diameter) were recorded at 0.5 m intervals along the 50 m transect. In addition, 13 Hobo Data Loggers (Onset Computer Corporation, Bourne MA) were placed in the Flint River and adjacent tributaries in July and August of 2007 to record temperature. Loggers were located at the following sites: 1, 3, 5, 7, 9, 12, 16, 17, 18, 19, 21 and 25. In addition, one logger was located on Ichawaynochaway Creek between sites 23 and 24. Loggers recorded temperature every hour and remained in the water through December of 2007. We calculated the mean, maximum and minimum temperatures at each location for four 30 day periods between August and February of 2007. Due to data loss from missing or malfunctioning loggers, data was only available for 10 out of the 13 temperature loggers during each time period. We compared mean, maximum, and minimum temperatures among upstream, downstream, and tributary locations using the GLM procedure and blocking to control for time period. Tukey's Studentized Range (HSD) test was used to determine which locations were significantly different in temperature. High flow and low flow periods were analyzed separately.

Triplicate water samples were collected at the location of each temperature logger in September-October of 2007 and again in November-December of 2007. Three samples were obtained from each location. Samples were placed on ice and analyzed using a Mettler DL 12 titrator within 24 h to determine total alkalinity and pH. Within each site, the mean of the three samples was used to estimate total alkalinity and pH. Alkalinity, pH, and temperature were compared to one another using regression analysis.

We explored how physicochemical characteristics varied among site locations using a principal components analysis (PCA) (Table 1.1). Temperature and total alkalinity collection sites were designed to obtain information about the overall trends of these characteristics within the lower Flint River basin and did not overlap with each crayfish sampling site. Crayfish sampling sites were assigned temperature and alkalinity values from nearby physicochemical data collection sites, most of which were located within 3 km. Although temperature and total alkalinity data were not collected at each site, both of these characteristics showed clear trends from upstream to downstream in the Flint River and tributaries. Estimated values, therefore, accurately reflect overall trends in these variables. Upstream, downstream, and tributary sites were labeled in the PCA, and these groups were examined to determine which physicochemical variables may be important in determining the relative abundance of crayfish species. To further examine which variables were significant in predicting the abundance of *O. palmeri* and *P. spiculifer*, we compared important variables to the abundance of each species in a correlation matrix.

Temperature preference of each species was assessed using a horizontal temperature gradient. The temperature gradient was constructed from a 25.4 cm diameter PVC pipe with a 15 cm wide strip removed from the top (Figure 2.2) design was modified from Peck (1985) and Kivivuori (1994). The gradient was divided into eight 28 cm sections which were separated by polycarbonate sheets with 6.5 cm by 5.5 cm portions removed to allow crayfish to move between sections. Submersible aquarium heaters were set at different temperatures to create the thermal gradient from 32 ± 1 °C to 21 ± 1 °C. Sections were between 1 and 2 °C different from adjacent sections. All sections contained heaters except for the coolest section of the gradient which contained an aquarium chiller. Temperatures in the gradient reflected temperatures found in the lower Flint River basin. Average daily temperature in the mainstem and tributaries ranged from 20 to 32 °C in the late summer and early fall of 2007. The bottom of the PVC pipe was covered in gravel and each section contained a piece of clear acrylic pipe for shelter. Acrylic pipes were partially covered in opaque tape so that crayfish used them for shelter and could be located visually. Water depth was roughly 20 cm. Water in each section of the temperature gradient was circulated with an airstone to prevent thermal stratification and to maintain high levels of dissolved oxygen.

O. palmeri and *P. spiculifer* were collected using hand nets from the Flint River in September and October of 2008. All crayfish were collected from the same 30 m stretch which was located in the Flint River above its confluence with Ichawaynochaway Creek. In 2007, average temperatures at this site ranged from approximately 20 to 30 °C in September and October. We collected 16 crayfish, 5 *O. palmeri* females, 4 *O. palmeri* males, 3 *P. spiculifer* females, and 4 *P. spiculifer* males. Carapace length ranged from 26 to 40 mm for *O. palmeri* and 27 to 34 mm for *P. spiculifer*. Average carapace length (± SE) was 33.3 ± 1.4 for *O. palmeri* and 29.3 ± 0.9 for *P. spiculifer*. According to 2007 visual surveys of the lower Flint River basin, *O. palmeri* are larger in the field than *P. spiculifer*. The crayfish sizes used in our study are similar to the average sizes of adult crayfish found in the field. Crayfish were stored in separate containers which contained gravel and a clear acrylic pipe for shelter. Water temperature in these containers was maintained at approximately 21 °C. Two to three times per week, crayfish were fed pellets made from algae, vegetables, and fish.

Initially, we conducted two thermal selection trials for each crayfish, one at night and one the next morning. Crayfish were placed in the thermal gradient during dark hours, and left for 30 min to adjust to their surroundings. Most crayfish stopped moving rapidly between sections and no longer attempted climbing up the sides of the sections after 30 min. After this period, we recorded the section and water temperature where the crayfish was located every 5 min for 1 h. Temperature was measured by digital thermometers, which were located in the center of each section near the gravel. Thermometers were accurate within 0.5 °C. Crayfish were observed using indirect light which was supplied by a lamp with a red light bulb and a red, light emitting diode headlamp, which was used to search for crayfish that were not easily located with the indirect light. The use of red light did not appear to alter crayfish behavior. We used the average temperature that each crayfish selected during the trial for our analyses. The first eight crayfish were left in the thermal gradient overnight and observed again for 1 h the next morning. We found that temperature selection of individual crayfish was similar (< 1 °C different) between night and morning selection trials; however, crayfish spent less time moving between sections in morning trials. In mid October, both species became more active. Crayfish did not acclimate within 30 minutes and attempted to escape from the gradient more often. For this reason, we chose to use only morning trials for most of our analyses. We analyzed night trials for three individuals as morning trials were unavailable because crayfish escaped from the gradient. We compared temperature selection between species and sexes using t-tests.

Results

P. spiculifer and *O. palmeri* were the most common crayfish species found during sampling. Additional native species, including *C. diogenes*, *P. gibbus*, and *P. paeninsulanus*, were found only at site 19 in Coolewahee Creek (Table 2.1). The abundance of crayfish was highly variable among sites. We were unable to detect crayfish at two sites within the Flint River, while at other sites in the Flint we observed as many as 49 individuals per 50 m survey. Crayfish were rare in the center portion of the study area and generally more abundant towards the upstream and downstream (Table 2.2). Abundance was also highly variable within the tributaries where we found from 13 to 53 individuals per site (Table 2.2).

Within the mainstem of the Flint, the relative abundance of *O. palmeri* and *P. spiculifer* varied from upstream to downstream (Figure 2.3). With the exception of four *P. spiculifer* individuals, *O. palmeri* was the sole species found at upstream sites. Downstream from Coolewahee Creek, *P. spiculifer* were more abundant than *O. palmeri* at six out of the eight sites where crayfish were present. *O. palmeri* were not detected in tributaries, even at sites close to the confluence of the tributaries and the Flint River (Figure 2.4). *P. spiculifer* were present at all tributary sites except for the site located within Coolewahee Creek.

On average, *O. palmeri* were larger than *P. spiculifer* (Figure 2.5). The carapaces of *O. palmeri* were significantly longer than those of *P. spiculifer* ($P < 0.0001$). In addition, the chelae of *O. palmeri* were significantly longer ($P < 0.0001$) and wider ($P < 0.0001$) than those of *P. spiculifer* even when carapace length was controlled for using ANCOVA. *O. palmeri* length varied from upstream to downstream. *O. palmeri* were significantly longer downstream from Coolewahee Creek ($P < 0.0001$) (Figure 2.6). *O. palmeri* collected downstream ranged from 28 mm to 42 mm in carapace length, while upstream *O. palmeri* ranged from 15 to 36 mm in carapace length. Sixty-three percent of upstream *O. palmeri* were smaller than 28 mm in carapace length, while all downstream *O. palmeri* were larger than 28 mm. *P. spiculifer* size could not be compared between upstream and downstream locations due to the small number of *P. spiculifer* found upstream. *P. spiculifer* carapace length within the Flint River was not significantly different than *P. spiculifer* carapace length within the tributaries (*P* = 0.5996) (Figure 2.7).

Both *O. palmeri* and *P. spiculifer* populations had similar numbers of males and females (*P* = 0.1455). Overall, 45% of *O. palmeri* and 54% of *P. spiculifer* collected were females. The percentage of *O. palmeri* that were female did not differ significantly between upstream and downstream sites within the Flint River, with 44% and 47% of captured crayfish respectively (*P* = 0.7364). There were more female *P. spiculifer* present in tributaries, 58%, than in the mainstem of the Flint, 46%, although this difference was not statistically significant (*P* = 0.1102).

PCA revealed clusters of upstream, downstream and tributary sites based on physicochemical variables (Figure 2.8). Axis 1 explained 25.4% of the variance and was negatively associated with temperature and medium particle sizes including gravel, pebbles and cobbles. Axis 1 was also positively associated with sand. Axis 2 explained 21.7% of the variance and was negatively associated with boulders, silt, clay and total alkalinity. Axis 2 was positively associated with bedrock and coarse wood. All physicochemical variables associated with axis 1 and axis 2 were compared among upstream, downstream and tributary sites using ANOVA and compared between mainstem and tributary sites using t-tests. Differences were not statistically significant (*P* > 0.1) unless otherwise specified.

Upstream sites and had relatively high percentages of medium particles including gravel, pebbles, cobbles and boulders. Sites 1 and 6 had a stronger negative association with axis 2 than other upstream sites. This association is attributed to the higher percentage of substrata made up by boulders and the lower percentage of substrata made up by gravel, pebbles, and cobbles at these two sites. Downstream sites in the mainstem typically had high percentages of medium substrata and of sand. Sites 15 and 16 were atypical of other downstream sites as they contained higher percentages of small particles. Site 15 had a higher percentage of clay and silt than any other site, and site 16 was almost exclusively made up of sand. These two sites were the closest to Lake Seminole and were likely influenced by the backwater effect of the reservoir. Site 16 was eliminated from ANOVA and t-test analyses comparing substrata among locations as the substrata at this site was atypical and no crayfish were detected there. Compared to mainstem sites, tributary sites had a significantly higher percentage of substrata made up of sand ($P = 0.0943$) and contained more coarse wood ($P < 0.0001$). Sites 22 through 25 in Ichawaynochaway and Chickasawhatchee Creeks also contained greater than 30% bedrock, which was less abundant at all other sites.

Temperatures were warmest in the upstream portion of the mainstem followed by the downstream portion of the mainstem and then the tributaries (Figures 2.9 and 2.10). During low flow periods, mean temperatures were significantly different among all three locations ($P < 0.0001$). Maximum and minimum temperatures differed between the mainstem and tributaries ($P < 0.0001$), but were not significantly different between upstream and downstream locations in the mainstem. During the period of high flow in January and February, temperatures were warmest downstream in the mainstem, followed by upstream in the mainstem and then the tributaries. Mean and maximum temperatures were not significantly different among upstream, downstream and tributary locations. Minimum temperatures were significantly lower in the tributaries ($P = 0.0001$).

Total alkalinity also varied among locations (Figure 2.11). Alkalinity increased from upstream to downstream within the mainstem of the Flint in both September-October and November-December of 2007. Alkalinity was more variable in the tributaries with values ranging from 13.1 to 107.4 ppm, and pH was positively correlated with total alkalinity during both sampling periods ($P = 0.0011$, $R^2 = 0.80$; $P < 0.0001$, $R^2 = 0.89$). Total alkalinity and temperature were negatively correlated throughout the Flint and tributaries. This trend was not apparent when the Flint and tributaries were analyzed together using temperature and alkalinity data from the first sampling period ($P = 0.2843$, $R^2 = 0.11$), but was apparent when data from the mainstem and tributaries were analyzed separately ($P = 0.1168$, $R^2 = 0.42$; $P = 0.3350$, $R^2 = 0.82$). Water was warmer in the mainstem of the Flint than in the tributaries even when total alkalinity values were similar.

There were a number of statistically significant correlations between *O. palmeri* abundance and physicochemical variables and *P. spiculifer* abundance and physicochemical variables. Correlations that were statistically significant at $\alpha = 0.1$ are mentioned below. Warmer temperatures and percent boulder were positively correlated with *O. palmeri* abundance ($P = 0.0068$, $R^2 = 0.29$; $P = 0.0339$, $R^2 = 0.19$). Coarse wood was negatively correlated with *O. palmeri* abundance ($P = 0.0452$, $R^2 = 0.17$) as was percent sand ($P = 0.0984$, $R^2 = 0.12$). *P. spiculifer* abundance was positively correlated with percent bedrock ($P = 0.0530$, $R^2 = 0.16$) and coarse wood ($P = 0.0002$, $R^2 = 0.47$). Warmer temperatures ($P = 0.0401$, $R^2 = 0.18$) and alkalinity ($P = 0.0693$, $R^2 = 0.14$) were negatively correlated with *P. spiculifer* abundance. *P.*

spiculifer abundance and *O. palmeri* abundance were negatively correlated with one another (P = 0.5300, R^2 = 0.16).

O. palmeri selected significantly warmer temperatures that *P. spiculifer* (P = 0.0043). *O. palmeri* selected an average temperature (± SE) of 26.2 ± 0.7 °C, while *P. spiculifer* selected an average temperature of 23.4 ± 0.3 °C (Figure 2.12). *P. spiculifer* males and females selected similar temperatures (P = 0.7237), while *O. palmeri* males selected warmer temperatures than *O. palmeri* females (P = 0.0893) (Figure 2.13). The date of the trial did not have an effect on temperature selected when both species were analyzed together (P = 0.2976, R^2 = 0.0772); however, there was a negative relationship between date and temperature selected by *P. spiculifer* (P = 0.0553, R^2 = 0.5532) and by *O. palmeri* (P = 0.0912, R^2 = 0.3536) (Figure 2.14). Carapace length had no significant effect on temperature selection *by O. palmeri* (P = 0.2697, R^2 = 0.1702) or by *P. spiculifer* (P = 0.5402, R^2 = 0.0795) (Figure 2.15).

Discussion

The observed trends in species distribution suggest that upstream, downstream and tributary habitat characteristics cause shifts in the relative abundance of *O. palmeri* and *P. spiculifer*. These distributions are more complex than can be explained by an invasion that is in process and has not fully expanded to all suitable locations. *O. palmeri* is well established above and below many of the tributaries and crayfish actively disperse by moving upstream, so it is unlikely that *O. palmeri* would remain undetectable in the tributaries if they contained suitable conditions. *P. gibbus*, *P. paeninsulanus* and *C. diogenes* were only located at a single site; however, previous research indicates that these species were historically found in small streams (Hobbs 1981), so we would not expect to find them at the other sites we surveyed. These species may be associated with the high percentage of groundwater, the low abundance of fish predators, or the flow characteristics associated with this small stream site.

Size differences between *O. palmeri* captured downstream and those captured upstream suggest that conditions are also less suitable for this species downstream in the Flint mainstem. A study of *O. palmeri palmeri* from western Tennessee found that second year specimens ranged in size from 26 to 35 mm (Payne and Price 1981). In this study, *O. palmeri* collected in the downstream area were 28 mm or larger, suggesting that these individuals were older than 1 year, and that young of the year (YOY) were undetectable in this area. In contrast YOY *O. palmeri* were abundant upstream, making up the largest size class. Very small *O. palmeri* were observed in the downstream area in early June, 2007 during preliminary sampling, but by the time formal sampling began in mid July, YOY *O. palmeri* were not detected downstream in the mainstem. This trend suggests that most YOY *O. palmeri* did not survive in the downstream area, but that larger *O. palmeri* were able to persist. One explanation for this observation is that small *O. palmeri* may be more vulnerable to predation than both *P. spiculifer* and large *O. palmeri*.

The PCA and correlation matrix revealed physicochemical variables that likely contribute to the relative abundance of one or both species. Some physicochemical variables are likely to shift within the lower Flint River basin along with the relative abundance of *O. palmeri* and *P. spiculifer* even if these variables were not responsible for differences crayfish abundance; therefore, we have considered the ecological implications of the significant correlations between physicochemical variables and crayfish abundance to evaluate their importance. *O. palmeri* abundance was positively correlated with percentage of boulder substrata. We would expect this result as shelter is important for predator avoidance, and we have found most *O. palmeri* under boulders, cobbles or pebbles. There was also a negative association between *O. palmeri* abundance and the percentage of sand and coarse wood at a site. There may be less shelter available in sandy areas and/or poor conditions for constructing burrows, or it may be that sandy substrate is simply more common in the tributaries and downstream in the Flint due to deeper incision of the channels in the aquifer bearing strata. Instream wood is also likely more common in the tributaries due to the narrower width of the channel. Coarse wood may be associated with fish predators that negatively affect *O. palmeri* populations. *P. spiculifer* abundance was positively associated with percentage of bedrock and coarse wood. Although we found *P. spiculifer* most abundantly under boulders, cobbles, and pebbles, we also found them under and around coarse wood. We did not find any *O. palmeri* associated with coarse wood. Coarse wood was generally more abundant at tributary sites, so it may be that wood is an important component of *P. spiculifer* habitat, or that it is simply more common in the tributaries. Highly fractured, exposed bedrock may provide refugia from predation. In addition, areas with exposed bedrock may be areas of aquifer seepage which would provide thermal refugia as well. Exposed bedrock may also be indicative of shallow riffle areas where *P. spiculifer* is more abundant due to other factors.

The abundance of both species was strongly correlated with temperature. *O. palmeri* were correlated with warmer temperatures and *P. spiculifer* were correlated with colder temperatures. Temperatures above 33.3°C and below 8.1°C are typically lethal for *P. spiculifer* when they are exposed for 24 hours (Caine 1978). To our knowledge, upper and lower temperature limits have not been determined for *Orconectes palmeri*. During the late summer, temperature loggers recorded maximum temperatures above 33.3°C for most locations within the Flint River. Crayfish may be able to survive periods of warm temperatures, if they are short in duration. In most locations, lethal temperatures lasted for one to two hours; however, at sites 3 and 4, lethal temperatures lasted periodically for up to six hours. No *P. spiculifer* were found at sites 3 and 4. Groundwater in this region varies between 19 and 21°C annually, so during the warm months, the percent of groundwater at a site is negatively correlated with water temperature. Upstream sites contain a lower percentage of groundwater than downstream sites. Diffuse groundwater seepage may create microrefugia in areas near the sediments with cooler water inflow and offer protection for brief intervals when water column temperatures exceed 33.3°C. The aquifer is thinner upstream and contributes less groundwater to baseflow, so a decline in refugia in this location may explain the reduction in *P. spiculifer* abundance. Upstream temperatures may provide *O. palmeri* with a competitive advantage over *P. spiculifer*, or these temperatures may be lethal to *P. spiculifer* in the summer. Tributaries remained cooler than sites within the mainstem, even if they had similar alkalinity, indicating a similar percentage of water from the aquifer. We attribute this difference to increased riparian shading in the tributaries. Previous studies have found riparian shading to be important in maintaining cooler water temperatures (Poole and Berman 2001; Dent et al. 2008). We expect that temperature plays an important role in this invasion and is responsible, at least in part, for the shift in the relative abundance of crayfish species in the lower Flint River.

The temperature selection experiment revealed that *O. palmeri* select warmer temperatures than *P. spiculifer*. Our results support previous research on temperature selection by *P. spiculifer*. Taylor (1984) found that *P. spiculifer* selected an average temperature of 23.4 ± 0.4 °C, which is very similar to our findings. *O. palmeri* selected a wider range of temperatures than *P. spiculifer*, suggesting that it may be a successful invader because its physicochemical requirements are relatively plastic. Differences in temperature selection between male and female *O. palmeri* suggest the importance of using both sexes when determining the temperature preference of a species. These differences may be reflected in microhabitat selection; however, we do not think that they reflect differences in distribution between males and females. The sex ratio of *O. palmeri* upstream is not significantly different from that of *O. palmeri* downstream. In addition, the trend between date of the experiment and temperature selected is supported by previous research. Crayfish temperature selection has been shown to vary by season, with crayfish selecting temperatures close to seasonal water temperatures in the habitat to which they are adapted (Taylor 1984). Temperature selection, in this case, works as an error detection system. As temperatures in laboratory crayfish tanks were kept constant throughout the study, it is probable that crayfish were detecting the change in seasons based on the natural light to which they were exposed. We expect that both species select warmer temperatures in the summer and cooler temperatures in the winter.

As both *O. palmeri* and *P. spiculifer* were collected from the same location in the Flint River and maintained at the same temperature in the laboratory, we attribute differences in temperature selection to differences in the thermal optima of each species. Taylor (1990) found that temperatures near the final temperature preferendum of *P. spiculifer* produce maximum growth and temperatures above and below the thermal preferendum produce slower growth. If this relationship is similar for *O. palmeri*, warmer temperatures would be more favorable for *O. palmeri* growth and cooler temperatures would be more favorable for *P. spiculifer* growth. Rapid growth is important for crayfish fitness, because larger crayfish are more likely to avoid fish predation and displace smaller crayfish from shelter (Garvey and Stein 1993; Hill et al. 1993; Mather and Stein 1993; Kuhlmann et al. 2008). Average fall temperatures in the mainstem are close to the thermal preferendum of *O. palmeri* and those in the tributaries are close to the thermal preferendum of *P. spiculifer* (Figure 2.10). Warmer temperatures in the upstream portion of the Flint River, therefore, are likely to associates with increased *O. palmeri* fitness, while cooler temperatures in the tributaries are likely to be associates with increased *P. spiculifer* fitness. We hypothesize that *O. palmeri* is undetectable in the tributaries because individuals are unlikely to actively disperse into the cooler water. Although we found many *P. spiculifer* in the downstream portion of the Flint River, the temperature in this location is closer to the thermal optimum of *O. palmeri* for much of the fall. These data suggest that *P. spiculifer* is able to compete with *O. palmeri* in this area, possibly through superior predator avoidance abilities, despite unfavorable temperatures.

Human induced changes to the temperature regime may have created warmer conditions in the upper Flint River that favor *O. palmeri* over *P. spiculifer*. Records indicate that *P. spiculifer* was historically located in the upstream portion of the lower Flint River (Hobbs 1981); however, it is unclear how abundant this species was in the past. Hicks and Golladay (2006) found that mean daily streamflow has declined in two of the tributaries to the lower Flint River since a dramatic increase in irrigation development in 1975. Although this region has recently experienced seasonal drought, differences in annual rainfall between pre- and post-irrigation periods were not significant. Altered stream flow is attributed to the increase in pumping of aquifer water for agriculture. While the impact of agricultural withdrawals has not been examined in the mainstem of the Flint, reductions in total groundwater discharge to the lower Flint River are observed during the summer months and are exaggerated during periods of drought (Opsahl et al. 2007).

Upstream reservoirs in the Flint River also increase temperatures. Temperatures above Lake Chehaw reservoir, located at the confluence of the Flint River and Kinchafoonee and Muckalee Creeks, were cooler on average than temperatures immediately below the reservoir.

The dam at Lake Chehaw is a run of the river dam used for electricity, creating a shallow reservoir with a low water residence time. The dam slows flow and creates a wider channel, so that there is increased surface area for heating from solar radiation and less shading from the riparian area. The reservoir is unlikely to stratify due to low water residence times and shallow depth. We hypothesize that water temperature has increased, on average, in the lower Flint River due to reduced groundwater inputs and the installation of upstream dams.

Although it is probable that temperatures are warmer in the Flint River than they were historically, we are unable to conclusively establish that the water temperature was significantly warmer due to a lack of historical temperature records. If water temperature had not changed significantly, *O. palmeri* may have succeeded in reducing *P. spiculifer* populations in the margins of the native range of *P. spiculifer*. Fausch (2008) proposed that some invasions may be successful because native species are not optimally adapted to conditions in certain portions of their ranges, typically at the edges, and these areas may be more readily invaded by non-native species that by chance have more suitable characteristics for these conditions. Temperature selection data suggest that *P. spiculifer* are not as well adapted to the current temperature regime in the upstream portion of the lower Flint River. It is possible that *O. palmeri* were able to successfully invade this area because it is located in the margins of the native range of *P. spiculifer*.

Drought alters the temperature regime in rivers, typically by causing reductions in flow, which reduces the stream's assimilative capacity for heat and warms the water (Caissie 2006). While yearly rainfall is not significantly different than it was historically, seasonal differences have been observed. There is often more rainfall in the winter months and less rainfall in the summer months than there was historically (Hicks and Golladay 2006). This region has experienced drought during the summers of 2006, 2007, and 2008. In addition to the reduced assimilative capacity for heat, there is reduced discharge from springs in the lower Flint River during periods of drought (Opsahl et al. 2007). This reduction in groundwater inflow likely warms water temperatures further. In contrast, there is less runoff during periods of drought, so while droughts reduce the volume of water at any given site, low flows may increase the percentage of water from the aquifer. This increase in groundwater would produce cooler water temperatures. It is difficult to determine how groundwater inflows to the river will be affected by drought due to spatial differences in the aquifer and propagation of drought through the groundwater system (Peters et al. 2006). These factors may interact in complex ways to influence water temperature during drought; it is possible that low flows further contribute to warmer temperatures in the Flint River.

Conclusions

O. palmeri has successfully invaded the portion of the Flint River between Coolewahee Creek and Lake Blackshear, and native species such as *P. spiculifer* are rare or absent at sites that are occupied by *O. palmeri*. Tributaries have not been colonized and may act as a refuge for native crayfishes such as *P. spiculifer*, *P. gibbus*, *P. paeninsulanus* and *C. diogenes*. This research supports previous findings that small streams protect native biodiversity by acting as a refuge from non-native species (Meyer et al. 2007).

It is likely that the success of this invasion is linked to human induced alteration of the temperature regime in the Flint River. Aquifer discharge may be creating a thermal refuge for *P. spiculifer* in the lower portion of the Flint. Increasing human water use is decreasing aquifer discharge throughout the basin, representing a long-term threat to populations of *P spiculifer* in this area. Maintaining coarse wood abundance and shading through protecting riparian woodlands may also be important in protecting *P. spiculifer* populations. Due to differences in size between upstream and downstream *O. palmeri*, we also propose that fish predation, in conjunction with physicochemical factors may be responsible for limiting the spread of this invasive crayfish.

It is possible that *O. palmeri* has helped to maintain ecosystem function in the upstream portion of the Flint River as *P. spiculifer* populations may have declined, irrespective of the invasion by *O. palmeri*, due to warmer water temperatures. The effect *O. palmeri* is having on the ecosystem is currently unclear and should be investigated through future research in the feeding behavior and trophic interactions of this species. This invasion demonstrates the importance of maintaining the temperature regime in the lower Flint River basin. It is essential that groundwater inputs to the Flint River and adjacent tributaries are maintained in the future to protect native species such as *P. spiculifer* and prevent further invasion by *O. palmeri*.

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Table 2.1. List of physicochemical characteristics measured for PCA. Coarse wood, macrophytes, and characteristics of substrata were measured between June and September of 2007, subsequent to each visual survey. Average temperature was collected between August 18th and September 17th. Total alkalinity was collected between November 29th and December 3rd.

PHYSICOCHEMCIAL VARIABLES
% clay
% silt
% sand
% gravel
% pebble
% cobble
% boulder
relative abundance of coarse wood
relative abundance of macrophytes
average temperature
total alkalinity

Table 2.2. Results of visual surveys for crayfish relative abundance indicating the number of each species observed at each site. Upstream and downstream locations refer to sites in the Flint River above and below Coolewahee Creek.

Site	Location	Species	Number Observed
1	Upstream	*O. palmeri*	47
		P. spiculifer	2
2		*O. palmeri*	12
3		*O. palmeri*	20
4		*O. palmeri*	8
5		*O. palmeri*	19
6		*O. palmeri*	14
		P. spiculifer	3
7	Downstream		
8		*O. palmeri*	1
		P. spiculifer	5
9		*O. palmeri*	14
		P. spiculifer	1
10		*O. palmeri*	7
		P. spiculifer	11
11		*O. palmeri*	11
		P. spiculifer	5
12		*O. palmeri*	11
		P. spiculifer	17
13		*O. palmeri*	3
		P. spiculifer	21
14		*O. palmeri*	10
		P. spiculifer	36
15		*O. palmeri*	13
		P. spiculifer	17
16			
17	Tributaries	*P. spiculifer*	22
18		*P. spiculifer*	18
19		*C. diogenes*	5
		P. gibbus	122
		P. paeninsulanus	3
20		*P. spiculifer*	13
21		*P. spiculifer*	24
22		*P. spiculifer*	36
23		*P. spiculifer*	33
24		*P. spiculifer*	37
25		*P. spiculifer*	18
26		*P. spiculifer*	53

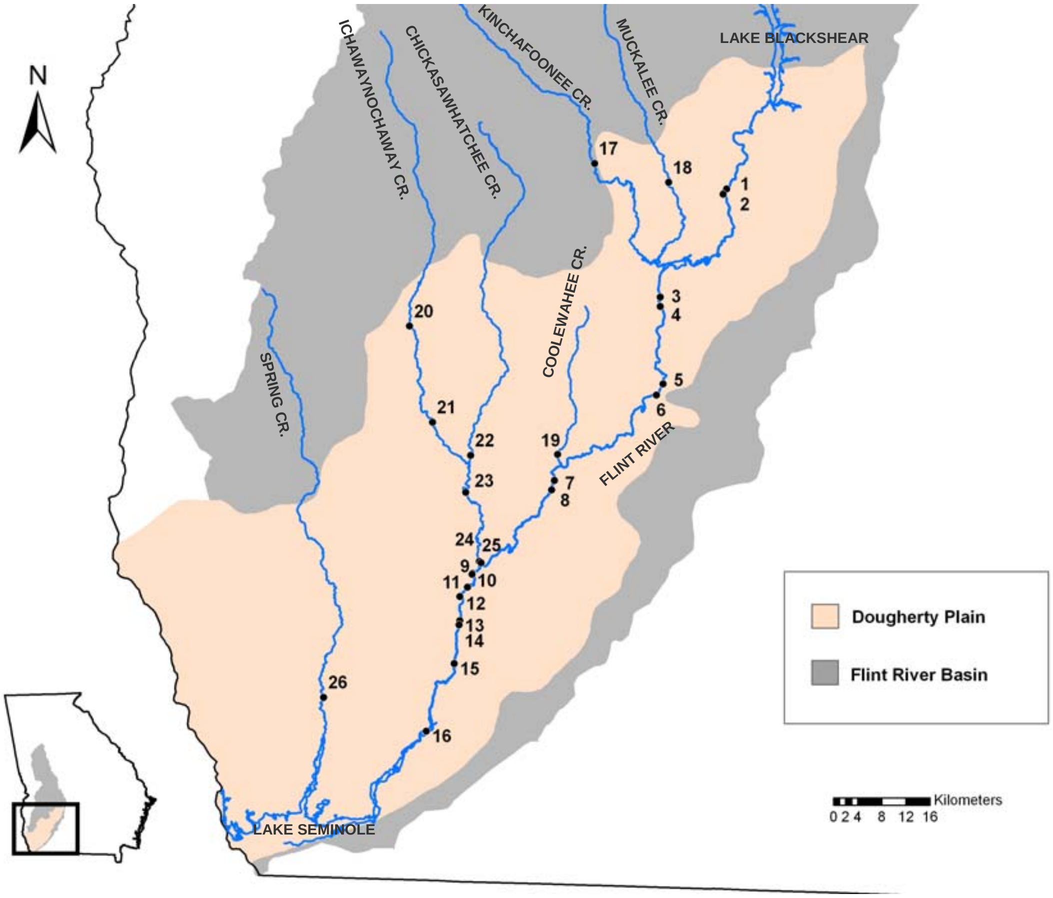

Figure 2.1. Study area including the lower Flint River and tributaries sampled. Site numbers and locations are indicated. Sites 1 through 16 are located within the mainstem of the Flint River, and sites 17 through 26 are located within surrounding tributaries.

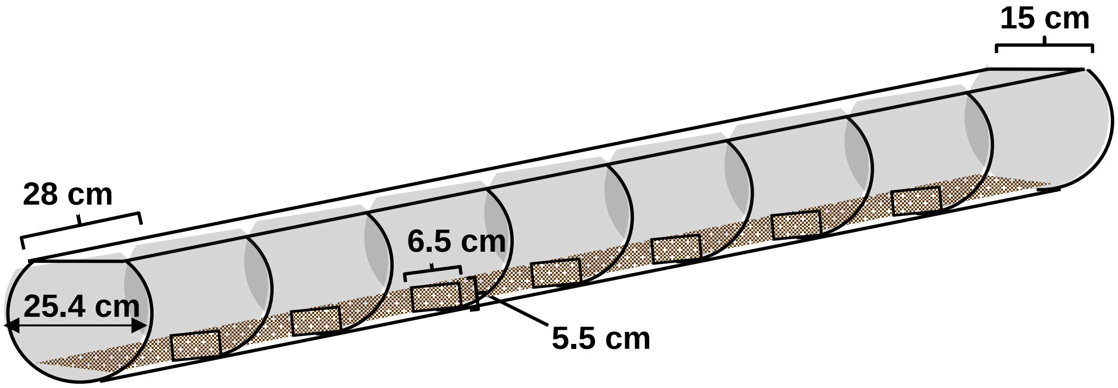

Figure 2.2. Depiction of the horizontal temperature gradient including dimensions.

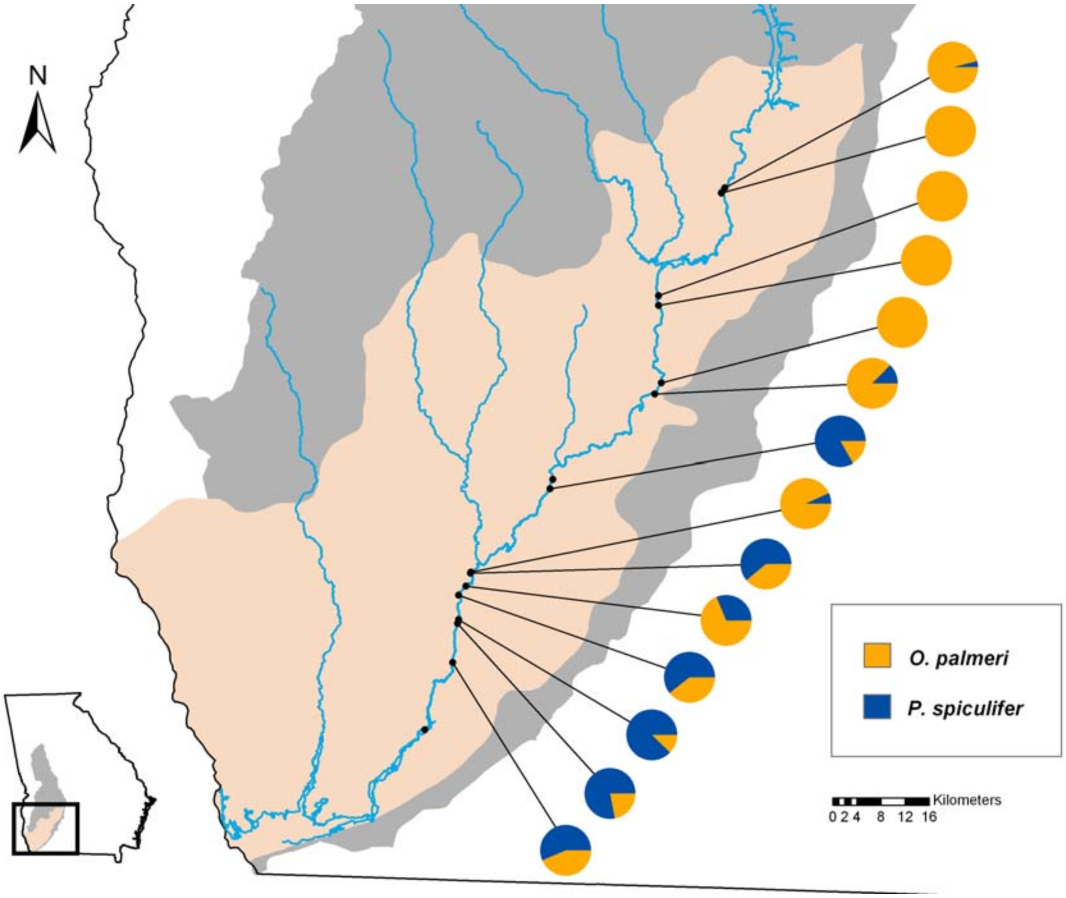

Figure 2.3. The relative abundance of *P. spiculifer* and *O. palmeri* at sites sampled in the mainstem of the Flint River. No crayfish were observed at the two sites that lack corresponding pie charts.

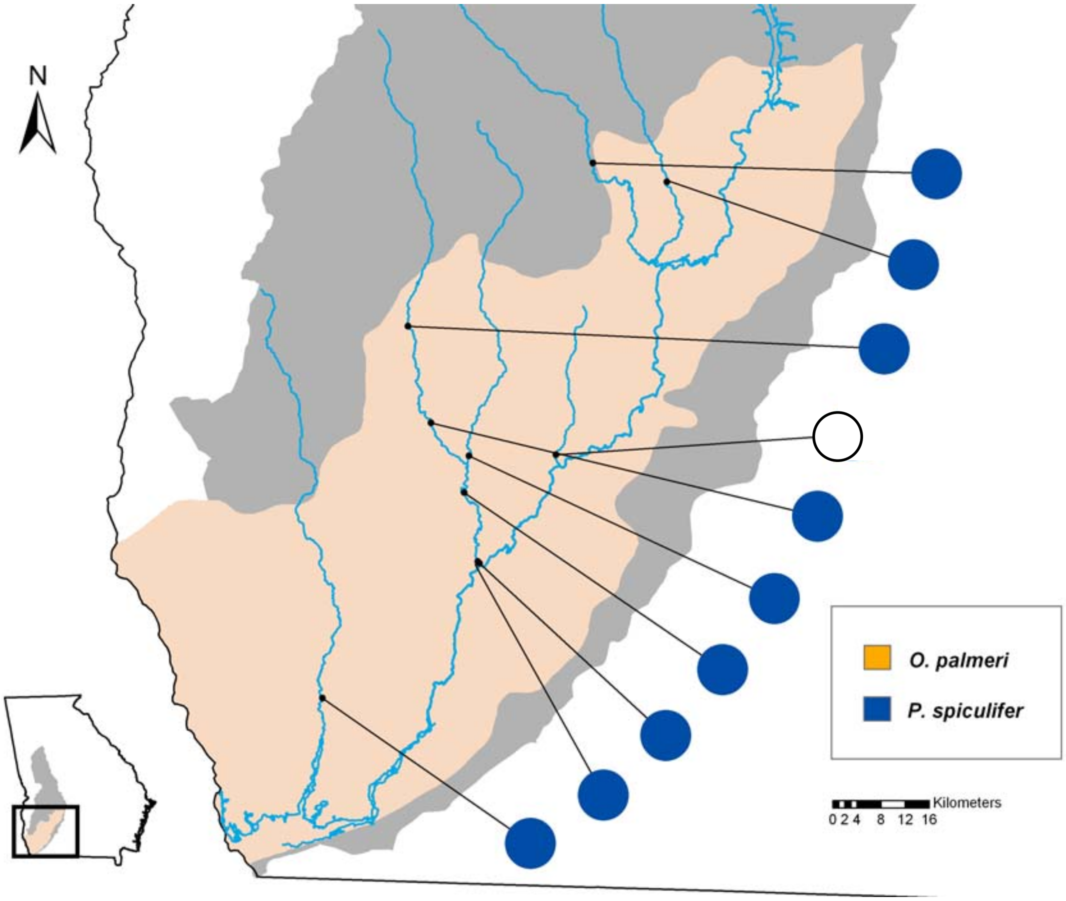

Figure 2.4. The relative abundance of *P. spiculifer* and *O. palmeri* at sites sampled in the tributaries to the Flint River.

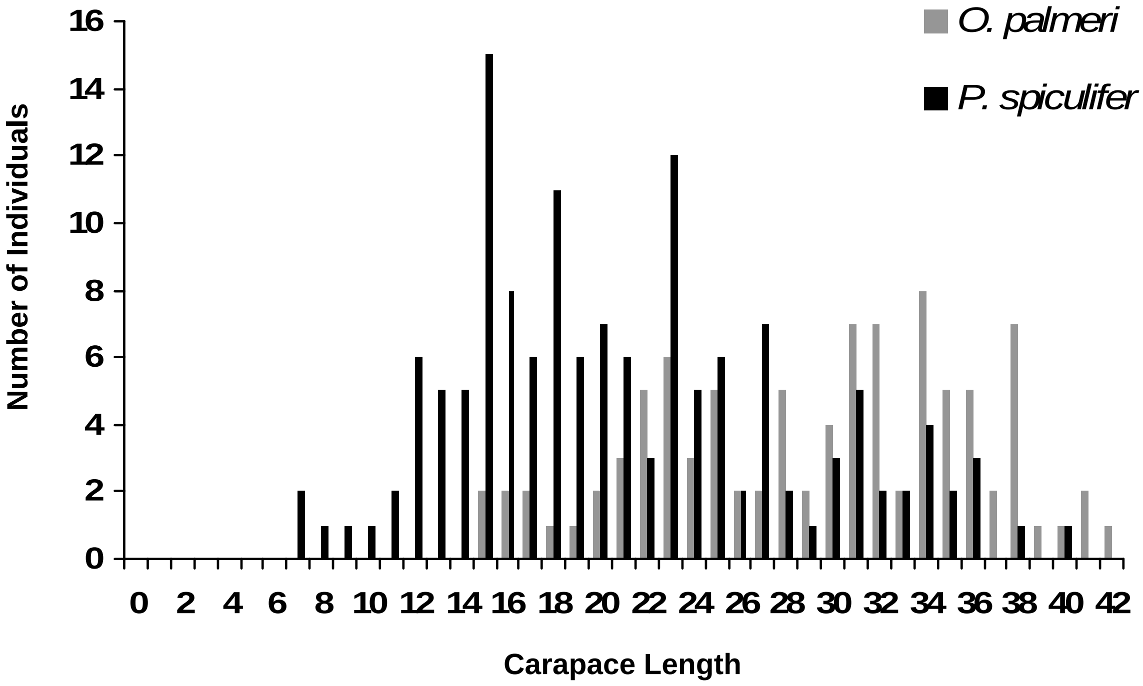

Figure 2.5. The size distribution of *O. palmeri* compared to that of *P. spiculifer*.

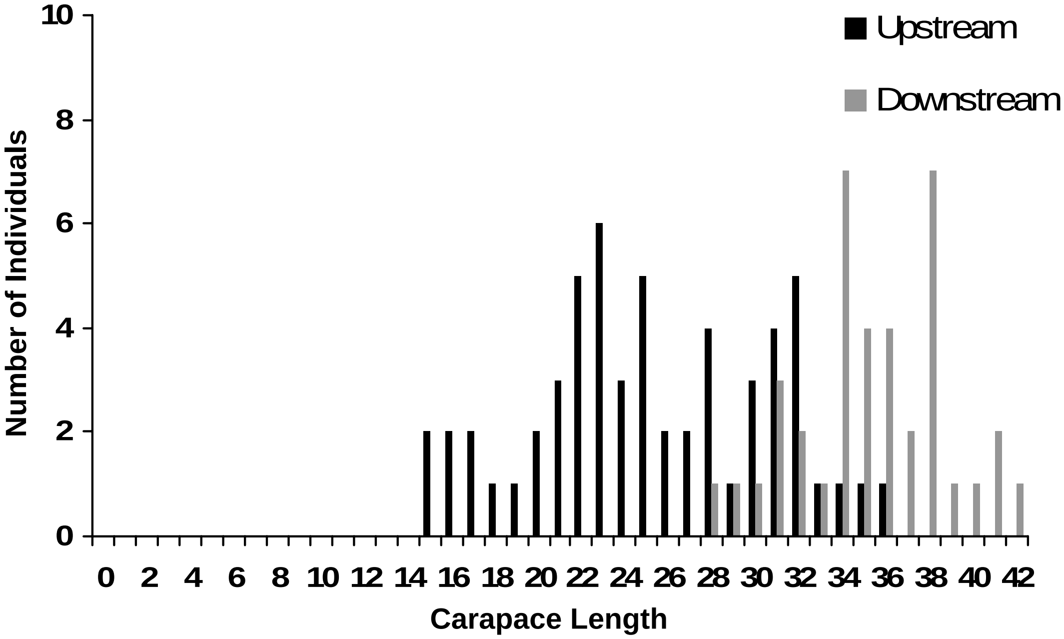

Figure 2.6. The size distribution of *O. palmeri* collected upstream in the mainstem of the Flint River compared to the size distribution of *O. palmeri* collected downstream in the mainstem of the Flint River.

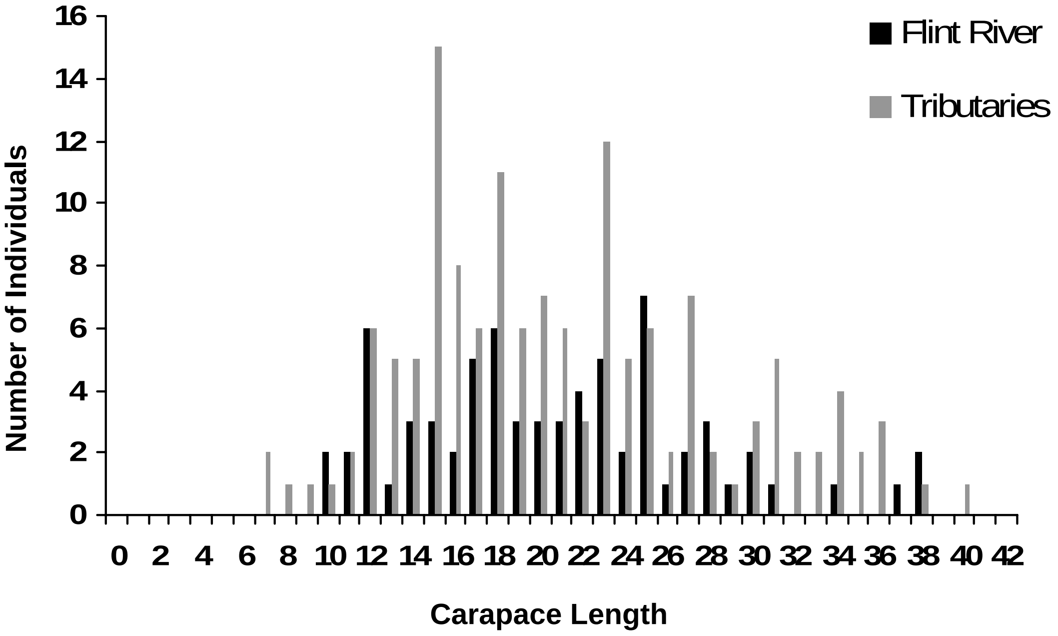

Figure 2.7. The size distribution of *P. spiculifer* collected in the mainstem of the Flint River compared to the size distribution of *P. spiculifer* collected in the tributaries.

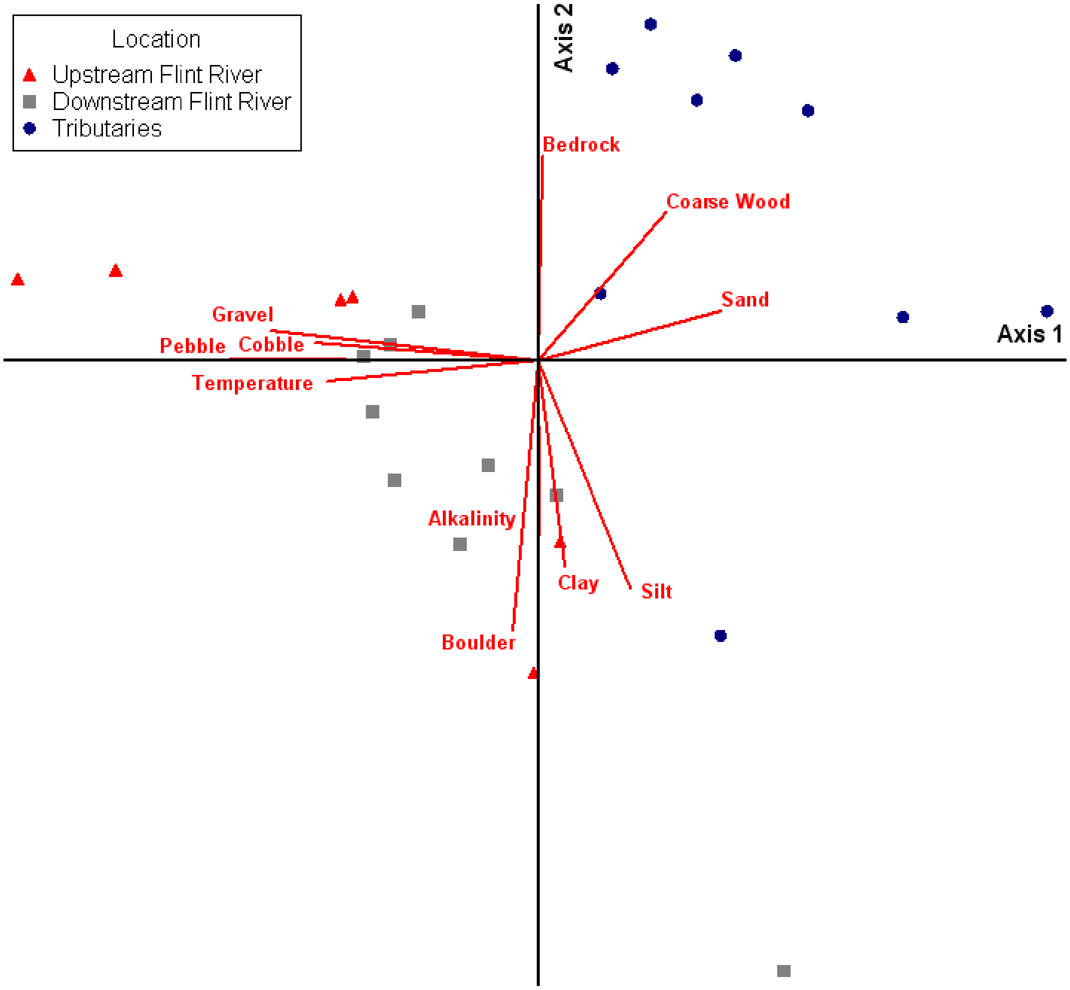

Figure 2.8. Axes 1 and 2 of the physicochemical principal components analysis.

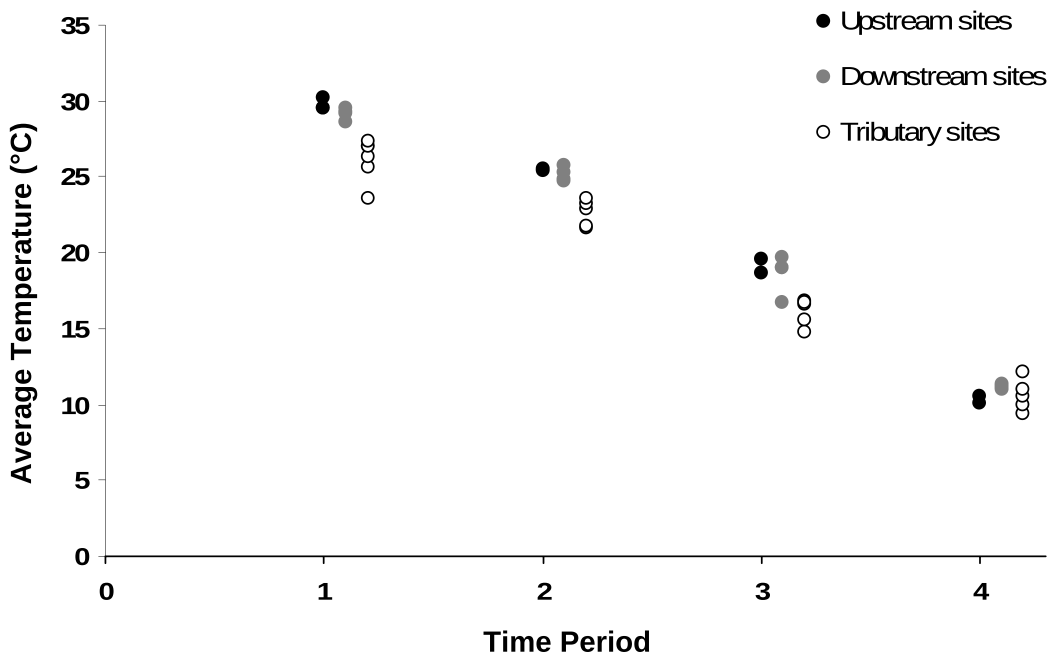

Figure 2.9. Average temperature for upstream, downstream, and tributary locations. Time period 1 is August 18th through September 17th. Time period 2 is September 20th through October 20th. Time period 3 is October 21st through November 20th, and time period 4 is January 10th through February 9th. Hobo data loggers recorded temperature hourly.

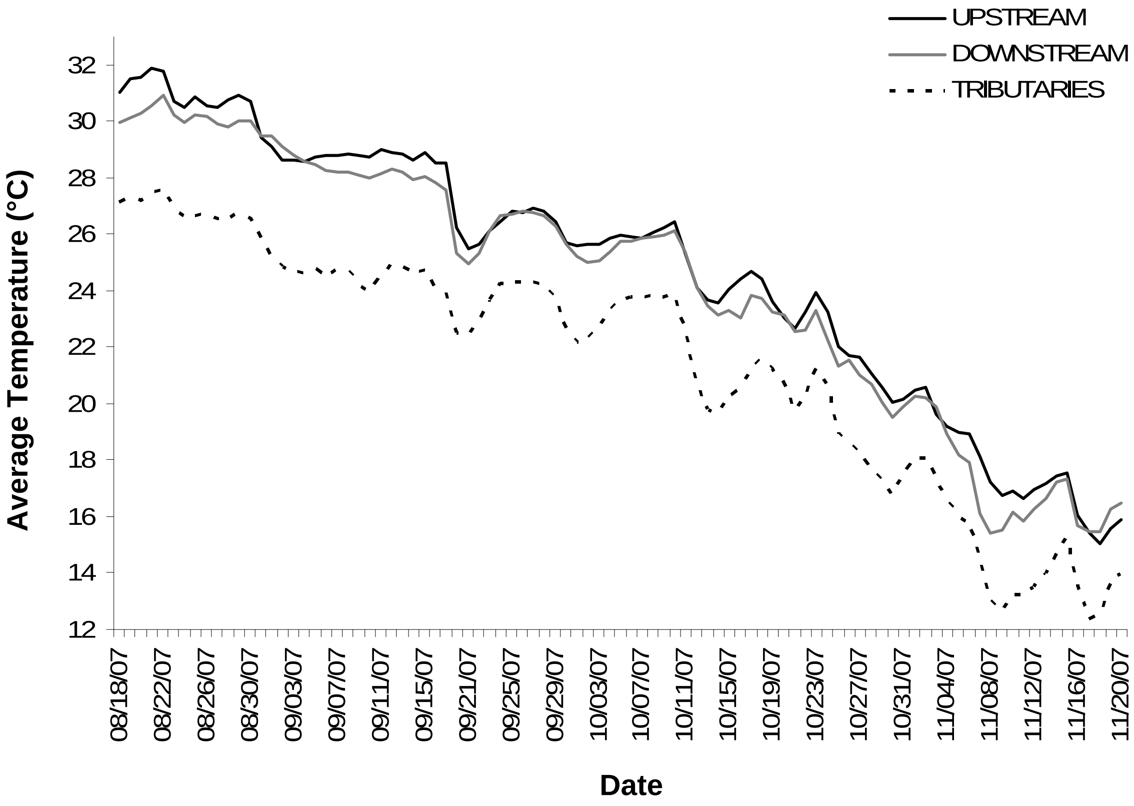

Figure 2.10. Average daily temperature collected by temperature loggers in upstream, downstream, and tributary sites.

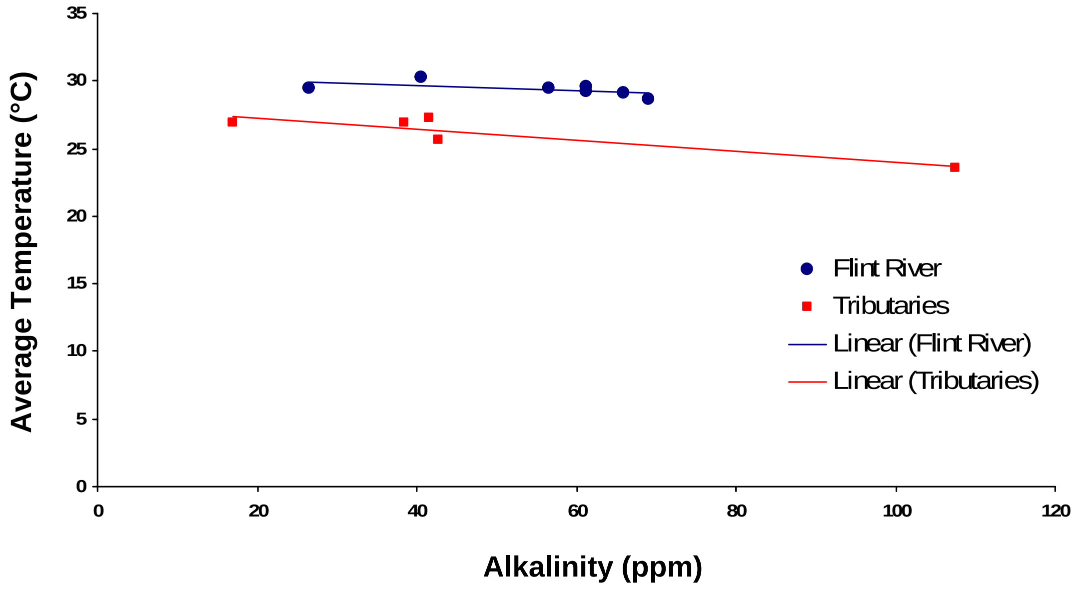

Figure 2.11. Average temperature vs. total alkalinity. Temperature is an average of hourly temperatures from period 1, August 18th through September 17th, and total alkalinity was collected from September through October of 2007. There is a negative trend between temperature and alkalinity in the Flint River and in the tributaries, although these trends are not significant.

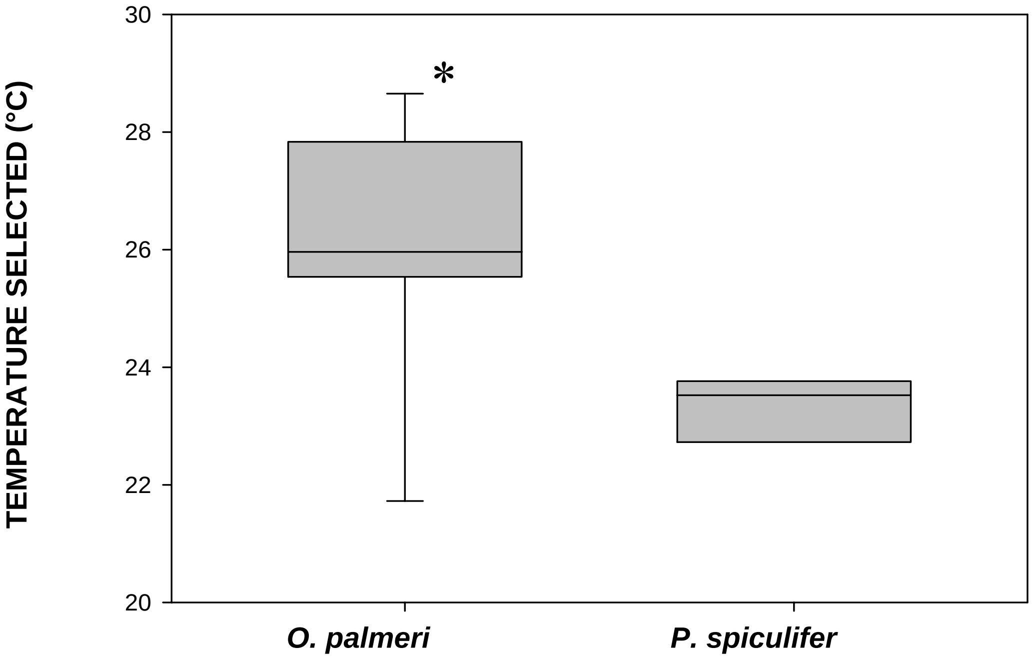

Figure 2.12. Box plot indicating temperatures selected by *O. palmeri* and *P. spiculifer*. Interquartile range, median, 10^{th}, and 90^{th} percentiles are indicated. Asterisk indicates significantly different means.

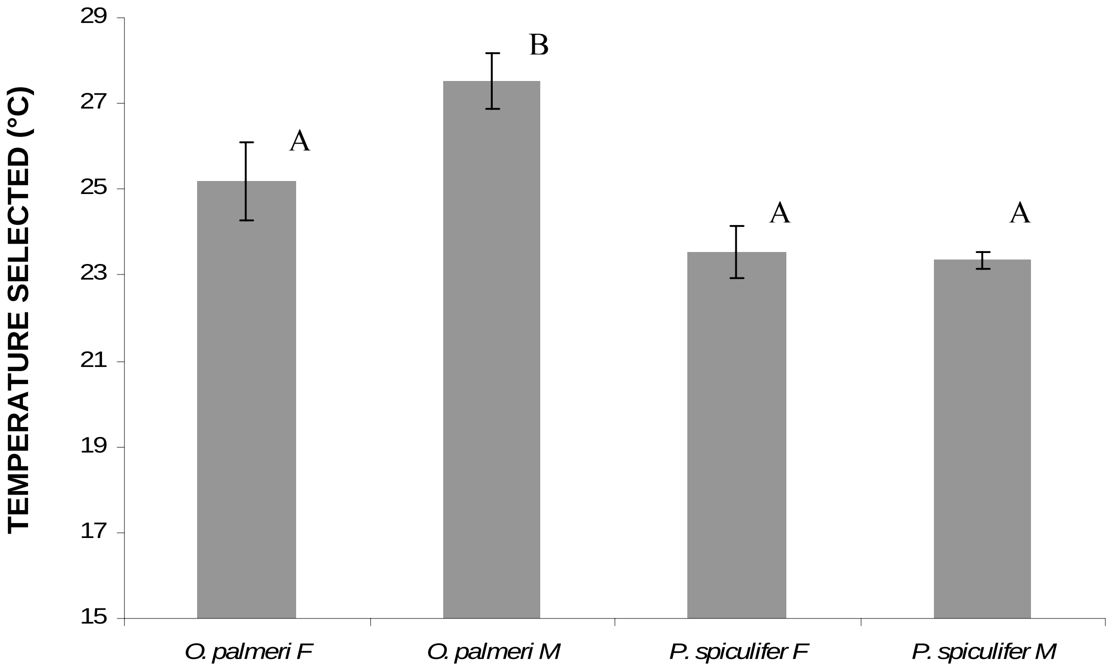

Figure 2.13. Temperature selection by each species and sex (± SE). Common letters indicate groups that selected statistically similar temperatures.

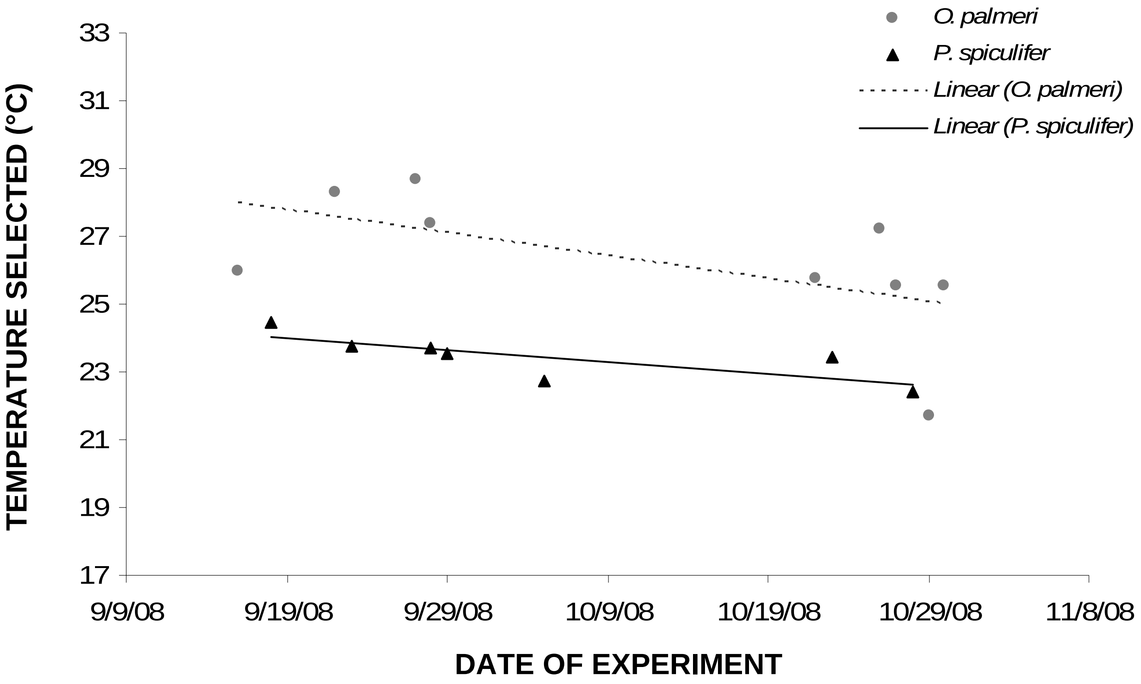

Figure 2.14. Date of temperature selection experiment vs. temperature selected by *O. palmeri* and *P. spiculifer*.

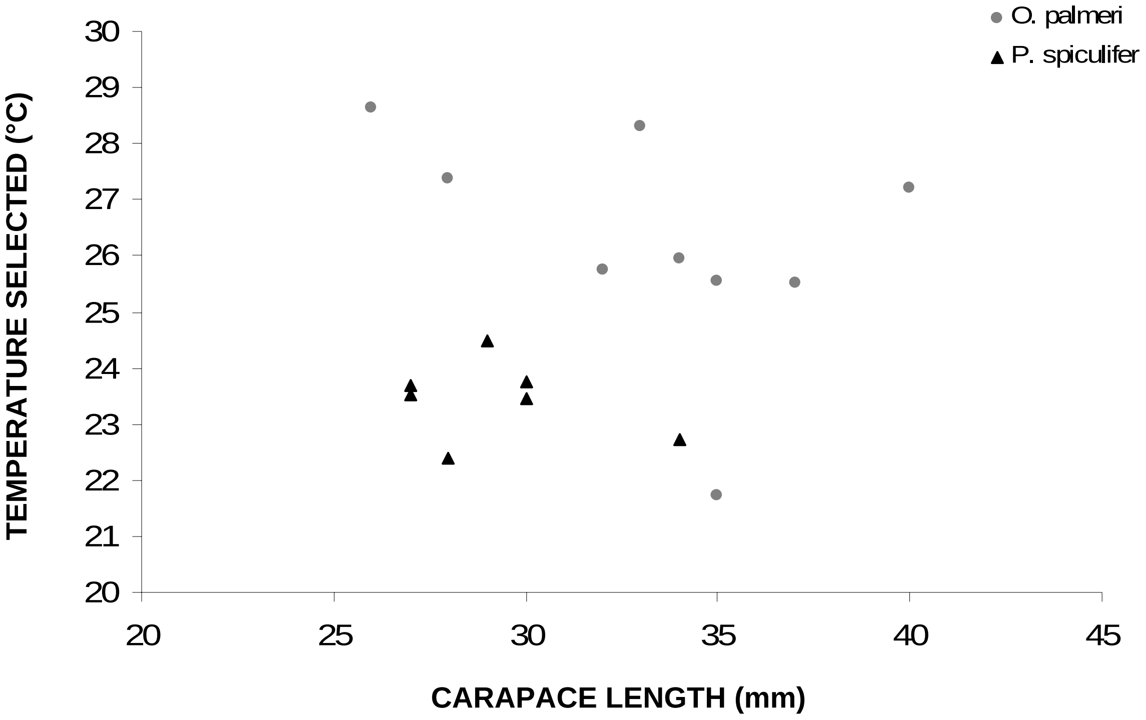

Figure 2.15. Carapace length of *O. palmeri* and *P. spiculifer* vs. temperature selected.

CHAPTER 3

PREDATOR AVOIDANCE ABILITY AND THE INVASION SUCCESS OF A NON-NATIVE CRAYFISH[1]

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[1] Sargent LW, Golladay SW, Covich AP, Opsahl SP and Rosemond AD. To be submitted to *Invasion Biology*.

Abstract

Examining species invasions can provide insight into ecological and evolutionary processes. Here we examine the impact of fish predation on the invasion success of *Orconectes palmeri*, a non-native crayfish in the lower Flint River Georgia, USA. Within in the lower Flint River, a native crayfish, *Procambarus spiculifer,* co-occurs with *O. palmeri*; however, the relative abundance of the two species shifts depending on location. *O. palmeri* is most abundant upstream, where *P. spiculifer* is rare, and both species are common downstream. The absence of juvenile *O. palmeri* downstream suggests that predation may be responsible for the observed shifts in relative abundance. We collected stomach contents from predatory fish and compared the relative abundance of crayfish species in fish stomach contents to the relative abundance of crayfish species in the Flint River. In the downstream portion of the river, we found that predatory fish were consuming a larger proportion of *O. palmeri* and a smaller proportion of *P. spiculifer* than were available in the environment. These findings suggest that *O. palmeri* are more susceptible to predation than *P. spiculifer* in areas where the species co-occur. Inferior predator avoidance ability may limit *O. palmeri* invasion success in areas where native crayfish populations are intact.

Introduction

Biological invasions are a principal human-induced threat to native biodiversity (Elton 1958; Coblentz 1990; Moyle and Williams 1990; Mooney et al. 2005). In addition, some invasive species can alter ecosystem processes (Vitousek 1990; Spencer et al. 1991; Lodge 1993; Dukes and Mooney 2004; Peters et al. 2008). Freshwater ecosystems are disproportionately impacted by invasive species (Ricciardi and Rasmussen 1999; Sala et al. 2000). It is crucial that we understand why invaders are successful in freshwater ecosystems in order to determine how to control invasions and identify which ecosystems are particularly vulnerable to invasion.

Within the freshwater fauna, crayfish are especially vulnerable to extinction along with fishes and mollusks (Master 1990; Taylor et al. 1996; Taylor et al. 2007; Crandall and Buhay 2008). Crayfish invaders are a major threat to native crayfishes (Lodge et al. 2000). Some invasive crayfish have caused declines of native crayfish due to their superior ability to compete for shelter (Garvey et al. 1994; Soderback 1994), interfere with native reproduction (Butler and Stein 1985; Perry et al. 2001), or avoid predation (Mather and Stein 1993; Gherardi et al. 2002; Kuhlmann et al. 2008). North America supports 75% of the world's crayfish species, and the southeastern U.S. has the highest species diversity of crayfish in North America (Hobbs 1981, 1991; Crandall and Buhay 2008). For example, GA has 45 native species of crayfish (Hobbs 1981). Due to the high species diversity of crayfish in the southeastern U.S. and extensive alteration of aquatic habitats, invasive crayfish pose a disproportionate threat to native crayfish diversity in this region. In this study, we examine whether selective fish predation is important in determining the relative abundance of an invasive crayfish, *Orconectes palmeri*, and a native crayfish, *Procambarus spiculifer,* in the lower Flint River, GA.

O. palmeri is native to the lower Mississippi River valley and was first found in the Flint River in 2001. *P. spiculifer* is native to Georgia, northern Florida, and eastern Alabama, and is widespread throughout the Flint River and surrounding tributaries. According to a 2007 visual survey, the relative abundance of *O. palmeri* and *P. spiculifer* varied from upstream to downstream in the lower Flint River (Chapter 2). *O. palmeri* was abundant and *P. spiculifer* was rare upstream. Downstream, the relative abundance of the two species was more variable, with *P. spiculifer* more abundant at most sites than *O. palmeri*. Juvenile *O. palmeri* were observed at upstream and downstream sites during sampling in early spring, but by mid-summer *O. palmeri* juveniles were not detected in the downstream area. This information suggests that young of the year (YOY) *O. palmeri* juveniles survived in the upstream area, but not in the downstream area where *P. spiculifer* are abundant.

Selective predation is one explanation for the lack of YOY *O. palmeri* observed downstream. Several recent studies have found fish predation to be important in structuring crayfish distributions and in affecting crayfish population sizes (Fortino and Creed 2007; Chucholl et al. 2008; Dorn 2008). Species replacements may result from the superior ability of one crayfish species to avoid fish predation. Differences in predator avoidance may be due to the competitive exclusion of one species from shelter by a larger or more aggressive species (Capelli and Munjal 1982; Soderback 1994; Nakata and Goshima 2003; Chucholl et al. 2008). Crayfish species may also differ in their predator-response behavior. Crayfish often modify their behavior in response to chemosensory cues from nearby predators, avoiding predation by spending more time in shelter and less time seeking food; however, all species do not respond with the same behavior and to the same extent (Willman et al. 1994; Gherardi et al. 2002; Hazlett et al. 2002; Acquistapace et al. 2004). Crayfish species have also been shown to have dissimilar responses to direct predator encounters when they are exposed. For example, some species are more likely to swim away from a predator and others are more likely to defend themselves with their chelae (Garvey et al. 1994; Gherardi and Daniels 2004; Fortino and Creed 2007). Over time, small differences in the ability of crayfish species to avoid fish predation can exclude the more vulnerable species from an area. In this case, we propose that *O. palmeri* may be more susceptible to fish predation in the lower Flint River basin than *P. spiculifer*.

Previous research indicates that small crayfish are more susceptible to fish predation than large crayfish (Didonato and Lodge 1993; Garvey and Stein 1993; Kuhlmann et al. 2008); thus sometimes YOY of one species are absent due to selective predation, even if the adults are present (Fortino and Creed 2007). In addition, in cases where more than one crayfish species are present in the field, the smaller or slower growing species may have a disadvantage in avoiding fish predation. Despite the smaller average size of *P. spiculifer* (Chapter 2), we hypothesize that YOY *P. spiculifer* are superior at avoiding predation in the downstream area of the Flint River, as we did not detect YOY *O. palmeri* in this area and we did detect YOY *P. spiculifer*. During the high flow period in the winter months, large *O. palmeri* may be passively dispersed by being washed downstream, so that the upstream area is a source for large downstream *O. palmeri*.

Materials and Methods

Selective fish predation was assessed throughout the lower Flint River from Lake Blackshear to Lake Seminole (Figure 3.1). The lower Flint River flows through the Dougherty Plain physiographic district, a region in the Coastal Plain characterized by karst topography and extensive subsurface aquifers. Limestone geology and substantial ground water inputs make the physicochemical characteristics of streams in this region distinct. We collected predatory fish from seven reaches along the Flint River using electrofishing (Figure 3.1). Each reach was 3 km in length and was sampled for 1 h for flathead catfish (*Pylodictis olivaris*) and 1 h for largemouth bass (*Micropterus salmoides*) and shoal bass (*Micropterus cataractae*). Sunfish (*Lepomis spp*.) may also consume small crayfish in the Flint River, but these fish are more likely to be gape limited, and were not examined in this study. Sampling for flathead catfish was conducted in August, 2007, and sampling for bass species was conducted in October, 2007. All sampling occurred during daylight between 0800 h and 1600 h. Flathead catfish were collected using boat-mounted low frequency electrofishing gear (18 Hz). Fish were measured for total length (TL) to the nearest 0.5 cm and placed on ice. Flathead catfish were transferred to a freezer within 8 h of collection. Fish were later thawed at room temperature, and stomach contents were removed by dissection. All stomach contents were identified using a dissecting microscope. Flathead catfish are non-native to the Flint River basin, so it was desirable to remove them from the river. To avoid mortality of native bass, we used non-lethal techniques to extract stomach contents from these fish. Bass were collected using boat-mounted, direct current electrofishing equipment. Fish were placed in a trough filled with river water until the end of the sampling period and then measured for TL to the nearest 0.5 cm. Stomach contents were removed using clear acrylic tubes and a flexible claw retriever (Van Den Avyle and Roussel 1980; Dimond 1985; Wheeler and Allen 2003). Bass stomach contents were preserved in 80% ethanol.

Whenever possible, crayfish from fish stomachs were identified to species and measured for carapace length, chelae length, and chelae width (Stein 1977). We created linear regression equations to predict carapace length from chela length or from chela length and width (Garvey and Stein 1993). Regressions were calculated from a sample of 210 *P. spiculifer* and 92 *O. palmeri* collected from the lower Flint River basin in the summer of 2007. We used the dominant, or larger, of the two chelae to create these regressions as this procedure controls for some of the inaccuracy associated with calculating carapace length from regenerated chelae. Separate regressions were created for each species and for males and females. We also created regressions for the combined sexes as sex could not always be identified in crayfish from stomach contents. Some chelae from crayfish in stomach contents may have been regenerated, leading to an underestimate of carapace length, but we expect that this possibility had relatively little effect on size estimates.

The relative abundance of crayfish species was assessed within each 3 km reach during a summer 2007 survey. Each crayfish sampling site consisted of a 50 m transect on one side of the stream that contained loose cobbles and boulders. Two experienced observers each sampled 25 m of the 50 m transect for 1 h by snorkeling and wading. Two 50 m transects were sampled within four of the seven reaches where fish were collected. Due to the variable relative abundance of crayfish species near reach 5, four transects were sampled in this area. We were unable to find crayfish in reach 7, so we used data from an upstream site within 15 km of the reach. The relative abundance of crayfish at this site was reflective of the overall relative abundance in the downstream area of the Flint River. In addition, reach 1 did not overlap with crayfish sampling sites, but was within 15 km downstream of the sites. We were unable to sample the same reach for fish as we had previously sampled for crayfish due to low water levels which could not accommodate our sampling equipment. The relative abundance of crayfish at these sites was reflective of the overall relative abundance of crayfish species in the upstream area of the lower Flint River.

We examined the relative abundance of crayfish in the environment and in fish stomach contents to determine whether either species of crayfish was consumed disproportionately to its relative abundance in the field. We used a t-test procedure to compare the percent of *O. palmeri* found at each site during visual surveys to the percent of *O. palmeri* found at each site in fish stomach contents. In addition, to assess size selection by fish predators, we divided crayfish into three size classes: small (< 19 mm carapace length), medium (20-29 mm carapace length), and large (> 30 mm carapace length). We used ranked data to compare the relative abundance of each size class of each species in fish stomach contents to the relative abundance of each size class of each species collected during visual surveys. The methods used were similar to those described by Johnson (1980). This non-parametric analysis is desirable as it does not require an exact measure of availability of a food item in the environment. In addition, there is no assumption of interdependence of experimental units, and locations where we did not find individuals of one crayfish species were not problematic as this analysis involved comparing ranked data and not ratios. We used the seven sampling reaches as our replicated experimental units.

Results

We collected 388 fish in the lower Flint River, including 208 flathead catfish, 150 largemouth bass, and 30 shoal bass (Table 3.1). Flathead catfish stomach contents contained 108 *O. palmeri* and 24 *P. spiculifer*. Largemouth bass stomach contents contained 54 *O. palmeri* and 14 *P. spiculifer*, and shoal bass stomach contents contained 7 *O. palmeri* and no *P. spiculifer*. Thirteen crayfish in stomach contents could not be identified to species due to poor condition. Shoal bass are difficult to collect using boat-mounted electrofishing equipment despite their abundance in the Flint River (Georgia Department of Natural Resources, personal communication, 2007). We combined the two bass species in many analyses due to the small number of crayfish found in shoal bass stomach contents. During crayfish visual surveys we found 168 *O. palmeri* and 95 *P. spiculifer* throughout the lower Flint River. Although we found a greater ratio of *P. spiculifer* to *O. palmeri* in the environment (117:190) than in fish stomach contents (38:169) (Figure 3.2), comparisons of relative abundance between visual surveys and fish stomach contents at each site did not reveal any significant trends. Neither flathead catfish (P = 0.3268) nor bass (P = 0.4902) consumed either crayfish species disproportionately to its relative abundance in the environment. The same was true when all fish species were analyzed together (P = 0.6293). If the downstream and upstream areas were examined as two groups, it was clear that *O. palmeri* was consumed downstream, particularly by flathead catfish, at a higher proportion than it was found in the environment (Figure 3.3). However, this cannot be assessed statistically due to a lack of replication. This trend was not apparent upstream (Figure 3.4).

Total length of fish ranged from 17.5 to 61 cm for largemouth bass, 16.5 to 53 cm for shoal bass, and 13.5 to 95.5 cm for flathead catfish. Mean lengths (± SE) were 31.8 ± 0.8 cm, 30.6 ± 1.8 cm, and 36.5 ± 1.0 cm respectively. Fish length was positively related to carapace length of crayfish in the stomach contents for both flathead catfish (P = 0.0014, R^2 = 0.0782) and bass (P = 0.1743, R^2 = 0.0129); however, fish length was a poor predictor of crayfish carapace length (Figures 3.5 and 3.6). Due to limitations in gape size, small fish consumed small crayfish, but larger fish consumed crayfish across the range of size classes. An analysis of species and size selection by flathead catfish and bass revealed that overall both groups of fish selected the larger size classes of *O. palmeri* and *P. spiculifer*. Medium and small *P. spiculifer* were selected least often (Table 3.2).

Discussion

We did not obtain significant results when sites were used as individual units to examine selective fish predation; however, results from the Flint River as a whole suggested that *P. spiculifer* may be superior to *O. palmeri* at avoiding fish predation in this environment. It is likely that when prey selection was examined by site, our sample size of fish and crayfish was too small for accurate estimates of availability and consumption. Clearly this was not a case where *O. palmeri* invasion success could be attributed to superior predator avoidance abilities. We attributed the reduction in *O. palmeri* abundance in areas with intact *P. spiculifer* populations to selective fish predation on the invasive crayfish, due to our data from the Flint River as a whole. A likely mechanism for the observed patterns of relative abundance would be that *P. spiculifer* excludes *O. palmeri* from shelter. If *P. spiculifer* was superior at avoiding predation by evicting *O. palmeri* from shelter, we would not observe selective predation in areas where *P. spiculifer* is rare, such as we observed in the upstream area. Differences in *P. spiculifer* abundance may explain why selective predation was not observed in the upstream area, but was observed downstream.

The ability of crayfish to avoid predation may differ based on size class. We found that both flathead catfish and bass species selected small and medium *O. palmeri* over small and medium *P. spiculifer.* Large *O. palmeri* may be superior to large *P. spiculifer* at avoiding fish predation, while the opposite trend is observed for small and medium size classes. Large *O. palmeri* may be able to avoid some predation by defending themselves with their large chelae, whereas small *O. palmeri* may more effectively avoid predation by seeking shelter. Because crayfish populations are often dominated by first year individuals, it is likely that predation on small size classes has a large impact on abundance of any species. This effect is especially apparent in the downstream portion of the Flint River, where small *O. palmeri* were not detected and relative abundance of *O. palmeri* was reduced.

Previous to the fish predation study, we predicted that predatory fish selectively consumed small crayfish, because we observed a lack of YOY *O. palmeri* in the downstream portion of the Flint River where *P. spiculifer* was abundant. Our finding that predatory fish selectively consumed large crayfish was unexpected. Previous studies have found that small crayfish are more susceptible to fish predation than large crayfish. When multiple sizes of crayfish are available, fish predators often select for small crayfish in the absence of structure due to reduced handling time (Didonato and Lodge 1993). Some research has also found that fish select for large crayfish on large substrata such as cobbles (Stein et al. 1977; Garvey et al. 2003). During preliminary crayfish surveys, we determined that crayfish in the Flint River utilized large substrata for shelter. All of the sites surveyed contained some cobble or boulder substrata, suggesting that fish may have selectively consumed large crayfish due to the size of substrata at these sites.

Alternatively, it is possible that our sampling methods underestimated the relative abundance of large crayfish in the river or overestimated their abundance in fish stomach contents. Large crayfish are often located in deeper water than small crayfish. This strategy of remaining in deeper water is effective because predatory fish are often gape limited and terrestrial predators are not (Power 1987; Schlosser and Ebel 1989), so small crayfish use shallow water to avoid fish predation and large crayfish use deeper water to avoid predation by terrestrial predators (Mather and Stein 1993; Englund and Krupa 2000; DiStefano et al. 2003; Flinders and Magoulick 2007). By snorkeling and wading along the bank of the Flint River, we may have missed some large crayfish located in deeper water that could not be thoroughly sampled using these methods. Our estimate of the abundance of small crayfish in the river, therefore, is likely more accurate than our estimate of the abundance of large crayfish. In addition, small crayfish molt more often than large crayfish. Recently molted crayfish are particularly vulnerable to predation, but are difficult to measure in fish stomach contents as they are quickly disintegrated by digestion. We expect that selective fish predation was responsible for the lack of small *O. palmeri* observed in the downstream area, and our finding that fish selected for larger individuals was a product of our sampling methods.

The observed distributions of *O. palmeri* and *P. spiculifer* cannot be explained by predation alone. It is likely that fish predation pressure did not change significantly by location. Productive locations where either species of crayfish was abundant also contained a large number of fish (L. Sargent, personal observation, 2007). In addition, shoal bass, largemouth bass, and flathead catfish were found throughout the lower Flint River. The relative abundance of crayfish species, however, did change by location as *O. palmeri* and *P. spiculifer* co-occurred downstream in the lower Flint River, while *O. palmeri* was found almost exclusively upstream. Warm temperatures were likely responsible for the lack of *P. spiculifer* in the upstream portion of the Flint River, while *O. palmeri* may have been more abundant in this area due to its tolerance of warm temperatures (Chapter 2). These data suggest that temperature may be important in limiting the distribution of the native crayfish species, while predation is important in limiting the spread of the invasive species. Local adaptation to the conditions in the Flint River may have provided *P. spiculifer* with more appropriate predator avoidance traits for this environment.

Monitoring of crayfish populations in the lower Flint River will determine whether selective predation is sufficient to control the spread of this invasion. In addition, as temperatures continue to increase due to urbanization, removal of groundwater for agriculture, and global climate change, *P. spiculifer* abundance may decline in the downstream portion of the lower Flint, allowing for further colonization by *O. palmeri*. Future research should focus on the traits and behaviors that make *P. spiculifer* superior to *O. palmeri* at avoiding fish predation. If the difference in predator avoidance ability is due to differences in predator response behavior, replacement of *P. spiculifer* by *O. palmeri* may impact the fish community as some species, such as flathead catfish, selectively consume *O. palmeri*. Selective predation may be less significant if the difference in predator avoidance is due to shelter competition as shelter will be available for *O. palmeri* in areas where *P. spiculifer* is rare. It is also important to study how *O. palmeri* interacts with the community and the physiochemical environment through feeding behavior, in order to predict the impact that replacement of *P. spiculifer* by *O. palmeri* may have on biota and ecosystem processes in the Flint River.

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Table 3.1. The number of flathead catfish, largemouth bass, and shoal bass collected through electrofishing. The total number of *O. palmeri* and *P. spiculifer* individuals found in the stomach contents for each fish species is also indicated.

Fish Species	**Number Collected**	***O. palmeri***	***P. spiculifer***
flathead catfish	**208**	**108**	**24**
largemouth bass	**150**	**54**	**14**
shoal bass	**30**	**7**	**0**

Table 3.2. Crayfish size selection by flathead catfish and bass. The multiple comparison procedure of Waller and Duncan (1969) was used to determine significant differences between sizes. Letters indicate significant differences at α = 0.1.

	P. olivaris				***Micropterus spp.***			
rank	**species**	**size**		**rank**	**species**	**size**		
1	*O. palmeri*	medium	a	1	*P. spiculifer*	large	a	
2	*P. spiculifer*	large	a	2	*O. palmeri*	large	a	b
3	*O. palmeri*	large	a	3	*O. palmeri*	small	a	b
4	*O. palmeri*	small	a	4	*O. palmeri*	medium		b
5	*P. spiculifer*	small	a	5	*P. spiculifer*	medium		b
6	*P. spiculifer*	medium	a	6	*P. spiculifer*	small		b

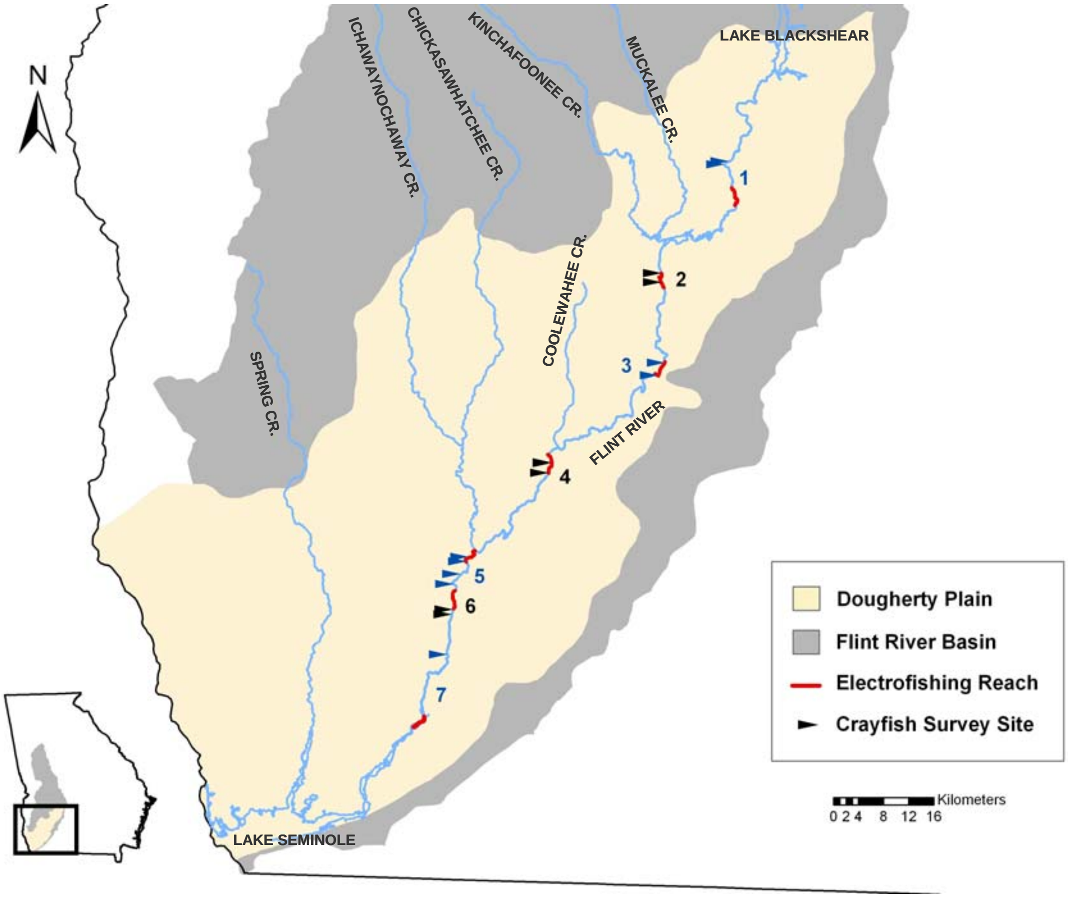

Figure 3.1. Study area including electrofishing reaches and crayfish survey sites. Numbers indicate electrofishing reaches. The crayfish survey sites are color coded to correspond with the numbered electrofishing reach in which they were used to estimate crayfish relative abundance.

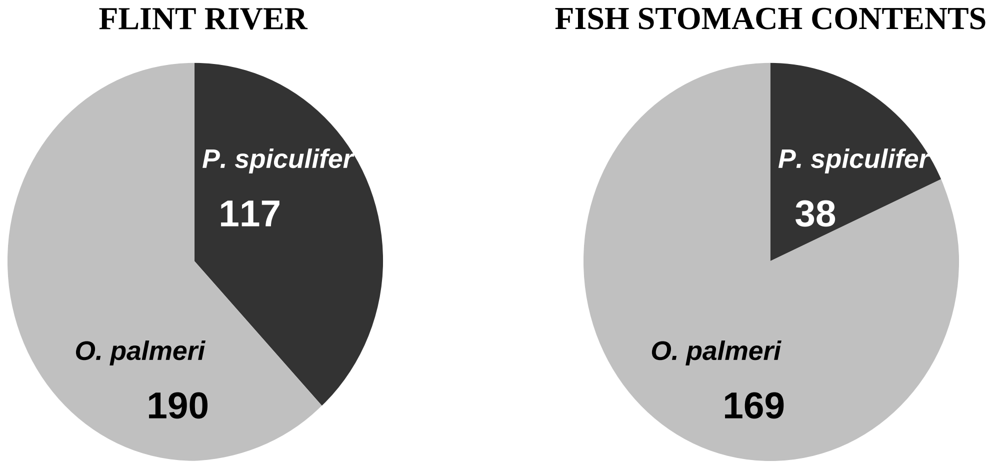

Figure 3.2. The relative abundance of *P. spiculifer* and *O. palmeri* found in the Flint River during visual surveys compared to the relative abundance found in flathead catfish and bass stomach contents. Numbers represent the total number of individuals found.

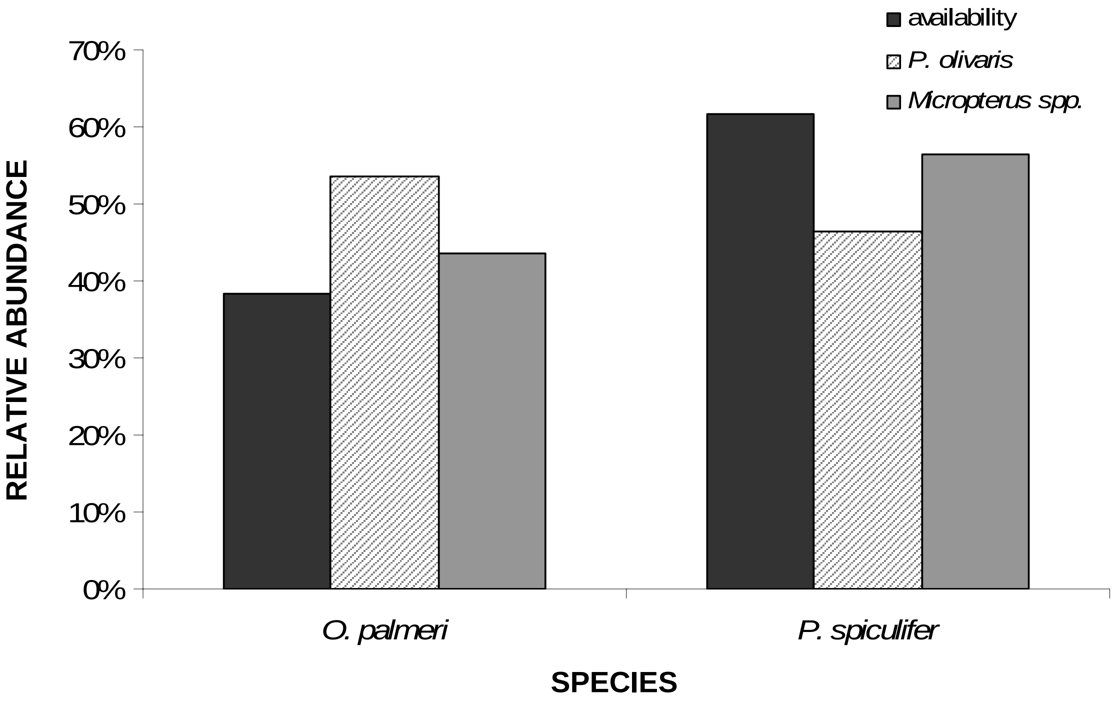

Figure 3.3. The relative abundance of *O. palmeri* and *P. spiculifer* in the downstream area of the lower Flint River. The relative abundance of each species found in the environment is compared to the relative abundance of each species in flathead catfish and bass stomach contents.

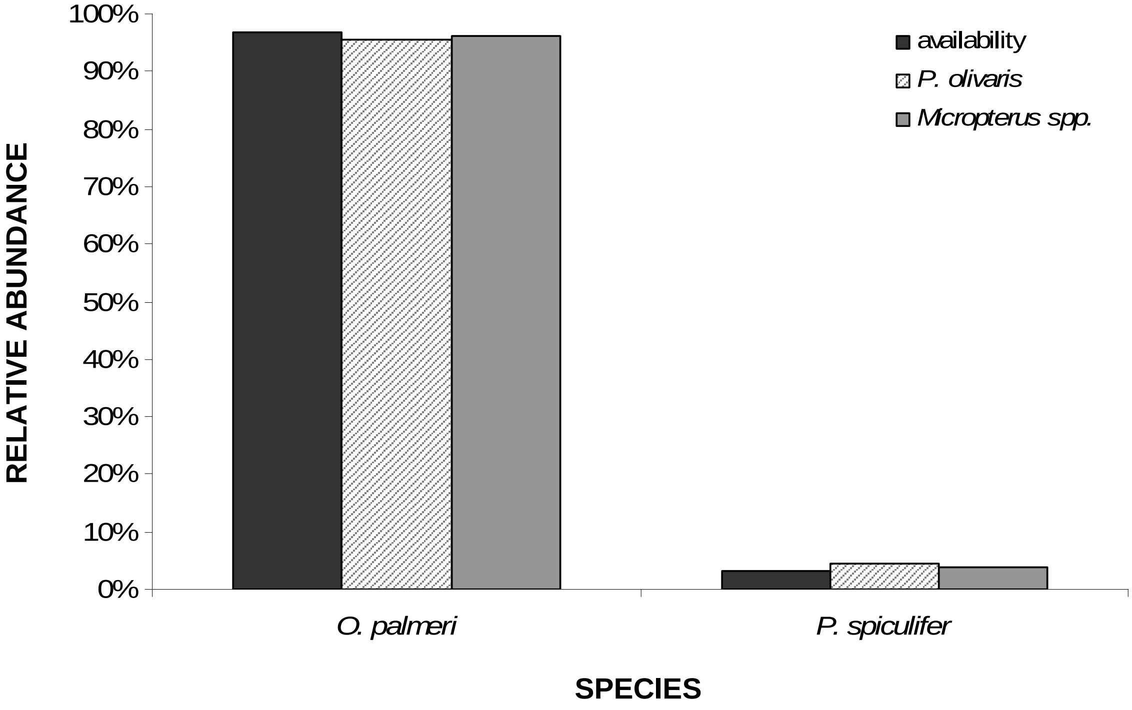

Figure 3.4. The relative abundance of *O. palmeri* and *P. spiculifer* in the upstream area of the lower Flint River. The relative abundance of each species found in the environment is compared to the relative abundance of each species in flathead catfish and bass stomach contents.

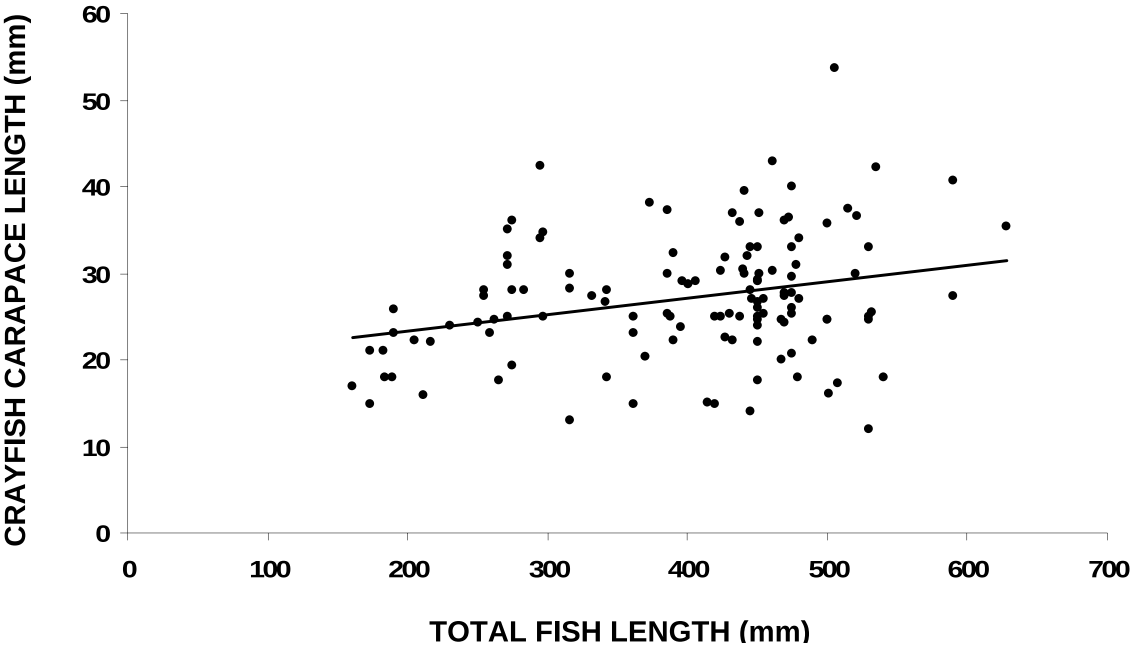

Figure 3.5. The relationship between total fish length and crayfish carapace length within flathead catfish stomach contents.

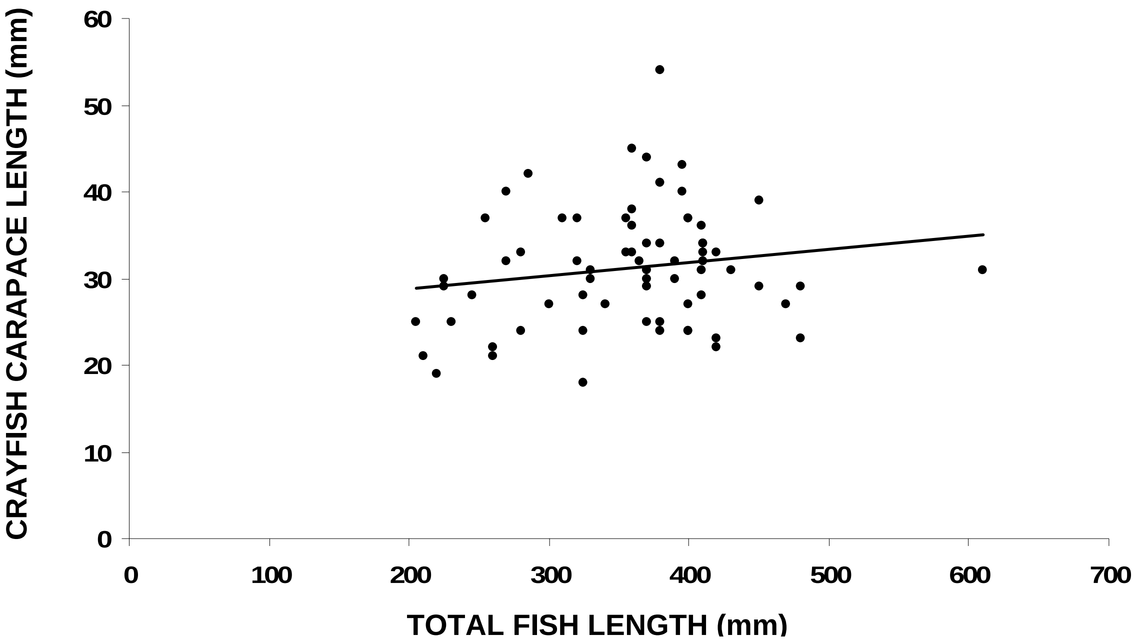

Figure 3.6. The relationship between total fish length and crayfish carapace length within bass stomach contents.

CHAPTER 4

CONCLUSIONS

Summary

Although native species are locally adapted to environmental conditions, they are sometimes outperformed by invasive species that did not evolve in the local habitat. Freshwater systems that are minimally altered by humans are less likely to be invaded as there are fewer niche opportunities for non-native species to exploit (Moyle and Light 1996a, Shea and Chesson 2002). We attribute the invasion success of *O. palmeri* in the lower Flint River basin to human induced alteration of the temperature regime.

The observed distribution of *O. palmeri* and *P. spiculifer* suggests that both physicochemical factors and interspecific competition influence the relative abundance of crayfish species in the lower Flint River basin. Each species is dominant in one section of the lower Flint River basin, *O. palmeri* upstream and *P. spiculifer* in the tributaries, and small *O. palmeri* are absent where the two species co-occur. Predatory fish consumed a higher proportion of small *O. palmeri* than were available in the river. This trend was apparent downstream where *P. spiculifer* were abundant, but not upstream where *P. spiculifer* were rare. This suggests that the presence of *P. spiculifer* increases the predation susceptibility of small *O. palmeri*, possibly because *P. spiculifer* evict *O. palmeri* from shelter.

The abundance of both species was highly correlated with temperature. *O. palmeri* was associated with warmer fall temperatures and *P. spiculifer* was associated with cooler fall temperatures. In addition, *O. palmeri* selected significantly warmer temperatures than *P.*

spiculifer when temperature preference was assessed in a thermal gradient. We expect that warmer water temperatures in the upstream portion of the lower Flint River give *O. palmeri* a competitive advantage over *P. spiculifer*, by increasing *O. palmeri* fitness and decreasing *P. spiculifer* fitness. It is also possible that upstream temperatures are lethal to some *P. spiculifer* in the summer months, allowing for *O. palmeri* to persist with little interspecific competition. Species may generally be well adapted to the conditions in the greater part of their native range, but not as well adapted to conditions near the boundaries. This may create conditions which favor species invasions due to low levels of competition (Fausch 2008). While *P. spiculifer* has historically been found in the upstream portion of the Flint River, it may be poorly adapted to the temperature regime in this portion of its range, allowing for the invasion success of *O. palmeri*.

Temperature varies throughout the lower Flint River basin due to increases in the percentage of groundwater in the river and tributaries as they flow downstream. Groundwater is cooler than streamwater in the summer months, so sites that contain a higher percentage of groundwater have cooler water temperatures. In addition, tributaries are cooler than the Flint River due to increased shading. Increased groundwater withdrawals for irrigation have reduced flows in at least two tributaries to the Flint River (Hicks and Golladay 2006). Although this trend has not been evaluated in the Flint River, we expect that the dramatic increase in groundwater usage in the Flint River basin has led to increased water temperatures in the Flint. *P. spiculifer* has been historically documented in the upstream portion of the lower Flint River, although the historic abundance of this species is undocumented (Hobbs 1981). We expect that *P. spiculifer* populations have declined due to warming of the water in the Flint River and/or invasion by *O. palmeri*. The success of this invasion is likely due to human induced changes to the thermal regime in the Flint River.

Management Implications

The results of this study underline the importance of maintaining the natural physicochemical characteristics of streams in order to protect native biodiversity and discourage species invasions. We found both temperature and coarse wood to be important for maintaining *P. spiculifer* populations. Much of the lower Flint River and adjacent tributaries are flanked by a forested buffer, which contributes coarse wood to the ecological system. In addition, a forested riparian area provides shading which helps maintain cooler water temperatures. It is important that natural inflows from the aquifer are preserved in order to protect cool water refugia in the river and maintain overall cooler temperatures provided by groundwater.

Future Research

Future research should focus on the ecological impacts of *O. palmeri* and the mechanism by which *P. spiculifer* is superior at avoiding fish predation. Information about the feeding behavior and trophic interactions of *O. palmeri* will help to determine the impact this species is having on the environment in areas where it is abundant. It is important to compare the ecological role of *O. palmeri* with that of *P. spiculifer* to predict changes to ecosystem processes that may accompany this invasion. Future research should also examine the behavioral adaptations that allow *P. spiculifer* to be superior to *O. palmeri* at avoiding fish predation. Our predation data suggest that *P. spiculifer* may evict *O. palmeri* from shelter, exposing them to fish predators. Finally, the lack of historical temperature data from the Flint River prevents us from conclusively examining how groundwater withdrawals have impacted the temperature regime and the organisms that depend on it. I would like to propose using a modeling approach to better understand the effect that groundwater pumping may have on the temperature regime.

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