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ABSTRACT OF DISSERTATION

Jennifer Schöpf Rehage

The Graduate School
University of Kentucky
2003

TRAITS UNDERLYING INVASIVENESS: A COMPARISON OF WIDESPREAD AND ENDEMIC SPECIES IN THE GENUS *GAMBUSIA* (POECILIIDAE)

ABSTRACT OF DISSERTATION

A dissertation submitted in partial fulfillment of the
requirements for the degree of Doctor of Philosophy in the
College of Arts and Sciences
at the University of Kentucky

By

Jennifer Schöpf Rehage
Lexington, Kentucky
Director: Dr. Andrew Sih, Professor of Biology
Lexington, Kentucky
2003
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ABSTRACT OF DISSERTATION

TRAITS UNDERLYING INVASIVENESS: A COMPARISON OF WIDESPREAD AND ENDEMIC SPECIES IN THE GENUS *GAMBUSIA* (POECILIIDAE)

Due to the irreversible nature of biological invasions, prediction has been a key area of emphasis in invasion biology. Specifically, the degree to which species-specific traits may help us predict invasion success is a core issue in the field. My research examined a series of traits and asked whether they were good predictors of invasion success, particularly establishment success. I compared traits among four species of the poeciliid fish *Gambusia*, two of them highly invasive (*G. affinis* and *G. holbrooki*) and two of them non-invasive (*G. hispaniolae* and *G. geiseri*).

I examined abiotic tolerances, feeding behavior, behavioral responses to novel predation and competition, life histories, and dispersal tendencies. I found the invasive *Gambusia* species to be more tolerant of low temperatures and to exhibit higher feeding rates and dispersal tendencies than non-invasives. Invasive species were more likely to respond appropriately to novel predation by reducing foraging and activity level and by increasing refuge use, and less likely to show lower foraging success when faced with competitors. Invasives exhibited higher fecundity and juvenile growth rates, and consequently reached maturity sooner than non-invasives. I found no differences in the species' diet breadth or aggressiveness.

I then simulated the invasions of simplified pond communities and measured establishment success (with and without novel competitors) and community impact by tracking population trajectories over several months. As predicted from the trait comparisons, I found that in both simulations invasive *Gambusia* outperformed non-invasives by achieving and

maintaining larger populations. In the first experiment, only invasive *Gambusia* were able to successfully establish (non-invasive populations had zero survival). In the second experiment, invasive *Gambusia* populations were better able to cope with competition and had greater community impact on lower trophic levels than the non-invasives.

Overall, species traits were good predictors of establishment success. A species' ability to cope with the abiotic conditions of the invaded community seemed particularly important to whether or not establishment occurred in the study communities. Life history traits and the species' ability to cope with biotic interactions were important to determining the level of establishment species achieved if invaders survived the novel abiotic element.

KEYWORDS: Biological invasions, invasiveness, traits, fish, *Gambusia*

Jennifer Schöpf Rehage

August 22, 2003

TRAITS UNDERLYING INVASIVENESS: A COMPARISON OF WIDESPREAD AND ENDEMIC SPECIES IN THE GENUS *GAMBUSIA* (POECILIIDAE)

By

Jennifer Schöpf Rehage

Andrew Sih, Director of Dissertation
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August 22, 2003

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DISSERTATION

Jennifer Schöpf Rehage

The Graduate School
University of Kentucky
2003

TRAITS UNDERLYING INVASION ABILITY: A COMPARISON OF WIDESPREAD AND ENDEMIC SPECIES IN THE GENUS *GAMBUSIA* (POECILIIDAE)

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Chapter One

Introduction

Biological invasions are widely recognized to be one of the most important agents of anthropogenic global change (Vitousek et al. 1996, Parker et al. 1999, Mack et al. 2000). Non-indigenous species are responsible for losses in biodiversity (Sala et al. 2000), changes in ecological processes and ecosystem function (D'Antonio and Vitousek 1992), and today they constitute one of the largest threats to natural communities (OTA 1993).

A core question in the study of biological invasions is: Do invasive species have certain traits in common that allow them to succeed while others fail? The fact that certain species have repeatedly invaded different areas of the world suggests that species are not successful invaders just because they have escaped biotic constraints in invaded habitats (Keane and Crawley 2002), but instead because something about the non-indigenous species itself allows it to become invasive. In fact, being a successful invader elsewhere is often a good predictor of invasion success in a given community (Daehler and Strong 1993, Reichard and Hamilton 1997, Marchetti et al. 2003). Certain key traits are then expected to allow a non-indigenous species to successfully invade (Vermeij 1991, Mack et al. 2000). Although the realized level of invasion success (or invasiveness) may often depend on community traits and interactions between invader traits and community traits (Reichard and Hamilton 1997, Kolar and Lodge 2001), certain key species-specific traits are generally expected to increase the probability of success of invasive species in any given community.

Ultimately, the goal is to use our knowledge of traits that contribute to invasion success to predict future invaders. This is a key issue since invasions are often permanent in ecological time (Coblentz 1990) and can be irreversible (Moyle 1999). Eradication may be almost impossible in many cases (Howarth 1991, Lodge et al. 1998), and control and mitigation are very expensive and difficult (Kolar and Lodge 2001), and not without significant disturbance to the environment (Coblentz 1990).

Prediction, however, has proven to be a difficult task not only because of the intrinsically complex nature of community assembly and the seemingly haphazard way in which arriving non-indigenous species fit in, but also because for any particular invasion, we lack detailed data

on the ecology of the invading species, how it is interacting with native biota, and the ecology of the invaded community. More importantly, we lack data on failed invasions and the factors or traits involved in those failures (Moulton and Pimm 1986, Lodge 1993a, Rejmanek and Richardson 1996). Furthermore, past efforts at understanding the mechanisms behind the invasion process have rarely been quantitative or systematic enough to elucidate patterns of invasiveness (Vermeij 1996, Kolar and Lodge 2001). This is especially true in aquatic systems (Lodge 1993b, Lodge et al. 1998; but see Kolar and Lodge 2002). For these reasons, many scientists have remained cautious and even pessimistic about our ability to predict which non-indigenous species will become invasive (Gilpin 1990, Enserink 1999).

Research has been largely unsuccessful at finding traits that convey invasiveness to all non-indigenous species across all invaded communities. This might not be surprising given that generalities that apply to all taxa may not exist (Kolar and Lodge 2002). However, great progress has been made when the scope of prediction has been more limited. Specifically, investigators have found some success identifying key traits that predict invasiveness within a taxonomic group (e.g., all species in the genus *Pinus*, Rejmanek and Richardson 1996), within an ecosystem or geographic area (e.g., all fish species invading the Great Lakes, Kolar and Lodge 2002), or along each stage of the invasion sequence (e.g., traits important to establishment versus spread in birds invading Australia, Duncan et al. 2001).

A comparative approach that promises to be a particularly powerful tool in invasion studies involves comparing species with common ancestry (Mack 1996a) such as congeners, where one species is a successful invader and the other is not invasive. This approach minimizes the potentially confounding effects of phylogeny (Clutton-Brock and Harvey 1991) and may clarify the importance of small differences in the ecology of related taxa in the context of invasions (Williamson 1996). This approach also allows us to test the idea that there might be taxonomic patterns in invasiveness (i.e., that some taxa (e.g., genera or families) might be generally highly invasive).

McKinney and Lockwood (1999) suggest that biodiversity is currently threatened by the biotic homogenization of local biotas. They suggest that non-indigenous species from a few ‘winning’ taxa are replacing natural diversity (lots of ‘loser’ species). In aquatic systems, the replacement of native species by a few widely introduced species is a major process shaping

communities (Rahel 2000). By comparing congeners, we can ask whether or not all the species in the family appear invasive, and thus test the idea that taxonomic patterns in invasiveness exist.

An ideal system for comparing traits that might explain relative invasiveness in closely related taxa is the poeciliid fish *Gambusia*. Species in this genus show great variation in their geographic distribution, abundance, and apparent invasiveness. At one end of the spectrum, the sister species *G. affinis* and *G. holbrooki* (western and eastern mosquitofish) have widespread distributions and are actively expanding their native North American ranges on their own (Lynch 1988). Mosquitofish have also been successfully introduced for mosquito control in over 40 countries with such significant spread from the points of introduction that they have acquired a panglobal distribution (Welcomme 1992, Lever 1996). In contrast, most of the other 43 species in the genus are rather restricted in their distributions, do not seem to be increasing their native ranges, and have been rarely translocated. The sizes of the species' ranges and their invasiveness differ despite strong similarities in morphology and adult size. These fishes are also ideal for experimental work because of their small size (2-5 cm long), short generation times, reproductive biology, and because they survive and reproduce readily in the laboratory and in field mesocosms.

G. affinis and *G. holbrooki* have been designated to be among the 100 worst invasive species worldwide (ISSG 2000). Not only have mosquitofish failed to control mosquitoes in invaded habitats (Courtenay and Meffe 1989), but they have also had serious negative effects on native biota. The reduction and/or elimination by mosquitofish of native fishes, amphibians, and invertebrates are well documented (Schoenherr 1981, Courtenay and Meffe 1989, Lloyd 1989, Gamradt and Kats 1996, Howe et al. 1997, Goodsell and Kats 1999). A major mechanism for impact seems to be predation of the eggs, fry, and larvae of native biota (Meffe 1985, Courtenay and Meffe 1989, Gamradt and Kats 1996). Competition, mediated by aggression and interference, with native fishes is also significant (Schoenherr 1981, Arthington and Lloyd 1989, Arthington 1991).

The success of mosquitofish in introduced habitats has often been attributed to their broad environmental tolerances, high tolerance of human-disturbed habitats, high population growth rate, aggressive feeding, and omnivorous diet (Myers 1965, Lloyd et al. 1986, Courtenay and Meffe 1989, Ehrlich 1989, Arthington 1991). Additionally, Courtenay and Meffe (1989) consider their livebearing reproduction to be an important element of their success. Furthermore,

female *Gambusia* can fertilize their eggs with stored sperm from multiple males, reducing the negative genetic impacts of founder effects (Chesser et al. 1984). However, no studies have looked at these traits in the more restricted *Gambusia* species. At first glance, the non-invasive *Gambusia* are also small, fast-growing, live-bearing, omnivorous fish. Quantitative comparisons of invasive versus non-invasive *Gambusia* under controlled, experimental conditions are required to identify key characters that might explain their differences in invasiveness.

For this dissertation, I focused on four species: the widespread and invasive sister species *G. affinis* and *G. holbrooki,* and two restricted and non-invasive species, *G. geiseri* and *G. hispaniolae*. *G. geiseri* is the sister species to the invasive species pair (partial phylogeny by Lydeard et al. 1995). It is native to spring-fed headwaters in central Texas, which are characterized by low environmental variation (Hubbs 1995). *G. geiseri* is usually limited to the springhead, while *G. affinis* is native downstream in the same drainages. *G. geiseri* has been introduced to at least seven springs habitats in western Texas (Fuller et al. 1999) with slightly more variable environmental conditions than their native springs (C. Hubbs, unpublished data). However, *G. geiseri* remains localized to the points of introduction and has never spread (C. Hubbs, personal communication), which indicates that this species is non-invasive. This research should shed light on the mechanisms for habitat partitioning between *G. geiseri* and *G. affinis* in their native habitat, and on the factors or traits that might limit the spread of *G. geiseri* in the introduced habitats. *G. geiseri* was also selected for this study because it is the only U.S. endemic *Gambusia* that is not protected at the state or federal level.

G. hispaniolae is native to the Neiba Valley/Cul de Sac region of the island of Hispaniola. Although it is the most widespread of the three endemic *Gambusia* species in Hispaniola, it is still quite restricted in its range (Burgess and Franz 1989). Even presently, when irrigations canals have increased the connectivity of the region, *G. hispaniolae* does not seem to be spreading its range (while other species are). The study population is native to a spring habitat that flows into a hypersaline lake, Lago Enriquillo. Very little is known about the ecology of *G. hispaniolae* as is the case for most restricted *Gambusia*. This species has not been reported to be introduced outside its native range, so nothing definitive is known about its potential invasiveness. However, its lack of spread throughout Hispaniola implies that it is less invasive than *G. affinis* or *G. holbrooki*. This comparative work should provide some understanding of the factors that limit its native distribution and its potential invasiveness.

Ideally, a comparative study aimed at understanding the relationship between traits and invasiveness would map all potentially important traits on a phylogeny that includes numerous species that represent multiple, evolutionarily-independent transitions between invasive and non-invasive states. However, if data on traits cannot be extracted from the literature (which is usually the case for most invasive species and their relatives), and experiments must be conducted to ascertain trait differences, then logistical constraints limit the number of species considered. This constraint explains why extensive comparative studies have rarely been conducted in the past. In the present study, I examined over 15 traits. Therefore, I was only able to consider four species. The four species included in the study represented a non-random sample of all possible *Gambusia*. In *Gambusia*, there are only two highly invasive species, and they are sister taxa. Despite this, there are important differences between them in both their traits (Scribner 1993) and potentially in their invasiveness (i.e., more invasions by *G. holbrooki* than *G. affinis*; Lever 1996); thus, both invasive species were included. For the non-invasive species, *G. geiseri* is the closest relative to the invasive species pair and is thus an obvious choice for the comparison. Finally, since so many species in this genus have a Caribbean distribution, I felt compelled to include a representative of those species - *G. hispaniolae*. Despite these limitations in species choice, I trust that important insights may still be gained from this comparative work.

Several studies have stressed the need to recognize different stages in the invasion process (Ashton and Mitchell 1989, Williamson and Fitter 1996a, Mack et al. 2000). A successfully invading non-indigenous species must first have the means to arrive to a new location (arrival) or be introduced, it must be able to survive and persist in that location (establishment), then it must increase in abudance and disperse into new areas (spread), and lastly it must interact with species in the new areas and cause some significant change in the invaded community and ecosystem (impact). Different traits are expected to allow invaders to succeed in each stage and thus move to the next stage in the invasion sequence (Carlton 1996, Vermeij 1996, Kolar and Lodge 2001).

The goal of this dissertation is to identify the key traits that might explain variation in invasiveness among closely related taxa. In particular, this work aims at gaining a predictive understanding of what traits are key to the establishment success of *G. affinis* and *G. holbrooki* (and to a lesser extent their success at spreading). In order to accomplish this objective, I compared a series of traits between the two invasive species and their two non-invasive relatives.

Then, based on the trait comparisons, I generated predictions on how species should differ in performance (at the population level) if introduced to a novel community. Lastly, I mimicked the invasion of standardized pond communities by both invasive and non-invasive *Gambusia* and compared whether the species were able to 'establish' in the novel community and the degree of establishment success (i.e., population size) achieved by each species.

Organisms arriving in a new community will encounter a certain level of ecological 'resistance' (Elton 1958) that will act to limit their ability to become established. Moyle and Light (1996b) envisioned such resistance as composed of three elements: abiotic, biotic, and demographic. We may then ask: what traits allow invaders to overcome each element of the resistance? Figure 1.1 shows a list of traits I hypothesize to be important in overcoming each element. Only organisms that are able to overcome all three elements will have some nonzero realized population growth in the invaded community. The population size invaders achieve should determine whether or not invading organisms establish populations in the invaded habitat and the level of establishment success (how locally abundant the species becomes).

Invading species with broader environmental tolerances should be better able to cope with novel abiotic conditions in an invaded habitat. For example, for non-indigenous fishes trying to become established in California streams, being able to cope with the novel abiotic conditions (e.g., fluctuating hydrologic regimes) is the most important factor limiting their success (i.e., biotic interactions are secondary; Moyle and Light 1996a, Brown and Moyle 1997). In invading fishes, temperature is often a key factor that limits their habitat use, degree of geographical spread, and reproductive success in invaded communities (Meffe 1991, Welcomme 1992, Crivelli 1995, Brown and Moyle 1997). I predicted that the invasive *Gambusia* should be more tolerant of stressful temperatures than the non-invasives. In particular, because all *Gambusia* are native to relatively warm, southern regions, while mosquitofish have spread north into cooler areas, I examined the low temperature tolerance of the four *Gambusia* species by simulating overwintering conditions in laboratory incubators (Chapter 5).

I expected the ability of invading organisms to cope with novel biotic factors to be largely dependent on three key traits: their ability to respond adaptively to novelty, their aggressiveness, and diet breadth. Invading organisms typically encounter a novel suite of competitors and predators in invaded communities; thus their invasiveness may be strongly affected by their ability to cope with these novel interactions. Plastic behavioral responses may

be critical in aiding organisms to respond appropriately to novelty. I examined the behavioral and foraging response of *Gambusia* to novel competition and predation in short-term laboratory assays (Chapter 3). I predicted *G. affinis* and *G. holbrooki* to be the only species to respond adaptively to novel predation risk (by decreasing foraging and activity and increasing refuge use) and novel competition (by increasing foraging efficiency).

Aggressiveness is suggested to be an important trait that allows species to have successful interactions in the novel habitat with potential competitors and predators (Moyle 1986, Townsend 1996). I tested whether invasive species were more aggressive than non-invasives in individual trials where *Gambusia* were paired with a novel competitor, *Pimephales promelas* (Chapter 3). I quantified the number of agonistic interactions initiated by either the *Gambusia* or the *Pimephales*, with the expectation that invasive *Gambusia* should be more aggressive than non-invasive *Gambusia*.

Organisms that have broad diet requirements should be more tolerant of novel biotic stress (Arthington and Mitchell 1986, Duncan et al. 2001), and probably have greater overall invasion success because prey resources will be rarely limiting (Moyle and Light 1996b). Invading species with wider diet breadth will also have greater impact on the invaded community (Ebenhard 1988, but see Bøhn and Amundsen 2001). I compared the diet breadth of the four *Gambusia* by offering individual females three different prey items at once and quantifying their feeding preferences (Chapter 2).

Beyond diet breadth, the foraging behavior of invaders will also be relevant to their ability to become abundant. All else the same, species or organisms that are more efficient at maximizing energy intake should have greater growth rates and fecundity (Weeks and Meffe 1996). These life history traits are, in turn, known to affect an invader's ability to increase in abundance and colonize a new area (Sakai et al. 2001). I expected invasive *Gambusia* to have higher feeding rates across a variety of prey than non-invasives. I tested maximum feeding rates of *Gambusia* species by offering individual wild females a randomized sequence of three novel prey items (Chapter 2). I also expected invasive *Gambusia* to have higher fecundity and reach sexual maturity at younger ages and smaller sizes than non-invasive *Gambusia*. I measured the number of offspring per brood of F1 females and the age and size at sexual maturity for their F2 offspring raised under standardized, favorable conditions (Chapter 5).

Non-indigenous species are generally defined as invasive only if they are able to spread (i.e., expand their range) beyond their original establishment in the new community (Richardson et al. 2000, Kolar and Lodge 2001). An organism's dispersal ability will then be a key factor to their spread success (Lodge 1993b, Sakai et al 2001). Specifically, a species' innate propensity to disperse and explore unfamiliar or novel space (dispersal tendency) may be important. *G. affinis* and *G. holbrooki* are documented to have high colonization rates and relatively fast spread in invaded habitats (Schoenherr 1981, Meffe et al. 1983, Brown 1987), presumably not only because of their ability to become abundant, but also due to their high dispersal. I expected the invasive species to exhibit greater dispersal tendencies than non-invasives. I assessed the dispersal tendency of the four species by comparing their movement and exploratory behavior in an experimental arena (Chapter 4).

Finally in Chapters 5 and 6, I examined whether the trait differences quantified in the laboratory between invasive and non-invasive species translated into differences in population performance. In two experiments I simulated the invasion of simplified pond communities by each *Gambusia* species and quantified whether or not the species became 'established' (i.e., whether they had some positive population growth) and the degree of establishment success achieved (i.e., how abundant they became). For both simulations, I constructed replicate pond communities in outdoor mesocosms by inoculating tanks with standardized amounts of pond biota (phytoplankton, periphyton, zooplankton, and benthic invertebrates). I then monitored population trajectories in each community for several months. In the first simulated invasion (Chapter 5), each species invaded a community on its own, in which it was the only predatory species. *Gambusia* populations were monitored for over 42 weeks, which included overwintering conditions and their effect on population performance. I expected both invasive species to achieve and maintain greater population that their non-invasive relatives.

In the second simulation (Chapter 6), *Gambusia* invaded communities that already had a top predator present (and a novel competitor), the red shiner, *Cyprenella lutrensis*. Because of logistic constraints, I only used two *Gambusia* species, *G. affinis* (invasive) and *G. geiseri* (non-invasive) in this simulation. To further address the effect of competition on each species' colonizing ability, I included a treatment simulating invasions by both the invasive and non-invasive *Gambusia* at once (without red shiners). Relative to *G. geiseri*, I expected *G. affinis* to exhibit greater population growth rates, both when it was the only fish species in the community

and when red shiners or *G. geiseri* already inhabited the community. I measured each species' population size over a period of 12 weeks. I also quantified Nitrogen and Carbon stable isotope signatures of *Gambusia* in competition and no competition treatments to test whether *G. geiseri* and *G. affinis* differ in their trophic placement, and whether competition resulted in trophic shifts in either species. The experiment also included a treatment where no fish were present, which allowed me to test whether the invasive and non-invasive species differed in their top-down community impacts. I hypothesized that *G. affinis* and *G. geiseri* should have functionally nonequivalent community roles and expected *G. affinis* to have more pronounced impacts on lower trophic levels than *G. geiseri*. I measured community impact by assessing the abundances of pond biota in tanks with *G. affinis* alone, *G. geiseri* alone, and no fish present.

The success or failure of biological invasions is at least partially dependent on the traits of the invading species. Only by studying a variety of traits can we gain insights into their relative relevance to the invasion process. By examining their relevance along each step of the invasion sequence, we can also better understand the exact mechanisms allowing species to invade. This dissertation is an unusually detailed study that provides valuable insight into the traits most relevant to establishment success.

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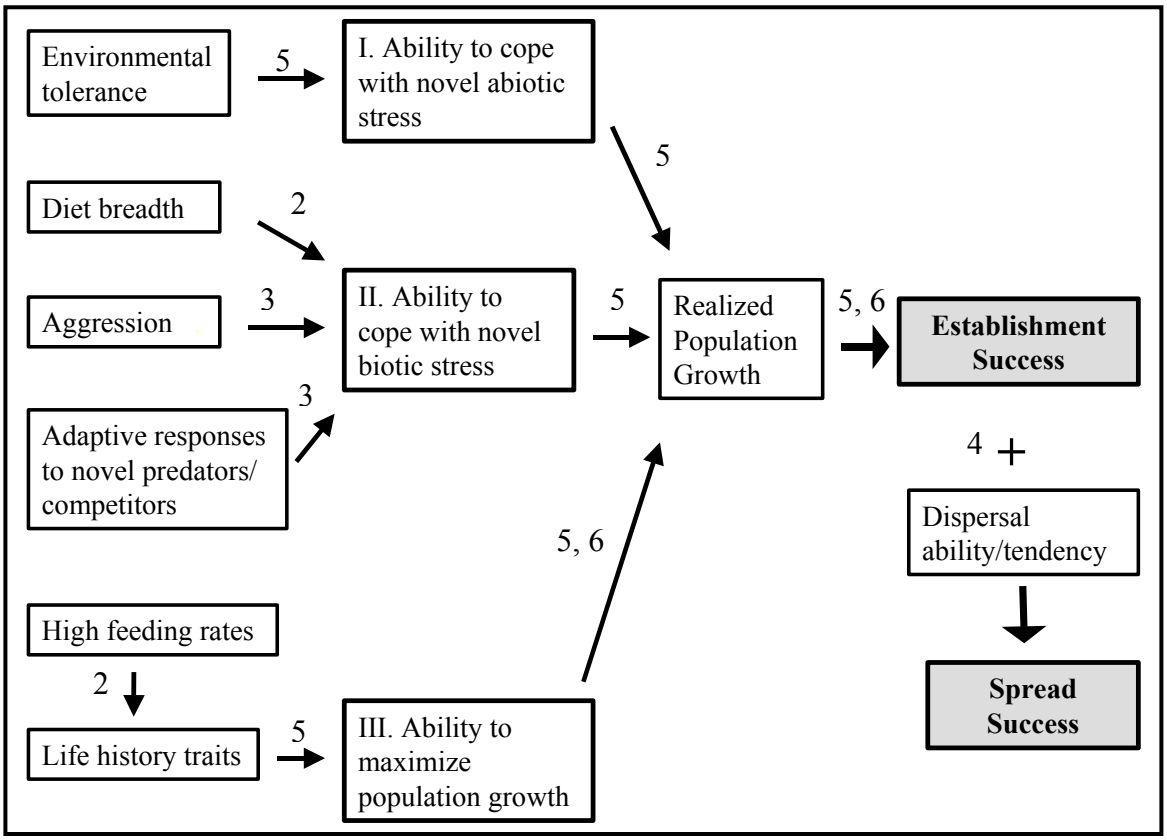
Figure 1.1: A conceptual overview of the key ecological, life history, and behavioral traits that may influence the abiotic, biotic, and demographic elements dictating abundance and thus invasion success in invaded habitats. The numbers correspond to chapters in the dissertation.



Chapter Two

Foraging behavior and invasiveness: Do invasive fish exhibit higher feeding rates and broader diets than non-invasive congeners?

SUMMARY

Serious impacts by non-indigenous species often occur via predation. The magnitude of impact should be closely tied to the invading species' niche breadth. Invaders with wider ecological niches will likely interact with and impact a greater number of species. For predatory impacts, diet breadth should be particularly important. I examined how foraging behavior relates to invasiveness and invader impact by comparing feeding rates, preferences, and diet breadth for four *Gambusia* species, two invasive and of high impact and two non-invasive. Individual feeding rates, feeding preferences, and diet breadths were tested across three different prey types in a sequence of four laboratory trials with wild caught females. In the first three trials, I measured maximum feeding rates on each prey individually, and in the fourth trial all prey were offered at once and I quantified total feeding rates, feeding preferences, and diet breadth. I found differences between invasive and non-invasive *Gambusia* in feeding rates, but there were no differences in the measures of diet preference or breadth. Invasive *Gambusia* fed at significantly higher rates on all prey and in all four trials. All species preferred *Daphnia*, avoided *Lirceus*, and consumed *Drosophila* in proportion to their availability. Female size affected feeding rates, prey preferences, and diet breadth. Larger fish of all species consumed more prey per unit time and were able to incorporate larger prey items into their diets, increasing diet breadth.

INTRODUCTION

Predation is a major force shaping natural communities, affecting species' abundances, distributions, and composition (Paine 1966, Connell 1975, Sih et al. 1985, Power 1992). The structuring role of predation is particularly evident when predators are introduced outside their native range (Thorp 1986). While only a minority of non-indigenous species significantly affect invaded biotas (Simberloff 1981, Williamson 1996), when deleterious impacts occur, they are often due to predation (Lodge 1993a). In particular, predation has been implicated as a major mechanism for native species extinctions (Simberloff 1981, Moyle and Light 1996a, Mooney and Cleland 2001). Classic examples of the large impact of predation involve the invasion of novel predators on to oceanic islands (Elton 1958). Among the most notorious cases are: the extinction of reptiles, amphibians, and mammals in the West Indies and Pacific islands caused by the voracious Indian mongoose *Herpestes javanicus* (Roots 1976, Case and Bolger 1991), the extinction of Australian marsupials and New Zealand birds caused by feral cats *Felis catus* (King 1985, Dickman 1996), the extinction of passerine birds in Guam due to the brown tree snake *Bioga irregularis* (Fritts and Rodda 1998), and the extinction of Pacific island snails driven by the carnivorous rosy wolfsnail *Euglandina rosea* (Cowie 1992).

In aquatic systems, top predators, in particular, have been shown to exert the greatest negative impact on invaded communities (Courtenay and Moyle 1992). The best-known example involves the introduction of the Nile perch *Lates niloticus* to Lake Victoria, which resulted in the extinction of over 200 of the 400 native haplochromine cichlids (Ogutu-Ohwayo 1999). In the United States, introductions of predatory centrarchids to western states have decimated native fish species already impacted by habitat alteration (Moyle 1976, Minckley 1991). In the Great Lakes, predation by introduced lamprey, alewife, and rainbow smelt has dramatically altered fish assemblages (Moyle 1986) facilitating further invasion of the ecosystem (presently more than 140 non-indigenous species) (Mills et al. 1993).

Serious predatory impacts, however, not only result from large piscivorous species, but also from smaller omnivorous fishes (Moyle and Light 1996b). Predation by the introduced mosquitofish, *Gambusia affinis* and *Gambusia holbrooki*, has been implicated in the extirpation of native fish, amphibians, and invertebrates (Schoenherr 1981, Courtenay and Meffe 1989, Lloyd 1989, Howe et al. 1997, Gamradt and Kats 1996, Goodsell and Kats 1999). Although

competitive interactions have been shown to be important, predation is the major mechanism for impact (Myers 1965, Meffe 1985, Courtenay and Meffe 1989, Gamradt and Kats 1996). Mosquitofish are aggressive foragers, feeding on a variety of prey, including the eggs, fry, and larvae of native biota (Goodell et al. 2000). This is presumably the reason why they were chosen as a biocontrol agent against mosquitoes (and mosquito-borne diseases) (Krumholz 1948). In this study, I examined the foraging behavior of these two highly invasive species in an effort to better understand their invasion success and impact.

The impact that invaders have on invaded communities should be intimately related to the invaders' niche breadth. Invading species with wider ecological niches will likely interact with a greater number of species (Goodell et al. 2000). For impacts via predation, diet breadth should be of particular interest. Studies have shown that diet generalists often have greater impacts than specialists (Ebenhard 1988, but see Bøhn and Amundsen 2001) and greater overall invasion success since prey resources are rarely limiting (Moyle and Light 1996b). To explore how diet breadth and feeding behavior in general relate to invasiveness and impact, I compared feeding rates, feeding preferences, and diet breadth of the highly invasive (and high impact) mosquitofish species to two non-invasive congeners. Comparisons of closely related species are an insightful approach to the identification of key traits conferring invasiveness (Mack 1996a, e.g., Rejmanek and Richardson 1996). Two *Gambusia* species of relatively low invasive potential, *G. geiseri* and *G. hispaniolae,* were chosen for comparison. In individual trials, I tested each species' maximum feeding rates, feeding preferences, and diet breadth across three different prey items. I hypothesized that the invasive species would exhibit both greater feeding rates and greater diet breadth (i.e., no preference) relative to their non-invasive relatives.

METHODS

I quantified maximum feeding rates, feeding preferences, and diet breadth for 12 wild females of each *Gambusia* species. Females used in the experiment were gravid adults collected from within each species' native range. In *Gambusia*, males typically reduce foraging and growth when sexually mature (Krumholz 1948); therefore, females have a greater potential to impact prey communities. Adult females are also the gender and age class of interest in an invasion context. Female *Gambusia* are multiply-inseminated and able to store sperm for long

periods of time (Zane et al. 1999), which allows them to start populations on their own without negative founder effects (Chesser et al. 1984).

Each female's feeding behavior was tested in a sequence of four laboratory trials. In the first three trials, maximum feeding rates on three different live prey items were quantified (one prey type per trial). The three prey chosen differed in microhabitat use and size. In each trial, I used the same prey biomass (approximately 0.020 g) but different numbers of prey: 40 *Daphnia pulex* (planktonic prey), 20 flightless *Drosophila melanogaster* (neustonic prey), and 15 isopods *Lirceus fontinalis* (benthic prey). Approximate prey lengths were as follows: 2.5 mm for the *Daphnia*, 3.5 mm for the *Drosophila,* and 4.5 mm for the *Lirceus*. Prey widths were more similar (under 2.5 mm for all prey) and more relevant since fish always consumed the largest prey (i.e., *Lirceus*) lengthwise. Small differences in gape size among *Gambusia* species are then not expected to have an overriding effect on preference measures. Experimental prey sizes are also within the range of sizes naturally consumed by *Gambusia* (Garcia-Berthou 1999). In the fourth trial, the three prey types (in the same quantities as in first three trials) were offered to fish simultaneously. In this last trial, I measured total feeding rates (on all prey combined), feeding preferences, and diet breadth.

Feeding trials were conducted in two nine-day time blocks in December 1999 and January 2000. For each block, *Gambusia* females were randomly chosen from stock tanks and placed individually in 6 L clear plastic containers. Females were given 48 hours (days 1 and 2) to acclimate to individual tanks during which time they were fed *ad libitum* Tetramin® flakes. On days 3, 5 and 7, I conducted the feeding trials with each single prey type. For each species, two females experience each of the six possible sequences for the three trials. To standardize hunger levels, I conducted *ad libitum* feedings in between trials (days 4, 6 and 8) followed by a starvation period. Fish were allowed to consume flakes *ad lib*. for a half hour, and then flakes were removed 23.5 hours prior to the next trial. On day 9, I conducted preference trials with all prey combined.

Trials ran for either 10 minutes or until all prey were consumed. From observations conducted during the trials, I calculated maximum feeding rates (number of prey consumed divided by trial duration). To quantify feeding preferences in the fourth trial, I computed a selection index for each prey (Manly et al. 1993). The selection index was simply the proportion of total prey consumed that were of a given type divided by the proportion of that prey type

available to the fish during the trial. Selection indices were then standardized (so that they add up to 1) by dividing each selection index by the sum of the three indices. Since there were three prey types in the experiment, a selection index above 0.33 indicated relative preference and values below 0.33 indicated relative avoidance. To assess diet breadth, I calculated Levins' (1968) measure of niche (or diet) breadth by squaring the proportions of each prey type consumed during the fourth trial (out of the total prey consumed), taking the sum and then the inverse. A value of 3 for Levins' measure indicated that organisms were consuming all three prey indiscriminately.

Fish used in the experiment were collected in the summer and fall of 1999. *G. geiseri* and *G. affinis* females were collected from Comal springs and the Comal river respectively in Comal County, TX. *G. holbrooki* females came from Leon Hines Lake, Escambia County, AL, and *G. hispaniolae* females were collected from La Azufrada spring, Lake Enriquillo, Dominican Republic. *Daphnia* and *Drosophila* were obtained from pond cultures at the University of Kentucky Ecological Research Facility and from laboratory colonies respectively. Isopods were collected from Glenns Creek, Woodford County, Kentucky. The experiment was conducted under room temperature (mean water temperature: 22.5 $\pm$ 0.77 º C) and long photoperiod conditions (14 hours light: 10 hours dark). Periodic water changes (50 %) were conducted in *Gambusia* tanks to maintain water quality instead of using aeration or filters that might interfere with prey. No refuges or substrate were provided for either the prey or the *Gambusia*. Fish standard length was measured at the end of trial sequences. Final sample size was 47 individuals due to one *G. affinis* mortality.

Statistical analyses

A repeated measures ANCOVA with female size as a covariate was used to test for species differences on the four feeding rates. To meet parametric test assumptions, all feeding rates were log transformed (Ln of observed value + 1) before analysis. No transformations were needed for the three selection indices or diet breadth since distributions were approximately normal and variances homogenous. Species differences on these four preference measures were tested with a MANOVA followed by four individual ANOVA's. If female size was found to be a significant covariate, ANCOVA results are reported instead. T-tests were utilized to test whether selection indices differed significantly from 0.33 (the no-preference value) and whether diet

breadth differed significantly from 3 (the broadest possible diet) for each *Gambusia* species. Simple linear regressions were used to test the nature of the relationship between female size and the foraging variables when size was found to be a significant covariate. Preliminary analyses indicated no effect of prey sequence or the blocking factor on any of the variables; thus sequence and blocking effects were left out of final analyses.

Orthogonal contrasts were used to test for species differences once a significant species effect was found. In three contrasts, I tested whether the two invasive species differed from the two non-invasive species and whether there were significant differences between species within the invasive category (*G. affinis* versus *G. holbrooki*) and between species within the non-invasive category (*G. geiseri* versus *G. hispaniolae*). P-values for comparisons of the two invasive against the two non-invasive species correspond to one-tailed tests. All other reported p-values reflect two-tailed tests. Statistical tests were conducted using SYSTAT® Version 10.

RESULTS

The repeated measures analysis of covariance detected a species effect on maximum feeding rates (Table 2.1). Orthogonal contrasts revealed that all four feeding rates were significantly higher in invasive *Gambusia* than the two non-invasive species (Figures 2.1 and 2.2a). On individual prey, invasive *Gambusia* foraged on average at rates 34 % higher than the non-invasives (*Daphnia*, $F_{1, 42} = 4.9$, $p = 0.02$; *Drosophila*, $F_{1, 42} = 3.3$, $p = 0.04$; *Lirceus,* $F_{1, 42} = 3.7$, $p = 0.03$). Feeding rates on all prey combined during trial 4 were 17 % higher for *G. affinis* and *G. holbrooki* relative to *G. geiseri* and *G. hispaniolae* ($F_{1, 42} = 5.3$, $p = 0.01$) (Figure 2.2a). I found no differences in feeding rates between the two invasive or between the two non-invasive *Gambusia.* Overall feeding rates in the first three trials seemed highest on *Daphnia*, lowest on *Lirceus*, and intermediate for the *Drosophila* prey (Figure 2.1); however no significant differences were found (Table 2.1).

The MANOVA comparing the four preference measures showed no species effect (Wilks' Lambda, $F = 0.7$, $p = 0.75$) indicating that invasive and non-invasive *Gambusia* exhibited similar feeding preferences and diet breadth. Individual ANOVA's and ANCOVA's confirmed this result (Table 2.2). In general, all species preferred *Daphnia*, consumed *Drosophila* in proportion to their availability, and avoided *Lirceus* (Figure 2.2b). For *Daphnia*,

selection indices of the four species were consistently greater than 0.33 (*G. affinis*: p = 0.001, *G. holbrooki*: p = 0.001, *G. hispaniolae*: p = 0.04, and *G. geiseri*: p = 0.06). For *Lirceus*, selection indices irrespective of species were significantly lower than 0.33 (p = 0.0001 for all species), and all four species ate *Drosophila* in proportion to their availability (no significant differences from 0.33). Diet breadths also did not differ significantly among the species (Table 2.2). Levins' measure of diet breadth averaged 1.7 for both invasive and non-invasive *Gambusia.* Mean diet breadths of all four species differed significantly from 3 ($p < 0.0001$) indicating that none of the species exhibited the broadest diet possible.

As may be expected, fish size had an effect on most measures of feeding behavior. A significant female size by feeding rate interaction was found in the repeated measures analysis of feeding rates (Tables 2.1). Female size was also a significant covariate for two of the selection indices and diet breadth (Table 2.2). The nature of the relationship between fish size and feeding rate was positive for all four rates but weakest for the isopods (*Daphnia,* p = 0.003; *Drosophila,* p = 0.0001; *Lirceus,* p = 0.05; all prey, p = 0.0001). R^2 values were also low for all regressions (0.16, 0.25, 0.06, and 0.38 respectively). Larger *Gambusia* fed at higher rates across all prey offered (either individually or combined). For the preference measures, there was no relationship between fish size and the smallest prey, *Daphnia*, but a positive relationship was found for the other two less preferred and larger prey items (*Drosophila,* p = 0.004, $R^2 = 0.15$; *Lirceus*, p = 0.009, $R^2 = 0.12$). Diet breadth was also positively related to fish size (p = 0.002, $R^2 = 0.19$). Only larger fish were able to incorporate the larger prey items into their diets, and thus exhibited a greater diet breadth.

DISCUSSION

The foraging behavior of predatory species is expected to be a key factor affecting their invasiveness and impact in invaded communities (Lodge 1993b). Species or organisms that are opportunistic foragers and diet generalists are typically expected to achieve greater invasion success and impact. However, few studies of foraging behavior (and of behavior in general) have tested this expectation (Holway and Suarez 1999). In invasive *Gambusia*, research on foraging behavior is clearly needed to better understand the impact of their introductions (Garcia-Berthou 1999). For this comparative study, I expected the invasive species, *G. affinis* and *G. holbrooki,* to

have greater feeding rates and broader diets than their non-invasive relatives (*G. geiseri* and *G. hispaniolae*). The results show some evidence that the two invasive *Gambusia* species are superior foragers compared to the non-invasive species. Feeding rates were higher for the invasives on all prey, either when offered individually or combined. However, diet preference and breadth results contradict the classic notion that invasive species, especially those with significant predatory impact, should have broad diets (reviewed by Lodge 1993b). Selection indices on the three prey used in the experiment and overall diet breadth were identical for invasive and non-invasive *Gambusia*. All *Gambusia* preferred *Daphnia,* the smallest prey item, exhibited no relative preference for *Drosophila,* and avoided *Lirceus*.

While little is known about the foraging behavior of non-invasive *Gambusia*, invasive mosquitofish are considered generalist predators (Farley 1980). The mosquitofish diet is composed of zooplankton, drifting and benthic invertebrates, and terrestrial and neustonic prey (Lloyd et al. 1986, Garcia-Berthou 1999). Stomach content analyses show that cladocerans including daphniids often constitute a significant proportion of the diet (Crivelli and Boy 1987, Blaustein and Karban 1990, Garcia-Berthou 1999). The higher feeding rates and strong preference for *Daphnia pulex* seen in the invasive species in this study concur with these dietary observations.

In invaded areas, mosquitofish feed opportunistically on naive prey that have no evolutionary history with mosquitofish predation. Naive prey, often eggs and larval stages of amphibians and fishes, either completely lack antipredator responses or show responses that are ineffective against novel predation by mosquitofish (e.g., Gamradt and Kats 1996). This trophic opportunism allows mosquitofish to decimate local prey populations and threatens the long-term persistence of many aquatic species in the invaded communities (Meffe 1985, Courtenay and Meffe 1989, Arthington 1991). The higher consumptive rates of invasive *Gambusia* across different prey noted in my trials may be revealing of this opportunistic foraging style.

Feeding preferences were highest for *Daphnia*, intermediate for *Drosophila*, and lowest for isopods. These results contradict the simple expectation that large prey should be preferred because of their higher energy content (Werner and Hall 1974). However, larger prey may not necessarily be more profitable. Prey profitability (energy per unit handling time) may decrease with increasing prey size if handling and/or digestion times become disproportionately large (Bence and Murdoch 1986, Kaiser et al. 1992). This may explain the strong preference for

Daphnia in all four *Gambusia* species. *Daphnia* may have been in fact the most profitable prey, since handling times (although not directly quantified) seemed virtually instantaneous for *Daphnia*, but were relatively long for *Drosophila* and even longer for *Lirceus*. Previous studies on invasive *Gambusia* foraging behavior have shown that these fish often preferentially consume the smallest prey available (Bence and Murdoch 1986, Arthington 1991).

The preference for *Daphnia* may have also resulted from a prey density effect: *Gambusia* were attacking and consuming the most frequently detected prey. Encounter rates were most likely higher and search times lower for *Daphnia* relative to the other two prey species, not only because *Daphnia* were in the highest density, but also because *Daphnia* were in the water column while flies and isopods were limited to either the water surface or the tank bottom. These differences in prey distribution may have resulted in distinct prey patches, in which case prey density may matter. While optimal foragers are typically expected to ignore density and choose prey according to profitability only (MacArthur and Pianka 1966), if prey are patchy, foragers should respond to density by spending more time in the most profitable patches (i.e., the water column) where prey are aggregated (Hassell and May 1974). Regardless of the mechanism and contrary to my predictions, invasive and non-invasive *Gambusia* seemed to be making identical foraging choices, and thus exhibiting equal diet preferences and breadths.

Not surprisingly, *Gambusia* size affected feeding rates, prey preferences, and overall diet breadth. The effect was similar for the invasive and non-invasive species. Larger fish consumed more prey per unit time and were able to include larger prey items into their diet, increasing diet breadth. This result may have important implications for *Gambusia* impact in light of recent research documenting body size changes in invaded communities. Several studies have reported greater body sizes for invaders in their invaded range relative to their native range (Crawley 1987, Willis and Blossey 1999, Leger and Rice 2003), presumably a response to a release from natural enemies in the introduced range (Keane and Crawley 2002). Introduced *Gambusia* have been shown to have lower loads of parasites than either ecologically similar native fishes in the invaded range or their native populations (Dove 2000). This enemy release may allow *Gambusia* to achieve greater sizes in invaded areas, and based on these results, greater feeding rates and diet breadth and perhaps greater impact.

A few studies have examined whether diet or diet breadth is a key correlate of invasion success and impact. Among them, studies on birds introduced to oceanic inlands provide some of

the best evidence on the relationship between species-specific traits and invasion success given that detailed records exits on both failed and successful introductions (Kolar and Lodge 2001). In such studies, the relevance of diet as a predictor of invasion success has mixed support. McLain et al. (1999) reported diet to be a significant predictor (among other traits) of introduction success for 132 bird species brought to nine oceanic islands. Bird species that incorporated both seeds and fruit into their diets were more likely to establish than more restricted foragers. Duncan et al. (2001) found that omnivorous and carnivorous diets were good predictors of establishment success for birds introduced to Australia but were unrelated to the amount of spread of these species. Diet breath was not correlated with establishment in birds introduced to New Zealand (Veltman et al. 1996).

Among fishes, evidence on the significance of diet breadth as an important ecological trait conferring invasiveness is also conflicting. Marchetti et al. (2003) showed that for non-indigenous fishes in California, diet breadth was not a good predictor of establishment, but it was positively correlated with spread and abundance (surrogates for impact) in invaded watersheds. Kolar and Lodge (2002) found that neither the establishment, spread, nor impact of non-indigenous fishes in the Great Lakes region was predicted by diet breadth. Overall, these differences seem to indicate that the predictive power of diet breadth in the context of invasion success and impact might be limited. This may be especially true when we compare diet breadth to life history characters (Sakai et al. 2001) and characteristics of the invasion effort (i.e., propagule pressure) (Williamson 1999), which seem to consistently correlate with measures of invasion success. Further studies on the foraging behavior of invasive versus non-invasive species might focus not just on diet breadth, but on other aspects of foraging, such as voracity per se and alternative measures of plasticity in foraging (e.g., foraging innovations, Sol et al. 2002).

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Table 2.1: Results from a repeated measures ANCOVA performed on feeding rates measured on the three prey items (*Daphnia*, *Drosophila*, and *Lirceus*) in trials 1-3 and on all prey combined in trial 4.

Effect	*df*	*F*	*p-value*
Species	3, 42	2.9	0.05
Female size	1, 42	13.2	0.001
Feeding rates	3, 126	1.4	0.25
Feeding rates x species	9, 126	1.3	0.25
Feeding rates x female size	3, 125	3.4	0.04

Table 2.2: Summary of statistical analyses of feeding preferences for each prey and overall diet breadth. Analyses of covariance are shown if covariate (*Gambusia* size) was significant.

Effects	Selection indices									Diet breadth		
	Daphnia			*Drosophila*			*Lirceus*					
	df	F	p	df	F	p	df	F	p	df	F	p
Species	3	0.7	0.54	3	0.5	0.72	3	0.6	0.62	3	0.5	0.68
Female size				1	4.5	0.04	1	7.9	0.007	1	7.9	0.008
Error	43			42			42			42		

Figure 2.1: Plots of feeding rates (FR) in numbers of prey consumed per minute on (a) *Daphnia*, and (b) *Drosophila* as a function of female standard length. Least-squares regression lines have been fitted separately to the invasive (solid line) and the non-invasive (dashed line) *Gambusia.*

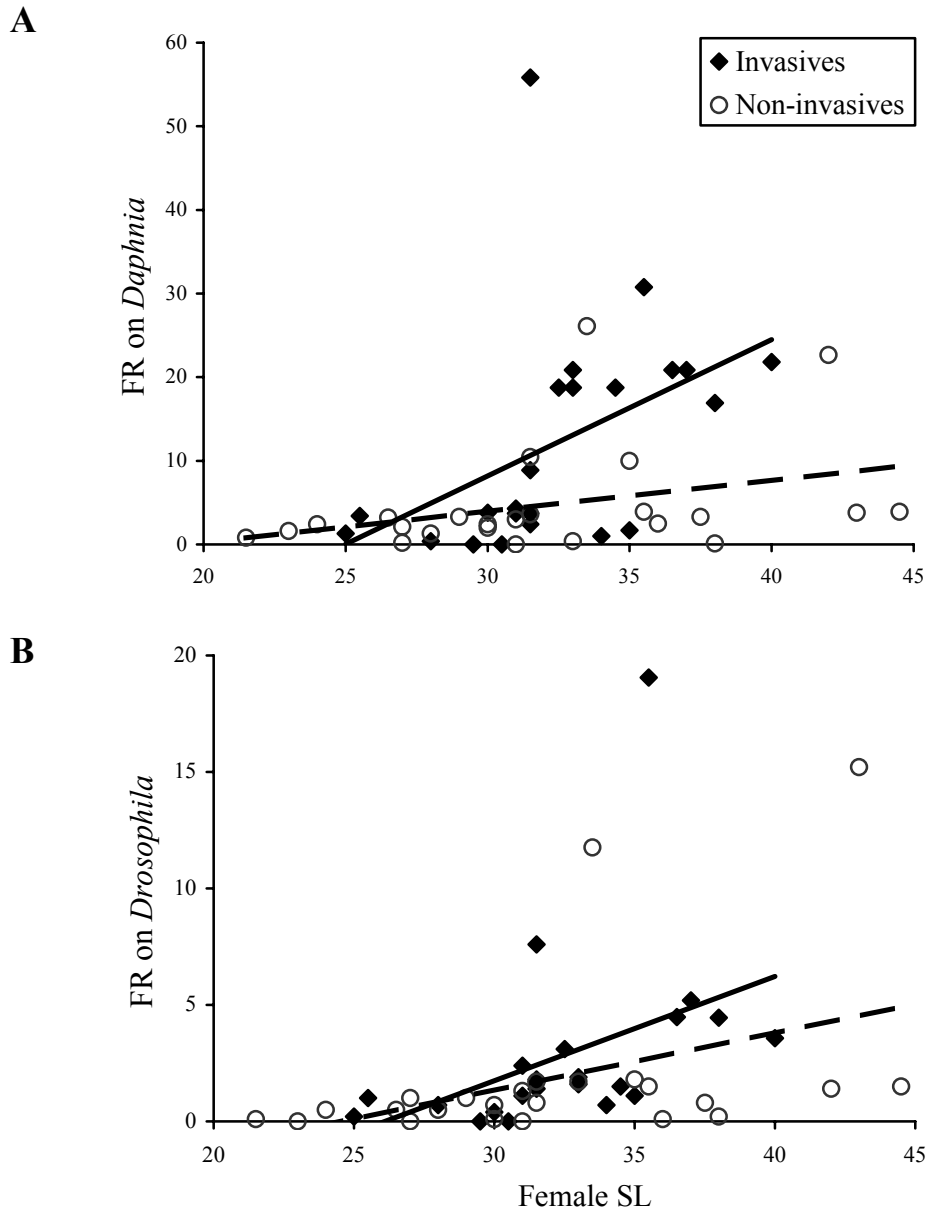


Figure 2.1: (c) Plot of feeding rate (FR) on *Lirceus* as a function of female standard length. Least-squares regression lines have been fitted separately to the invasive (solid line) and the non-invasive (dashed line) *Gambusia.*

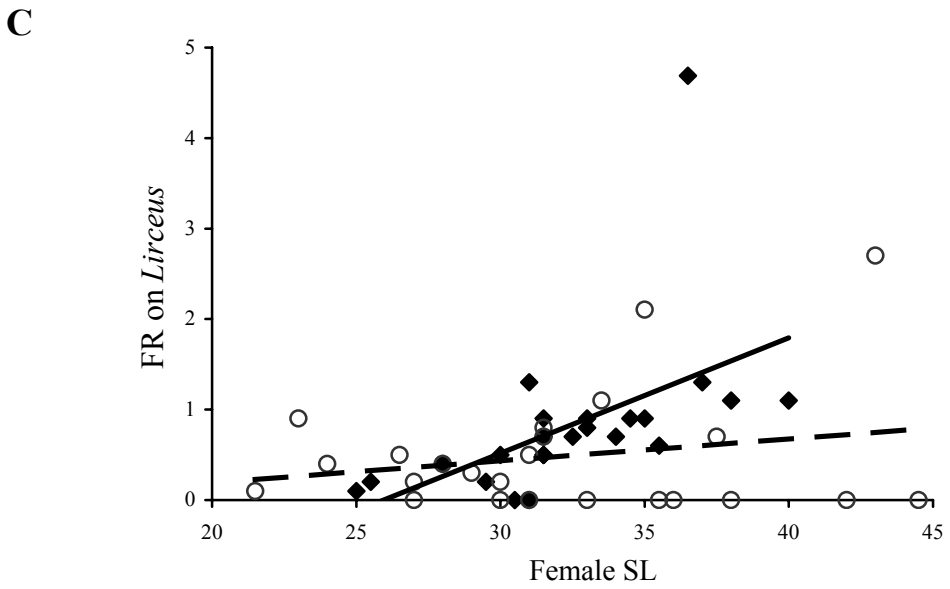
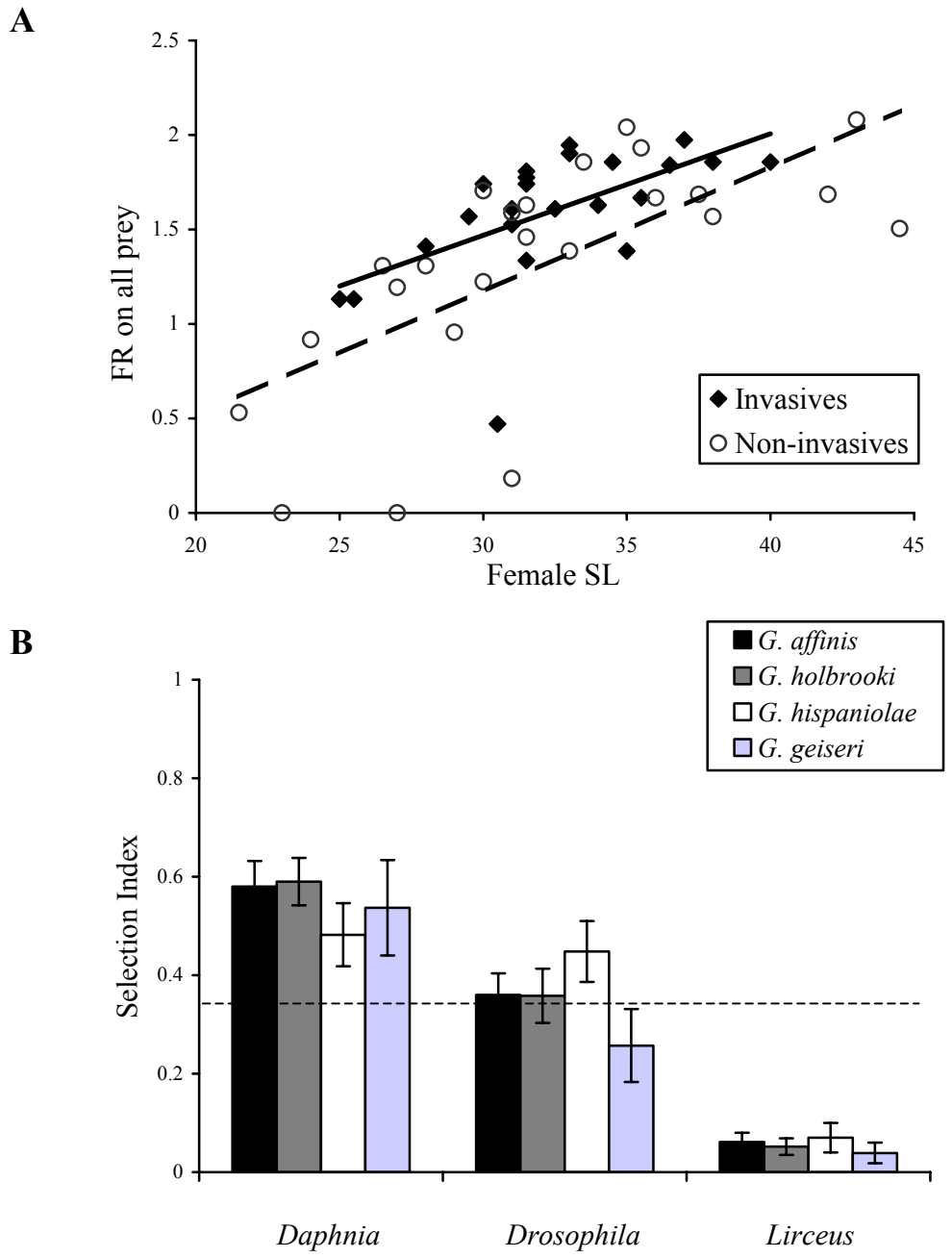


Figure 2.2: (a) Feeding rates on all three prey combined during trial 4 as a function of female size. Regressions were fitted separately to the invasive (solid line) and non-invasive (dashed line) *Gambusia.* (b) Preference measures (means ± 1 SE) for each prey type by the four *Gambusia* species. The dotted line indicates no preference (0.33).



Chapter Three

Behavioral responses to novel predation and competition: A comparison of invasive mosquitofish and their non-invasive relatives

SUMMARY

Attributes of invaded communities are known to affect the invasion success of arriving non-indigenous organisms. In particular, biotic interactions (competition and predation) can enhance the resistance of recipient communities to invasion. Invading organisms typically encounter a novel suite of competitors and predators in invaded communities, and thus their invasiveness may be strongly affected by their ability to cope with these novel interactions. Plastic behavioral responses may be critical in aiding organisms to respond appropriately to novelty. I examined the responses of highly invasive mosquitofish to representative novel competitors and predators that they might encounter as they spread through North America. To elucidate whether these responses to novelty may in fact relate to invasiveness, I conducted short-term, laboratory behavioral assays to compare the responses of these two invasive species to those of two closely related species that are much less invasive. Adult wild females of each species were paired with a novel competitor, the fathead minnow, and a novel predator, smallmouth bass, and their responses measured in terms of foraging success, foraging efficiency, activity, refuge use, predator inspection behavior, and interspecific aggression. The results provided evidence that invasive species generally respond more appropriately to novel biotic challenges than non-invasive species. In addition, I observed significant differences in responses between the two invasive species and between the two non-invasive ones.

INTRODUCTION

While all communities appear to be susceptible to invasion (Usher 1988, Williamson 1996, Lonsdale 1999), the level of invasion success achieved by non-indigenous species may vary depending on characteristics of the invaded community. In particular, trophic structure and the strength of species interactions seem to significantly affect the invasibility of communities (Mack et al. 2000, Sakai et al. 2001). Biotic interactions (i.e., competition and predation) are thought to enhance the 'resistance' of recipient communities to invasion (Elton 1958, Moyle and Light 1996). Predation on non-indigenous species often results in the prevention of establishment and the deceleration of spread (Lodge 1993a, Jaksic 1998, Reusch 1998). Competition with native species can lower resources available to invading species and may also contribute to invasion failure (Tilman 1997). Communities with higher diversity (often with more intense competition or more predators) have been generally found to be more resistant to invasion (Vermeij 1991, Stachowicz et al. 1999, Tilman 1999, Kennedy et al. 2002, but see Stohlgren et al. 1999). Similarly, areas where human impact has disrupted species assemblages and community structure (i.e., those with empty niches) often appear more prone to invasion (Moyle and Light 1996, Vitousek et al. 1996, Williamson 1996).

Because coevolved enemies will likely be absent (Mitchell and Power 2003, Torchin et al. 2003), invaders into novel communities generally encounter a suite of novel enemies (Strong et al. 1984). A species' ability to invade should be affected by how invaders respond to these novel enemies (Shea and Chesson 2002). If novel enemies are similar to natural enemies, organisms may respond adaptively because they are essentially 'preadapted' to new conditions (Sakai et al. 2001). If, however, enemies in the new community are truly novel, phenotypic plasticity may allow organisms to respond adaptively (Vermeij 1996, Hänfling and Kollman 2002). Thus, plastic behavioral responses should often be important in aiding organisms to respond appropriately to novelty (Schlaepfer et al. 2002). In birds, the best-studied invasive taxon (Kolar and Lodge 2001), behavioral flexibility is strongly correlated with invasion success. Bird species with a higher frequency of foraging innovations achieve greater invasion success (Sol and Lefebvre 2000, Sol et al. 2002).

In the present study, I used laboratory assays of behavioral responses to novel competitors and predators to test the idea that ability to cope well with novel biotic challenges is

a component of species invasiveness. Short-term behavioral assays are a useful tool in ecology, often yielding important insights into species interactions (e.g., Milinski and Heller 1978, Gilliam and Fraser 1987, Abrahams and Dill 1989). Short-term assays are also helpful in generating predictions about longer-term population level responses to novel species interactions (tested in Chapter 6). In particular, I quantified the foraging and behavioral responses of the two invasive mosquitofish species (*Gambusia affinis* and *Gambusia holbrooki*) to novel predators and competitors, and compared them to responses exhibited by two closely related species of lower invasion success, *G. geiseri* and *G. hispaniolae*. Smallmouth bass (*Micropterus dolomieu*) were used as novel predators and fathead minnows (*Pimephales promelas*) as novel competitors. I measured the four species' responses to novelty by comparing their foraging success, foraging efficiency, activity, and refuge use in the presence and absence of novel predators and competitors. I also quantified whether invasive and non-invasive *Gambusia* differed in predator inspection behavior or agonistic interactions with novel competitors.

SYSTEM

Because of their ability to spread widely and their negative impacts on aquatic communities, mosquitofish, *Gambusia affinis* and *G. holbrooki,* have been designated to be among the 100 worst invasive species worldwide (ISSG 2000). Efforts to reduce the incidence of malaria early last century resulted in the widespread introduction of mosquitofish to over 40 countries as biocontrol agents against mosquitoes (Krumholz 1948, Welcomme 1992, Lever 1996). While mosquitofish have often been released in highly disturbed or artificial habitats, they eventually spread into pristine areas (Arthington and Lloyd 1989) where they severely impact native fish, amphibians, and invertebrates (Schoenherr 1981, Lloyd 1989, Howe et al. 1997, Webb and Joss 1997, Gamradt and Kats 1996, Goodsell and Kats 1999). Their impact is primarily through predation, usually of the eggs, fry, and larvae of native biota (Meffe 1985, Courtenay and Meffe 1989, Gamradt and Kats 1996); thus my focus on how novel interactions may affect their foraging success and underlying behaviors.

The congeners *G. geiseri* and *G. hispaniolae* were selected for comparison because of their seemingly low invasive potential. *G. geiseri* is native to spring habitats of the Southwestern U.S. (Hubbs and Springer 1957) and appears to be a sister taxon to the invasive species pair

(based on a partial phylogeny by Lydeard et al. 1995). While several introductions of *G. geiseri* have been conducted in spring habitats similar to its own (Fuller et al. 1999), *G. geiseri* has failed to spread from points of introduction (C. Hubbs, personal communication). *G. hispaniolae* is native to the southwest region of the Dominican Republic, and although it is the most widespread of the three endemic *Gambusia* in Hispaniola, it is still rather restricted in its range (Burgess and Franz 1989). Even presently, when irrigation canals have increased the connectivity of the region and allowed other fish species to spread, *G. hispaniolae* has not spread.

METHODS

To examine how invasive and non-invasive *Gambusia* species responded to novelty, I exposed wild, gravid *Gambusia* females to a novel predator and a novel competitor in separate laboratory experiments. Females were collected from within each species' native ranges (not their invaded range). Novel predators and competitors were species that have not coevolved with any of the four *Gambusia* species. Because of my interest in exploring correlations in feeding performance and behavioral responses across predation and competition contexts (these correlations are reported elsewhere), I used the same individual females for both experiments. I chose adult females because they are the gender and age class that is most relevant for initial invasions. *Gambusia* females can store sperm from multiple males; thus a single female can initiate a population with minimal negative inbreeding effects (Chesser et al. 1984, Zane et al. 1999).

G. geiseri and *G. affinis* females were collected from Comal springs and the Comal river respectively in Comal County, TX. *G. holbrooki* females came from Leon Hines Lake, Escambia County, AL, and *G. hispaniolae* females were collected from La Azufrada spring, Lake Enriquillo, Dominican Republic. All collections were made in the late summer and fall of 1999.

Novel Competition

Fathead minnows (*Pimephales promelas)* were chosen as novel competitors because they exhibit significant overlap in resource use with *Gambusia* (i.e., they are potential competitors),

but do not co-occur with focal *Gambusia* populations. Fathead minnows are very similar to invasive mosquitofish in their habitat requirements (usually shallow and vegetated portions of lakes and streams), broad diets, and wide physiological tolerances (Moyle 2002). Fathead minnows are themselves widespread invaders (introduced as a bait and forage fish) (Fuller et al. 1999) that would likely be encountered by spreading *Gambusia*. Minnows used in the experiment were obtained from the Frankfort Fish Hatchery in Frankfort, KY.

I conducted timed videotaped trials (10-minute duration) where I measured the feeding behavior and activity of individual *Gambusia* females in the presence and absence of fathead minnows. Twelve females from each *Gambusia* species were chosen at random from stock tanks and paired with a randomly chosen but size-matched (within 1 mm standard length) fathead minnow. Because *Gambusia* maximum size is typically around 6.5 cm and fathead minnow maximum size is closer to 10 cm (Fuller et al. 1999), all minnows used in the experiment were juveniles.

Trials were conducted in 6 L plastic containers in two time blocks during April, 2000. Trials without the competitor (referred to as individual trials) were conducted first (April 8 to 13), followed by trials with minnows (competition trials) (April 19 to 24). *Gambusia* were housed in the same plastic containers for the duration of the experiment. In competition trials, twenty-four hours prior to a trial, a translucent, perforated partition was placed in the center of each container. A minnow was then introduced on the side without the *Gambusia*. This partition allowed visual and chemical cues to be transmitted between the minnow and *Gambusia* without physical contact. Trials started 5 minutes after the partition was removed and when 40 live waterflea prey (*Daphnia pulex*) were added to the tank. The same number of prey was used for individual and competition trials. All fish were starved 24 hours prior to trials but were fed *ad libitum* commercial fish flakes in between trials. All trials were videotaped using a Sony® High 8 camcorder mounted on a tripod and positioned above tanks.

Trials ended either after 10 minutes, or after all 40 prey were consumed. From observations conducted during trials, I assayed the number of prey consumed (foraging success) by each competitor. For the competition trials, I calculated the proportion of the total prey consumed by the *Gambusia* out of the total consumption during the trial (prey eaten by *Gambusia*/(prey eaten by *Gambusia* + prey eaten by minnow). From analyses of taped trials, I extracted measures of *Gambusia* foraging efficiency and activity with and without competition;

and when minnows were present, I recorded aggressive acts between the two fish. Foraging efficiency was calculated as the number of prey consumed divided by the amount of time spent foraging. Foraging time included the time encountering, attacking, capturing, and consuming prey, but it did not include prey search time (hard to measure in the confinement of small tanks), or time spent inactive and interacting with minnows. Because time spent foraging could vary substantially across trials, patterns of foraging efficiency did not necessarily mirror patterns of foraging success. Inactivity was recorded as the proportion of time *Gambusia* spent motionless (time spent motionless divided by trial duration). For interspecific aggression, I calculated a rate of agonistic interactions (number per minute) by dividing the number of interactions initiated by the *Gambusia* or the minnow separately by trial duration. These interactions included approaches, chases, and chases with physical contact (usually a bump or bite).

Novel Predation

Smallmouth bass (*Micropterus dolomieu)* were chosen as the novel predator species because they are widespread and abundant, and like fathead minnows, do not co-occur with study populations. Smallmouth bass occur naturally in the upper Mississippi River drainage, but like other predatory centrarchids they have been widely introduced (Fuller et al. 1999) and are presently a common predator in North American streams and lakes. Because other centrarchid species (e.g., largemouth bass, sunfishes) are likely predators of three of the four *Gambusia* species, *G. affinis, G. holbrooki* and *G. geiseri,* but not *G. hispaniolae* (cichlids are their natural predators), the different *Gambusia* species arguably differ in the degree to which smallmouth bass represent a completely novel threat. However, even closely related centrarchids, like smallmouth and largemouth bass, differ in their predatory behavior, foraging efficiency, and prey selection (Winemiller and Taylor 1987, Hodgson et al. 1997), making smallmouth a relatively novel threat to all *Gambusia*, even to those which have experienced other centrarchid predators in their native habitats. For this experiment, juvenile smallmouth bass (average total length of 15.7 cm) were collected from the confluence of the north and south forks of Elkhorn Creek, Franklin County, KY.

As in the competition experiment, trials in this experiment lasted 10 minutes and were videotaped for later analysis. The same 12 females of each *Gambusia* species used in the competition trials were subjected to two consecutive trials, a no predator trial (also referred to as

the individual trial) followed by a trial with a smallmouth bass present (predation trial). Trials were conducted in September 2000 in six three-day blocks. On day 1 of each block, female *Gambusia* were introduced to 38 L aquaria and starved for 24 hours. On day 2, individual trials were conducted utilizing 20 flightless live fruitflies (*Drosophila melanogaster*) as surface prey for the *Gambusia*. Prey left unconsumed at the end of individual trials were counted and left in the tank for an additional 15 minutes to allow fish to continue feeding in order to better standardize hunger levels prior to predation trials. *Gambusia* were then starved for the next 23.5 hours. On day 3, predation trials were conducted with the same number of flies, but in the presence of a predatory smallmouth bass. Trials were randomized so that three females of each species experienced each of four individual predators used in the experiment.

For predation trials, I divided tanks into two equal sides using an opaque, plastic partition and placed the bass in one half of the tank one hour before trials (half without refuge). Partitions were later removed; and after a 5-minute acclimation period, *Drosophila* were added and trials started. Tanks were provided with a refuge for the *Gambusia* consisting of a piece of PVC tubing (10 cm length by 5 cm diameter) glued to the side of the tank 1 cm below the water line. Refuges were placed high in the water column because *Gambusia* are typically found close to the surface and under predation risk they often move to shallow areas where predators are excluded. I considered *Gambusia* to be 'in refuge' if they were found either on top, inside, behind (between partition and back wall of tank) or right underneath the PVC tubing. Predators were allowed to move freely in the tanks and approach *Gambusia*. Predators were fed *ad libitum Gambusia* for the duration of the experiment but were starved 24 hours prior to trials. After the first three-day block, three of the four predators died and had to be replaced. There were three actual predation events during the experiment, but I was able to replace only one *Gambusia* (final sample size is 46 *Gambusia*).

Trials were terminated either when all flies were consumed or after 10 min. From direct observations during the trials, I quantified the number of prey consumed in the presence and absence of predators. From tapes, I quantified *Gambusia* foraging efficiency, activity, and refuge use in the absence and presence of smallmouth bass, and predator inspections when bass were present. Foraging efficiency and activity were calculated in the same manner as in the competition trials. Refuge use was the proportion of the trial period spent in refuge. Predator inspections involved cautious approaches by the *Gambusia* towards the predator and were scored

as a rate (number of approaches divided by trial duration). Inspections were distinguished from normal swimming because inspecting *Gambusia* were visually fixated on the predator, and when done inspecting they backed away from the predator without losing sight of it. I also quantified the activity of predators as the proportion of trial time predators spent swimming.

Statistical analyses

For both experiments, I examined species differences in behavioral and foraging responses to novel competitors and predators by running repeated measures these ANOVA's with species as the between subject effect and competition (or predation) and the competition (or predation) by species interaction as the within subject effects. Focal variables included: the number of prey consumed (foraging success), foraging efficiency, proportion of time inactive, and proportion of time in refuge (only measured in predation trials). Prior to running ANOVA's, a MANOVA was performed to test for an overall species effect on mean response variables (with and without competition and predation). Preliminary repeated measures analyses included two covariates, predator activity and *Gambusia* size; however, these covariates were generally not significant and their inclusion did not alter results. Consequently, they were omitted from the analyses shown here. The effect of time as a blocking factor was also omitted from final analyses because it did not have a significant effect on any of the competition and predation response variables.

Simple one-way ANOVA's with species as a main effect (neither covariate was significant) were used to analyze behavioral variables that could only be quantified in the presence of competitors or predators: proportion of prey eaten by *Gambusia* (as opposed to minnows) in competition trials, rates of agonistic interactions by *Gambusia* and minnows, and rates of predator inspections.

To examine how variation in foraging success might be explained by variation in the other behavioral responses measured in the study (foraging efficiency, inactivity, refuge use, agonistic interactions, and predator inspections), I ran multiple regressions with foraging success as the dependent variable and these behavioral responses as independent variables. Separate regressions were done for the presence and absence of competition and predation risk, pooling data for fish from all four *Gambusia* species.

To meet parametric test assumptions of homogeneity of variances, I conducted Cochran's test on all variables, and transformed all those where evidence of heterogeneity was found. For the competition experiment, proportion of time inactive, proportion of prey consumed by *Gambusia,* and rates of agonistic interactions were transformed; while for the predation experiment, foraging efficiency, proportion of time in refuge, and predator inspection rates were transformed. Transformations were log (Ln of observed value + 1) for rates and arcsine square root for proportions.

Orthogonal contrasts were used to test for species differences once a significant species effect was found. In three contrasts, I tested whether the two invasive species differed from the two non-invasive species and whether there were significant differences between the two invasive species (*G. affinis* versus *G. holbrooki*) and between the two non-invasive species (*G. geiseri* versus *G. hispaniolae*). P-values for comparisons of the two invasive against the two non-invasive species correspond to one-tailed tests. All other reported p-values reflect two-tailed tests. For significant species by predation/competition interactions in the repeated measures, comparisons between species means were done with the same contrasts once a dummy variable was created with all the species by competition/predation treatment combinations. All statistical tests were conducted using SYSTAT® Version 10.

RESULTS

The MANOVA performed on behavioral and foraging variables averaged in the absence and presence of novel competition and predation showed a strong species effect (Wilks's Lambda, $F = 2.5$, $p = 0.002$). This result indicates that *Gambusia* species, in general, differed in their behavior, even when ignoring how species might differentially respond to predation risk and competitors and averaging values across contexts.

Novel competition

In the absence of competition, invasive *G. holbrooki* and *G. affinis* achieved greater foraging success (number of prey eaten during the trial) than the non-invasive *G. hispaniolae* and *G. geiseri* (Figure 3.1a). On average, invasive *Gambusia* consumed 35 % more prey that non-invasives ($F_{1, 44} = 9.1$, $p = 0.002$). Of the two invaders, *G. affinis* tended to be the species with

the greatest overall consumption (compared to *G. holbrooki*; $F_{1, 44} = 3.3$, $p = 0.06$). The addition of novel competition had a large impact on the foraging success of *Gambusia*. On average, *Gambusia* consumed 40 % fewer *Daphnia* when fathead minnows were present. The four *Gambusia* species responded equally to competition; they all reduced consumption in the presence of minnows (no significant species by competition interaction) (Table 3.1, Figure 3.1a).

However, when considering the proportion of prey consumed by *Gambusia* in competition trials (relative to the minnows), a species effect was found (Table 3.1). With competition, the non-invasive species secured a lower proportion of the total prey compared to the invasive species ($F_{1, 44} = 3.1$, $p = 0.04$). In particular, while invasive *Gambusia* and *G. geiseri* secured about half of the prey eaten in competition trials (47 % compared to 53 % by minnows), *G. hispaniolae* only secured 22 % of the prey ($F_{1, 44} = 8.3$, $p = 0.006$) (Figure 3.1b).

Regressions of behavioral variables on the number of prey consumed across all species revealed that in the absence of competition, foraging success was solely dependent on foraging efficiency (Table 3.3). With novel competition, variation in *Gambusia* foraging success was due to variation in not only foraging efficiency but also activity level and aggression by minnows (but not aggression by *Gambusia*). *Gambusia* that were more efficient foragers (consumed more prey per unit time spent foraging), more active, and experienced lower rates of agonistic interactions with minnows consumed more prey in competition.

Repeated measures analyses of foraging efficiencies detected a competition effect, a species effect, and a trend for a species by competition interaction (Table 3.1). When competitors were absent, invasive *Gambusia* were significantly more efficient than the two non-invasive species ($F_{1, 44} = 13.3$, $p = 0.0005$). On average, invasive *Gambusia* consumed the same number of *Daphnia* in 46 % less time than non-invasive *Gambusia*. Interestingly, competition caused an overall increase in *Gambusia* foraging efficiency (i.e., all fish generally foraged more intensely when a competitor was present; Figure 3.1c). This effect was significant for *G. affinis*: ($F_{1, 88} = 7.7$, $p = 0.007$), and *G. geiseri* ($F_{1, 88} = 5.4$, $p = 0.02$), but not quite significant for *G. holbrooki*: ($F_{1, 88} = 2.9$, $p = 0.09$). *G. hispaniolae*, the species that ate only 22% of the prey consumed in the presence of minnows, showed no tendency to increase its foraging efficiency in the presence of competitors ($F_{1, 88} = 0.01$, $p = 0.91$). With competition, invasive *Gambusia* remained more efficient than non-invasives ($F_{1, 44} = 11.2$, $p = 0.001$).

Gambusia tended to be very active in the competition experiment. On average, females spent only about 10 % of trial time completely immobile, although there were some species differences. In competitor-free trials, inactivity for non-invasive *Gambusia* was more than double that of the invasives ($F_{1, 44} = 7.4$, $p = 0.005$). This effect was completely due to *G. hispaniolae* being highly inactive in the absence of competition (even when compared to *G. geiseri*; $F_{1, 44} = 33.8$, $p = 0.0001$) (Figure 3.2a). In response to competition from fathead minnows, the non-invasive species were the only ones to change their activity. *G. hispaniolae* responded by cutting in half its time spent immobile ($F_{1, 88} = 9.8$, $p = 0.002$), while *G. geiseri* tended to become more inactive ($F_{1, 88} = 2.9$, $p = 0.09$).

Contrary to expectations, I found that fathead minnows were significantly more aggressive than *Gambusia* (Figure 3.2b). The number of agonistic interactions initiated over all trials totaled 629 for minnows compared to only 282 for *Gambusia*, and interaction initiation rates (adjusted for trial duration) were on average three times greater for minnows relative to *Gambusia* (Two sample t-test: $t = -3.8$, $p = 0.0001$). *Gambusia* species did not differ in their rates of either initiating or receiving agonistic interactions with minnows (Table 3.1); i.e., invasive and non-invasive *Gambusia* appeared equally aggressive.

Novel predation

Predation risk from smallmouth bass resulted in, on average, a 21 % decrease in the number of *Drosophila* consumed by *Gambusia*. The response to predation differed, however, between invasive and non-invasive species (Table 3.2). Invasive *Gambusia* responded to predation risk by lowering their consumption ($F_{1, 84} = 5.1$, $p = 0.03$), while no effect was detected for the non-invasive *Gambusia* ($F_{1, 84} = 0.05$, $p = 0.83$) (Figure 3.3a). In particular, invasive *G. holbrooki* exhibited a 46 % decrease in foraging success in response to predation risk ($F_{1, 84} = 5.6$, $p = 0.02$) compared to only a 14 % decrease in *G. affinis* ($F_{1, 84} = 0.6$, $p = 0.44$).

In the absence of predators, the two invasive *Gambusia* consumed over 50 % more prey than the two non-invasive *Gambusia* ($F_{1, 42} = 13.3$, $p = 0.0005$). In contrast, in the presence of predators (because of the decrease in foraging success seen for the invasives), there was only a trend for invasives to have higher consumption than the non-invasives ($F_{1, 42} = 2.3$, $p = 0.07$). Across all species, regressing the behavioral variables on foraging success (both in the presence and absence of predation risk) revealed that variation in foraging success was primarily due to

variation in foraging efficiency (Table 3.3). Fish that were more efficient foragers consumed more prey. Inactivity, refuge use, and predator inspection rates were not significant predictors of foraging success either in the presence or absence of predation risk.

There was a trend for foraging efficiency to differ among *Gambusia* species (Table 3.2). Invasive *Gambusia* tended to be more efficient foragers (more prey consumed per minute spent foraging) than non-invasives in the absence of predation risk (Figure 3.3b). The significant species by predation interaction for foraging efficiency was due perhaps to a tendency for *G. hispaniolae* to increase its foraging efficiency with predation risk ($F_{1, 84} = 2.5$, $p = 0.12$), while for the other three species, efficiencies were similar with and without predation risk.

Unlike competition, predation risk had a strong effect on activity level, and this effect varied significantly among species (Table 3.2). While no response was observed for other *Gambusia*, *G. holbrooki* females more than doubled their time spent immobile in trials where predators were present ($F_{1, 84} = 5.2$, $p = 0.03$) (Figure 3.4a). Under predation risk, *G. holbrooki* females were also four times more inactive than *G. affinis* females ($F_{1, 42} = 12.2$, $p = 0.001$).

Predation risk had a similar effect on refuge use (Table 3.2). On average, predation risk caused fish to almost triple their refuge use, but this effect was largely due to the invasive species' response ($F_{1, 84} = 10.6$, $p = 0.002$), and in particular, *G. holbrooki* ($F_{1, 84} = 16.6$, $p = 0.0001$) (Figure 3.4b). *G. holbrooki*'s refuge use increased by four-fold, resulting in a significant difference between the two invasives ($F_{1, 42} = 12.3$, $p = 0.001$). The proportion of time spent in refuge for *G. holbrooki* was more than seven times greater than for *G. affinis*.

Although I found species differences in activity and refuge use in response to the presence of predatory smallmouth bass, I found no species effects on the rate of predator inspections (number per minute of trial duration) (Table 3.2). Overall, a relatively small number of predator inspections (total of 56) were recorded during predation trials. This may be due to the fact that trials were conducted in relatively small tanks where *Gambusia* might have been able to assess predators and risk well without expressly approaching them.

DISCUSSION

When a non-indigenous species invades a new community, both the invader and the invaded community experience novel species interactions. It is widely accepted that non-

indigenous species often have particularly large impacts on the invaded community if the invader performs a novel function in the invaded range (Elton 1958, Simberloff 1991). This novelty in function has been linked to species extinctions, shifts in community structure (e.g., predators on oceanic islands and lakes; Fritts and Rodda 1998, Ogutu-Ohwayo 1999) and even changes in key ecosystem processes (e.g., nutrient cycling; Vitousek et al. 1997). In contrast, how non-indigenous species themselves deal with novel species interactions associated with invading new communities is less well understood.

Predators and competitors in a recipient community may act to resist an invasion (Elton 1958) simply because there are novel and invading species lack adaptive responses. Invading species that respond adaptively to these novel interactions may be expected to have greater invasion success. Results from my short-term behavioral assays provide evidence that species that successfully invade may in fact be better than non-invasive congeners at coping with novel predation and competition. However, results also show that even species that are closely-related, ecologically similar, and thought of as equally invasive or non-invasive may differ in how they respond to novelty.

In the absence of biotic interactions, *G. affinis* and *G. holbrooki* (invasive) had greater foraging success than *G. geiseri* and *G. hispaniolae* (non-invasive). Invasive fish consumed more *Daphnia* and *Drosophila* than non-invasives. Variation in foraging success was primarily explained by variation in foraging efficiency. This greater foraging success and efficiency might be a key factor explaining the large predatory impact of mosquitofish in their introduced range (Courtenay and Meffe 1989). Previous work on foraging behavior (Chapter 2) also showed that the invasives *Gambusia* exhibit greater feeding rates (prey consumed per minute) than non-invasives on *Daphnia, Drosophila*, and even on larger prey (*Lirceus* sp.).

Novel competition with fathead minnows affected *Gambusia* foraging success and behavior via agonistic interactions (i.e., interference competition) and through the direct consumption of resources (i.e., exploitation). Unexpectedly, fathead minnows proved to be more aggressive competitors than invasive *Gambusia*. Minnows were more likely to chase and contact *Gambusia* than vice versa, and they significantly lowered *Gambusia* foraging success. Minnows also did not discriminate among the four *Gambusia* species; they were equally aggressive toward invasive and non-invasive species. Several authors make reference to high aggression as another key element explaining the invasion success and impact in mosquitofish (Myers 1965, Meffe

1985, Courtenay and Meffe 1989); however, this study found that invasive and non-invasive *Gambusia* showed equally low levels of aggression, at least toward fathead minnows.

Despite being more aggressive than *Gambusia*, fathead minnows did not generally monopolize prey resources. Invasive *Gambusia* species faced with competition reduced foraging success (relative to when competitors were absent), but competition for prey between invasives and fathead minnows was highly symmetrical. In paired interactions, prey consumption was 52 % by minnows and 48 % by invasive *Gambusia*. An increase in foraging efficiency allowed the invasive species to cope with competition rather well. This increase in foraging efficiency may be considered an adaptive response to novel competition. Surprisingly, non-invasive *G. geiseri* showed the same adaptive response to novel competition seen in invasive *Gambusia*. *G. geiseri* also increased foraging efficiency and thus secured 45 % of the prey consumed. Only *G. hispaniolae* showed a poor response to competition; since females of this species did not increase foraging efficiency, they secured only 22% of the prey.

With regard to predations risk, studies on other prey show that prey typically respond by decreasing activity, foraging behavior, and/or by altering habitat use (i.e., increasing refuge use or use of predator-free microhabitats) (Sih 1987, Lima and Dill 1990). These responses should be adaptive since they often reduce conspicuousness to predators and encounter rates. This is especially true with ambush predators like smallmouth bass that respond to prey movement. In this study, invasive *Gambusia* responded adaptively to the presence of novel smallmouth bass by reducing foraging activity and increasing refuge use, but this effect was largely due to the response of just one of the invasive species. *G. holbrooki*, the eastern mosquitofish, responded strongly to predation risk by reducing food consumption and activity and increasing refuge use. In contrast, predation risk had little or no effect on the foraging success or behavior of the other three *Gambusia* species, including invasive *G. affinis*. The only detectable response by non-invasive *Gambusia* was a tendency to increase foraging efficiency. This response may be considered inappropriate since foraging and foraging at a faster rate may make prey more conspicuous to predators, and may reduce prey vigilance and escape success.

Since invading species are likely to encounter both novel predators and novel competitors as they colonize new habitats, invasion success could depend on the ability of invading organisms to respond adaptively to both (as well as other novel enemies; e.g., pathogens, parasites, etc.). Behavioral mechanisms should enhance the ability of organisms to cope with

multiple, new selection pressures (Schlaepfer et al. 2002). My results show that of the four species tested, only one species responded adaptively to both competition and predation. Invasive *G. holbrooki* increased foraging efficiency in competition, and when faced with predation decreased consumption and activity and increased refuge use. *G. holbrooki* might inherently have greater behavioral plasticity than its sister species, *G. affinis*, and than other less invasive *Gambusia*. It is also possible that the particular competition and predation regime experienced by *G. holbrooki* in the behavioral assays resembled native conditions enough to allow females to respond adaptively in both contexts. Further testing with a larger number of novel predators and competitors is needed to determine if *G. holbrooki* in fact generally exhibits greater plasticity in response to novelty than other *Gambusia*.

Interestingly, I found disparities between species of seemingly equal invasiveness in their response to novelty. In the competition trials, the non-invasive *Gambusia* species responded differently to fathead minnows, and in the predation experiment invasive species differed from each other in response to smallmouth bass. While there might be many reasons for this variation in response, a simple explanation might be that my assumption that these species are equally invasive or non-invasive is incorrect. Previous studies show that *G. holbrooki* has a life history that leads to higher population growth rates than *G. affinis* (Scribner 1993, but see Chapter 5) and that *G. holbrooki* exhibits directional introgression when *G. holbrooki* and *G. affinis* are sympatric (Scribner and Avise 1994). This evidence suggests that *G. holbrooki* might be in fact a superior invader to *G. affinis*. Indeed, most successful introductions of mosquitofish outside their native range have involved *G. holbrooki* (Lever 1996). However, while this could be due to greater success by *G. holbrooki* outside its native range, it might also only reflect greater invasion opportunities (i.e., more introductions of *G. holbrooki* than *G. affinis*).

It is also plausible that differences between species of apparently equal invasiveness relate to differences among species in their mechanisms for invasion success or failure. Among invaders such as *G. holbrooki* and *G. affinis* where invasions have occurred repeatedly across myriad habitats and communities, it is expected that species-specific attributes play an important role in determining invasion success (Mack et al. 2000). Appropriately responding to novel biotic interactions might be an important behavioral 'trait' favoring invasion success for *G. holbrooki* but not *G. affinis*. *G. affinis* may rely instead on other traits such as its greater dispersal tendency to successfully invade new habitats (Chapter 4). Indeed, recent studies provide

evidence that multiple traits (not just single traits) tend to account for variation in invasiveness (e.g., Rejmanek and Richardson 1996, Reichard and Hamilton 1997 for plants; Veltman et al. 1996, Green 1997, Sol et al. 2002 for birds). In fish and aquatic invaders in general, more studies are needed to carefully examine which traits might be important to invasion success (e.g., Kolar and Lodge 2002).

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Table 3.1: Summary of repeated measures and one-way ANOVA results for effects of *Gambusia* species and competition with minnows on number of prey consumed, foraging efficiency, inactivity, proportion of prey consumed by *Gambusia* in the presence of minnows, and agonistic interactions by both fish.

Variable	*Effect*	*df*	*F*	*p-value*
Prey consumed	Species	3, 44	4.6	0.007
	Competition	1, 44	25.3	0.0001
	Species x competition	3, 44	1.1	0.37
Foraging efficiency	Species	3, 44	6.3	0.001
	Competition	1, 44	17.1	0.0001
	Species x competition	3, 44	2.4	0.08
Inactivity	Species	3, 44	5.1	0.004
	Competition	1, 44	0.03	0.86
	Species x competition	3, 44	8.7	0.0001
Proportion of prey consumed by *Gambusia*	Species	3, 44	3.0	0.04
Agonistic interactions by *Gambusia*	Species	3, 44	1.3	0.28
Agonistic interactions by fathead minnow	Species	3, 44	1.2	0.32

Table 3.2: Summary of repeated measures and one-way ANOVA results for effects of *Gambusia* species and the presence of predatory bass on number of prey consumed by *Gambusia*, foraging efficiency, inactivity, refuge use, and predator inspections.

Variable	*Effect*	*df*	*F*	*p-value*
Prey consumed	Species	3, 42	3.2	0.03
	Predation	1, 42	8.4	0.006
	Species x predation	3, 42	3.3	0.03
Foraging efficiency	Species	1, 42	2.6	0.07
	Predation	3, 42	0.6	0.43
	Species x predation	3, 42	3.2	0.04
Inactivity	Species	3, 42	3.1	0.04
	Predation	1, 42	1.6	0.23
	Species x predation	3, 42	3.0	0.04
Refuge Use	Species	3, 42	3.6	0.02
	Predation	1, 42	14.7	0.0001
	Species x predation	3, 42	4.9	0.005
Predator inspections	Species	3, 42	0.88	0.46

Table 3.3: Multiple linear regression equations for effects of various behaviors on the number of prey consumed in trials for all species pooled. Separate regressions were run in the presence and absence of competition and predation.

Model	*Adj. R^2*	*F*	*df*	*p-value*	*Terms in model*	*Standard Coefficient*	*p-value*
In absence of competition	0.69	20.7	2, 44	0.0001	Foraging efficiency	0.65	0.0001
					Inactivity	-0.11	0.34
In presence of competition	0.81	19.7	4, 43	0.0001	Foraging efficiency	0.56	0.0001
					Inactivity	-0.31	0.003
					Interactions by M	-0.20	0.04
					Interactions by G	0.14	0.14
In absence of predation	0.75	46.7	3, 42	0.0001	Foraging efficiency	0.80	0.0001
					Inactivity	-0.16	0.13
					Refuge use	0.05	0.60
In presence of predation	0.69	26.2	4, 41	0.0001	Foraging efficiency	0.73	0.0001
					Inactivity	-0.08	0.22
					Refuge use	-0.19	0.60
					Predator inspections	0.01	0.90

Note: Abbreviations are as follows: G = *Gambusia*, M = Minnow.

Figure 3.1: (a) Number of prey consumed in the presence and absence of novel competition, and (b) proportion of prey consumed out of total consumption during competition trials for each *Gambusia* species, the invasive *G. holbrooki* and *G. affinis*, and the non-invasives *G. geiseri* and *G. hispaniolae*. Data are means ± 1 SE.

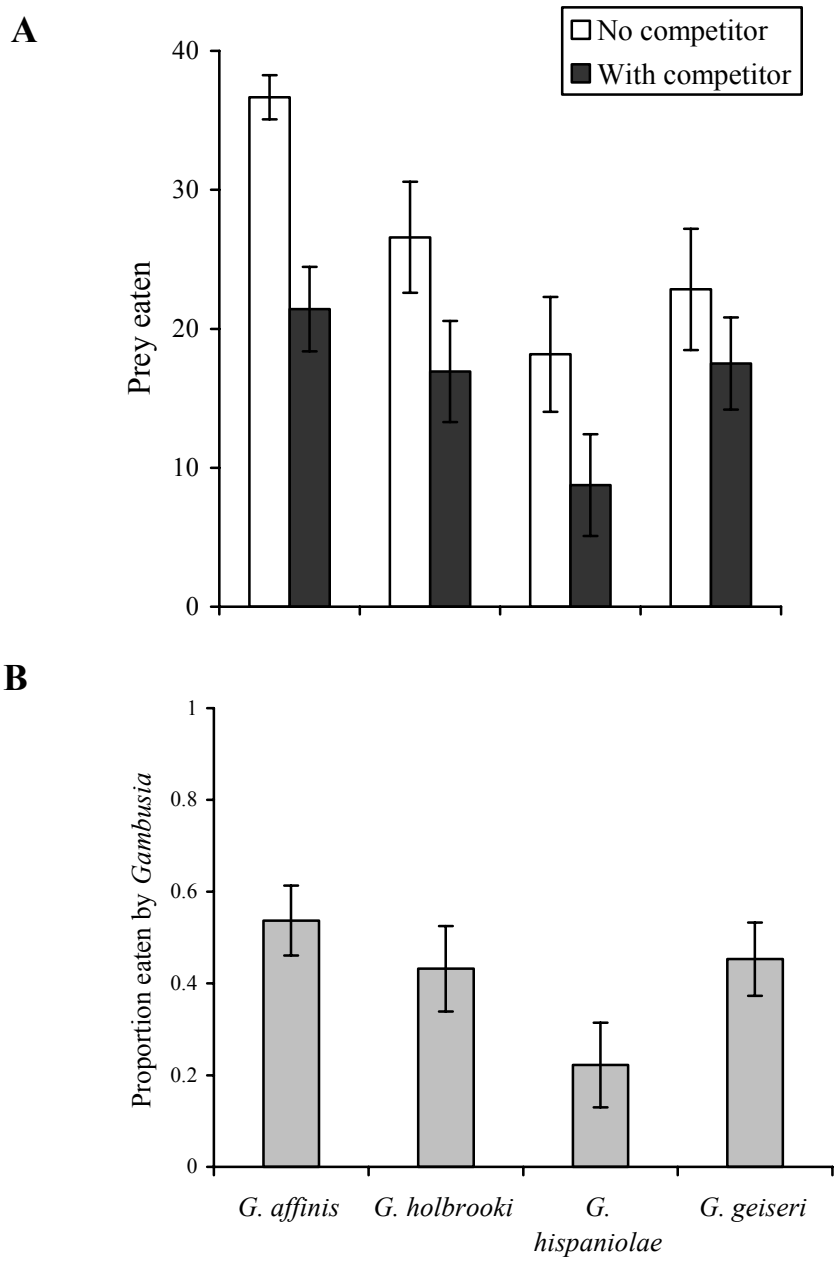


Figure 3.1: (c) Foraging efficiency (prey consumed per minute spent foraging) in the presence and absence of competition for each *Gambusia* species, the invasive *G. holbrooki* and *G. affinis*, and the non-invasives *G. geiseri* and *G. hispaniolae*.

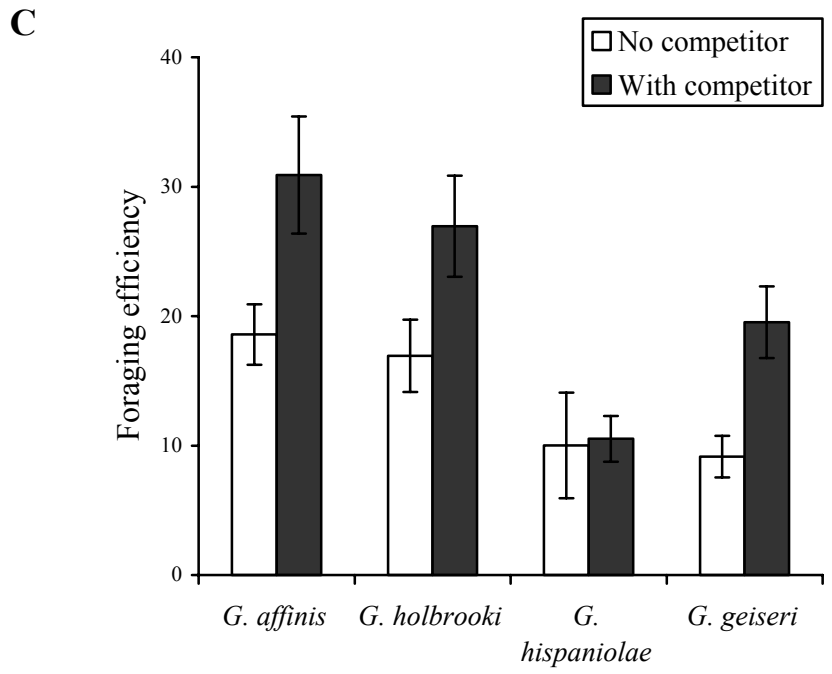


Figure 3.2: (a) Proportion of time spent inactive during trial by each *Gambusia* species in the presence and absence of competition. (b) Number of agonistic interactions per minute initiated by either the *Gambusia* species or by the minnow (toward each *Gambusia* species) in competition trials. Data are means ± 1 SE.

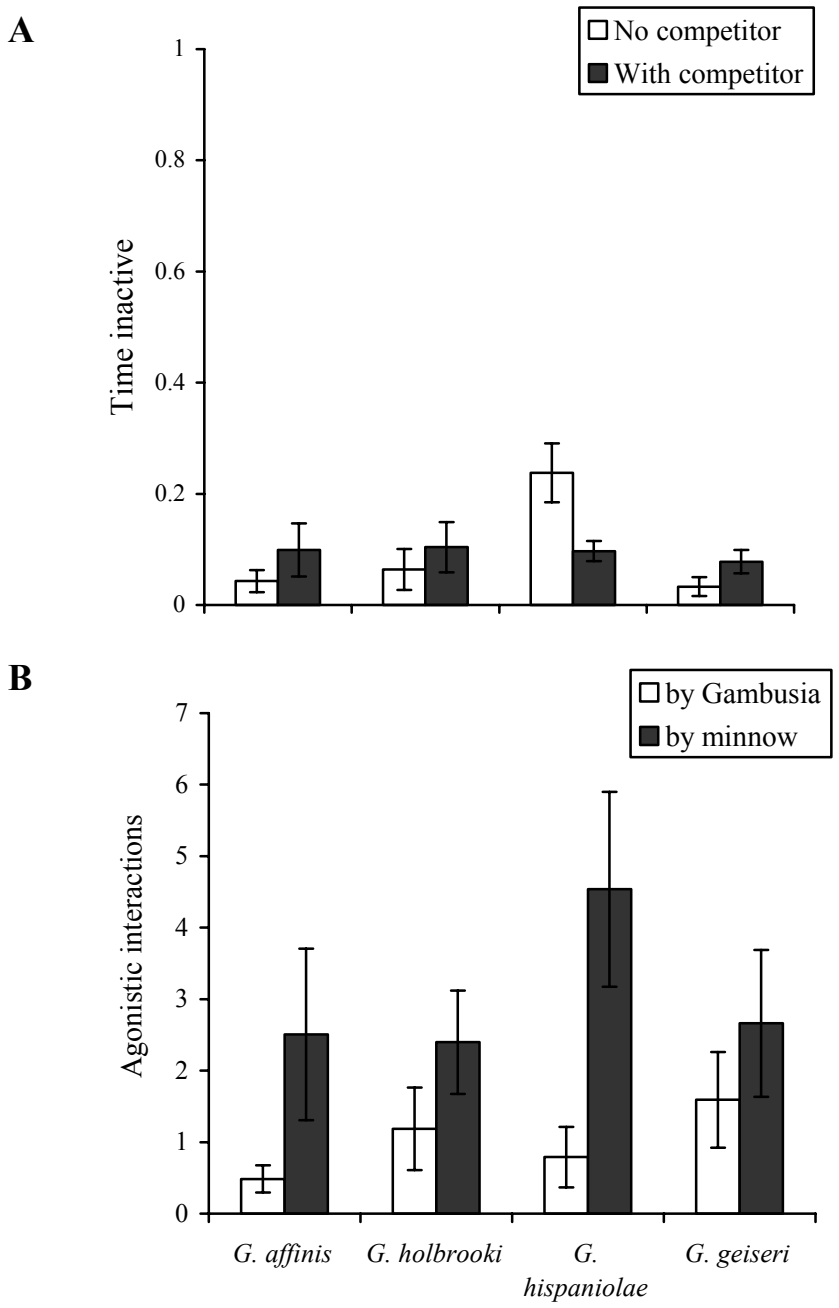


Figure 3.3: (a) Number of prey consumed and (b) foraging efficiency (prey consumed per minute spent foraging) for the four *Gambusia* species in the presence and absence of predation risk. Data are means ± 1 SE.

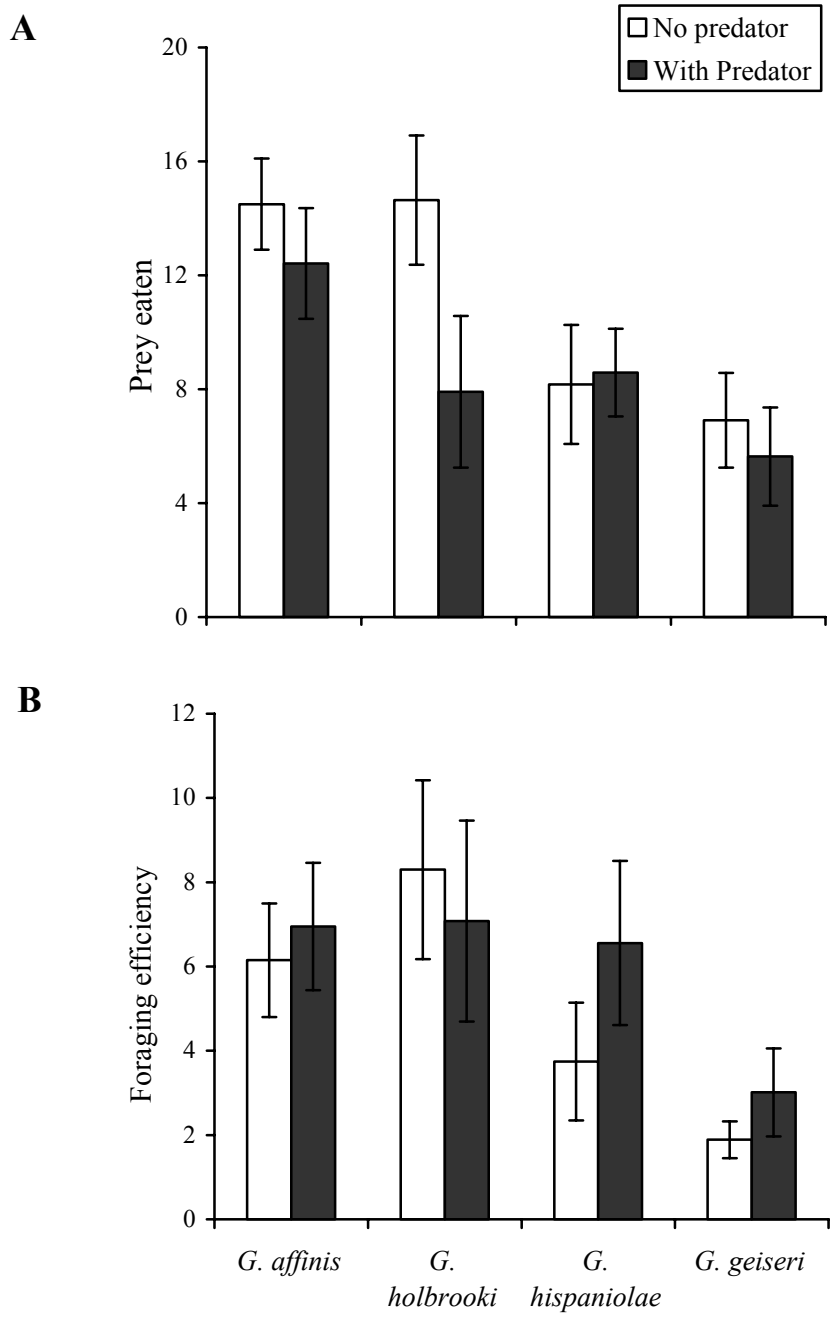
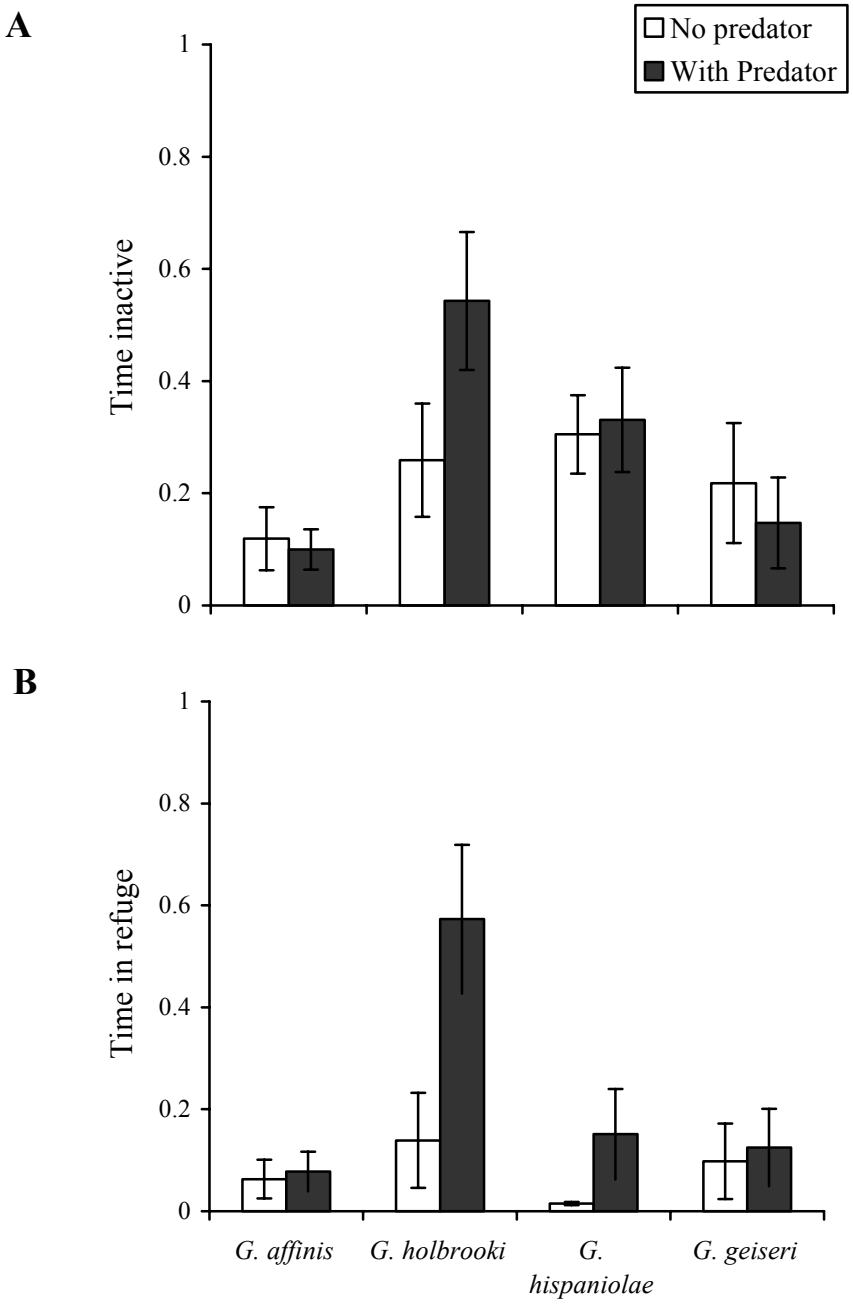


Figure 3.4: (a) Proportion of time spent immobile and (b) proportion of time spent in refuge for each *Gambusia* species measured in the presence and absence of predation risk. Data are means ± 1 SE.



Chapter Four

Dispersal characteristics and boldness: A comparison of *Gambusia* species of varying invasiveness

SUMMARY

The dispersal ability and/or tendency of organisms is expected to not only influence the pattern of spatial spread of invading organisms but also to be a key factor in overall invasion success. Intraspecific and interspecific variation in dispersal distances and rates may be linked to variation in an underlying behavioral trait, boldness. Species that have a high propensity to explore unfamiliar space may be better dispersers than species wary of novel space. This study examined the link between dispersal and invasiveness by comparing dispersal characteristics among invasive and non-invasive *Gambusia* species in experimental streams. I also explored whether variation in dispersal among two invasive and two non-invasive species is related to differences in boldness. While I found differences between invasive and non-invasive dispersal that largely fit my predictions, the results also indicate that species identity matters. Of the four species examined, two fit my prediction very well, while two fit but not as well. I suspect this result indicates variation in the relative invasiveness or non-invasiveness of species. I also found that the measure of boldness chosen (proportion of fish out of refuge) correlated strongly with dispersal. These results argue for the greater incorporation of experimental approaches and behavioral mechanisms in the study of invasive species.

INTRODUCTION

Non-indigenous species are generally defined as invasive only if they are able to spread (i.e., expand their range) beyond their point of initial arrival or introduction (Richardson et al. 2000, Kolar and Lodge 2001). Thus, dispersal ability is generally thought to be a key factor determining invasion success (Ehrlich 1986, Lodge 1993b, Sakai et al. 2001). In particular, the rate of spatial spread of invasions is strongly dependent on the dispersal rates or distances of the invading organisms (Parker and Reichard 1998). Dispersal is also a fundamental component of ecological processes in natural populations, affecting gene flow, population structure, and metapopulation dynamics that have important consequences for species distributions, abundances, and persistence (Endler 1977, Kareiva 1990, Hanski and Gilpin 1991, Tilman 1994).

In aquatic systems, the striking invasion success of some of the best-known invaders has been largely attributed to their dispersal abilities. The classic example comes from the invasion of the Great Lakes by the zebra mussel, *Dreissena polimorpha*. Unlike native bivalves, zebra mussels can disperse quickly and broadly as free swimming larvae and as adults that can attach themselves to submerged moving substrates (Lodge 1993a, Johnson and Carlton 1996). Another example involves common and grass carps, *Cyprinus carpio* and *Ctenopharyngodon idella*, two of the most widespread fish invaders worldwide, whose invasion success, at least in North America, is strongly linked to their ability to disperse rapidly (Moyle 1986). However, beyond these case-studies little comparative evidence exists to show that successful invasive species have, in fact, greater dispersal tendency, ability or dispersal rates than species that are either not as successful, have failed to spread, or are being displaced by invasives.

The spatial spread of invasions has traditionally been modeled by reaction-diffusion models (e.g., Skellam 1951) where dispersal is treated as either a constant or a normally distributed parameter. However, dispersal patterns are often highly variable (Kot et al. 1996), and field data show that dispersal distances are generally leptokurtically distributed (high frequency of values near the center and tails of distribution) (Okubo 1980, Howe and Westley 1986, Paradis et al. 1998). Indeed, high intraspecific variation in dispersal distances or rates has been documented repeatedly for a variety of taxa (Greenwood and Harvey 1976, Gaines and McClenaghan 1980, Swingland 1983, Bengtsson et al. 1994, O'Riain et al. 1996, Bradford and

Taylor 1997). While some of this variation may be attributed to differences among organisms in age, size, condition, or gender, Fraser et al. (2001) argued that this variation might also be the result of variation in an underlying behavioral trait that affects dispersal. Specifically, Fraser et al. (2001) suggested that boldness, defined as the propensity to move through and explore unfamiliar space (Wilson et al. 1993), might be an important source of intraspecifc variation in dispersal. Whether individuals are bold or shy might determine whether they disperse or remain sedentary, or if individuals are short versus long distance dispersers. Greenberg (1995) suggested that individual responses to novelty might also be species specific. Species that are bolder than their congeners respond to novelty in a less neophobic manner (Greenberg 1983, 1989), and might be better dispersers.

A major objective of my study is to examine the link between dispersal and invasiveness by determining whether dispersal characteristics, in fact, differ between invasive and non-invasive congeneric species. All else being equal, I expect successful invasive species to exhibit greater dispersal than non-invasives. A second objective is to determine whether variation in dispersal among invasive and non-invasive species is related to differences in boldness. Boldness could be highly advantageous for species arriving and spreading through novel habitat. I then expect successful invasive species to be significantly bolder than non-invasive species. To address these issues, I compared dispersal characteristics and underlying behavior in an experimental stream for four closely related *Gambusia* species, two invasive and two non-invasive. For dispersal, I quantified whether individuals dispersed or not (from an initial introduction point), their time until dispersal (dispersal rate), dispersal distance, dispersal endpoint (pools versus stream), and direction (upstream versus downstream pools). I then asked whether dispersal could be predicted by a measure of boldness, time spent out of refuge.

Gambusia are a group of about 45 species of small livebearing fishes (Poeciliidae). Most of what we know about this genus comes from the rather extensive study of the two most temperate, most widely distributed, and highly invasive species, *G. holbrooki* and *G. affinis*. These two sister species (both known as mosquitofish) have been introduced for mosquito control purposes worldwide and have spread successfully on their own to over 40 countries (Welcomme 1992, Lever 1996). Their invasion success and impact on native communities have been notable (Lloyd et al. 1986, Courtenay and Meffe 1989, Gamradt and Kats 1996, Webb and

Joss 1997, Goodsell and Kats 1999), such that they have been designated to be among the 100 worst invasive species worldwide (ISSG 2000).

In contrast, despite strong similarities in their ecology, morphology, and body size, most of the other species in the genus have restricted geographic distributions, have rarely been introduced, and when they have been introduced have failed to spread. For this study, I focused on two such non-invasive species, *G. geiseri* and *G. hispaniolae*. *G. geiseri* is endemic to spring habitats of the Southwestern U.S. (Hubbs and Springer 1957) and appears to be a sister taxon to the invasive species pair (partial phylogeny by Lydeard et al. 1995). Outside its native range, the success of *G. geiseri* has been very limited. While this species has been introduced to several habitats similar to its own (Fuller et al. 1999), *G. geiseri* has not spread and remains localized to the points of introduction (C. Hubbs, personal communication). *G. hispaniolae* is a Caribbean species native to the Neiba Valley and Cul de Sac region of the island of Hispaniola. Although it is the most widespread of the three endemic *Gambusia* species in Hispaniola, it is still rather restricted in its range (Burgess and Franz 1989). Even presently, when irrigations canals have increased the connectivity of the region, *G. hispaniolae* does not seem to be spreading out of its native range, which suggests that its potential invasiveness is low; therefore, I treat it as a non-invasive.

METHODS

Study organisms

For my study, I focused on adult gravid females that are likely to be the most important stage and gender in an invasion context. Previous studies suggest that female invasive *Gambusia* exhibit greater dispersal rates (Robbins et al. 1987, Congdon 1994) and perhaps greater dispersal success (Brown 1987) than either males or juveniles. Females have higher overwintering survival than males (Winkler 1975) – a key trait for invading more northern habitats. In addition, females *Gambusia* can retain sperm from multiple males for several months and even across breeding seasons (Chesser et al. 1984, Zane et al. 1999). Thus, individual females are capable of founding populations in the absence of males without negative genetic founder effects (Chesser et al. 1984, Robbins et al. 1987). Indeed, levels of genetic variation in newly founded populations are comparable to levels in more established populations (Brown 1985, Scribner et

al. 1992, but see Congdon 1995). Additionally, populations founded by females alone versus males and females do not differ in either population growth rates or population structure (Resetarits 2000). Therefore, the dispersal and underlying behavioral traits of *Gambusia* females are probably keys to understanding the relationship between dispersal and invasion success or failure in this taxon.

The two invasive species, *G. affinis* and *G. holbrooki*, were collected from populations within their native range, not their invaded range. Collections of *G. affinis* were made in the Comal River, Comal County, TX, and for *G. holbrooki*, collections were made in Leon Hines Lake, Escambia County, AL. *G. geiseri* were collected from Comal Springs also in Comal County, TX, and *G. hispaniolae* came from La Azufrada, a freshwater spring flowing into Lake Enriquillo, Dominican Republic. All collections were conducted in summer and early fall of 1999. In order to remove variation due to different experiences in their natural environments, I studied F2 fish that were raised in the laboratory under standardized conditions. For all four species, fish were raised under similar densities in 76 L aquaria at 22-26° C, at a 14 L: 10 D photoperiod and fed *ad libitum* a combination of Tetramin flakes, freshly hatched brine shrimp nauplii, and a calf liver and spinach frozen paste enriched with minerals and vitamins. Males and females were housed together to allow matings to occur. To prevent cannibalism of young, gravid females were isolated just prior to parturition in brood chambers that allow newborns to escape.

Experimental streams

The experiment was conducted in two artificial streams set up outdoors under direct sunlight at the Putah Creek Aquatic Facility at the University of California, Davis, CA. Each stream consisted of a series of three pools connected to a large PVC channel via three smaller PVC channels (Figure 4.1). In each set-up, a ¾ HP pump circulated water producing substantial flow in the large channel, but no detectable flow in the three side pools. The large channel simulated a flowing stream while the pools simulated slow moving backwaters that are typically inhabited by *Gambusia*. Maximum flow velocities at the upstream outflow in the PVC channel averaged 1.19 m/s (n = 10, S.E. = 0.041) for both set-ups, and decreased away from the outflow, so that flow velocities for the two entire PVC stream (averaged over four locations) were 0.51 m/s (n = 10, S.E. = 0.018).

The substrate for the channel and all pools consisted of a 2 cm layer of sand. Refugia were provided for the fish throughout each artificial stream (Figure 4.1). Clumps of the macrophyte *Elodea canadensis* comprising about 15 % of the pool area were used as water column refuges in the three pools. Due to concerns with pump clogging, refuges in the river portion of the setup consisted of floating artificial aquarium plants secured to the side of the PVC. Additional benthic refugia were provided by small pieces of PVC conduit (two per pool and four in the main channel, 2.5 cm diameter x 10 cm); however, fish rarely used these. *Gambusia* should prefer pools over the channels because pools have little or no flow and more refuge available.

Fish were introduced into the second of the three pools. From there, they had free access to the channel and the other two pools. Small funnel traps placed in the upstream and downstream pools where each pool met the connecting channel (Figure 4.1) allowed fish to disperse into the pools, but restricted their return back to the channel. These traps facilitated the ease of measuring colonization of these pools. No traps were placed in the middle pool where fish were introduced to the setup.

Dispersal trials

I compared the dispersal characteristics of the four *Gambusia* species by measuring the amount, timing, and direction of movement of replicate groups of three gravid females of each species in the artificial streams. I focused on small groups (rather than single individuals or larger schools) for three reasons: (1) individuals in small groups appear much less stressed than solitary individuals, (2) small groups allowed us to get detailed behavioral data on individuals that would be difficult to record for larger schools, and (3) because I suspect that successful dispersal in an invasion front often involves small numbers of individuals.

The three randomly selected females in each group were fed *ad libitum* Tetramin flakes in their respective source tanks and then placed in clear, plastic containers (900 mL) with mesh sides the evening before the trial day. Groups were moved in these plastic containers to the middle pool of the experimental streams and acclimated for 5 minutes before being released. All groups were released in the side of the pool opposing the opening to the connecting channel (Figure 4.1). Water temperature was recorded just before fish were released. Each group was then given 1 hour to disperse out of the middle pool and move into one of three locations: the

main channel, the upstream pool, or the downstream pool. Every 10 minutes, observations noted both the location and activity of each of the three members in a group. For each group, I also recorded distance traveled in the channel over three two minute periods. All observations were made with binoculars from an observation tower where the entire setup was visible and fish were undisturbed. Each hour, I ran an invasive species in one stream system and a non-invasive in the other system; systems were alternated the following hour. Overall, over the course of three consecutive days in September 2002, I ran 11 groups of three females for the two invasive species and 9 groups for the two non-invasive species, for a total of 40 experimental groups and 120 fish.

Response variables

To examine potential differences in female dispersal, I compared the following variables for the four *Gambusia* species: (a) whether dispersal occurred, (b) the timing (rate) of dispersal, (c) the distance traveled, (d) dispersal endpoint, and (e) dispersal direction. The first three measures describe dispersal per se, whereas the latter two measures are aspects of habitat choice. I expected invasive and non-invasive *Gambusia* to differ in their habitat choice. In particular, I hypothesized that non-invasive *Gambusia* species would be more likely to colonize the larger refuge and slower moving pools compared to the invasives. I also expected non-invasives, because of a potentially lower ability to cope with high flow, to preferentially colonize the downstream pool compared to the invasive *Gambusia*.

I defined dispersal as the movement of fish out of the introductory pool. My most basic measure of whether a species was a good disperser or not was the proportion of fish per trial that moved out of the introductory pool by the end of the 60-minute period. For fish that dispersed, a second dispersal characteristic of interest was dispersal rate, which I defined as the speed of movement out of the introductory pool. Individuals that dispersed immediately (before the first observation) were assigned the highest score (6), while individuals that dispersed between the first and second observations were assigned a 5, and so on down to a score of 1 for individuals that left between the next to last and last observations) (I conducted 6 observations, one every 10 minutes for 1 hour). The third measure of dispersal was the distance traveled (meters/min) by fish in the channel averaged over three two-minute observation periods. In a few cases, fish left

the introduction pool and moved quickly into another pool; and therefore, I was unable to record distance. These groups were excluded from the distance analysis.

Note that these variables are, in principle, independent. In particular, because my measures of dispersal rate and distance traveled only considered individuals that actually dispersed, it is possible for a trial to get a low score for proportion dispersing and to get a high score for dispersal rate (when in the trial dispersal occurred) or distance traveled if those few dispersers left early and swam a great deal in the channel. It is also possible for a trial to have all individuals disperse quickly (high scores for proportion dispersing and dispersal rate) and yet exhibit little movement while in the channel. If a species exhibits high values for proportion dispersing, dispersal rate, and distance traveled, this really represents three separate measures of high dispersal. To provide an overall measure of dispersal for each group of three females, I took the product of the proportion of individuals dispersing in 60 min., the dispersal rate score, and the mean travel distance while in the channel. I refer to this product as the dispersal tendency. Groups for which no dispersal distance was recorded were excluded from this analysis.

For dispersers, I also calculated the proportion of fish that colonized a new pool versus the proportion that remained in the channel. Finally, for fish that entered pools, I calculated the proportions entering the upstream versus downstream pools. In all cases, I averaged data for the three fish in each group to yield one value for each variable for each trial group. To assess variation in female boldness and its relationship to dispersal, I quantified refuge use of fish before dispersal. Boldness was measured as the proportion of fish out of refuge averaged for all observations where fish remained in the introductory pool. For fish that dispersed before the first observation at 10 min., I have no data on their refuge use, and they were excluded from the analysis.

Statistical analyses

Differences among the four *Gambusia* species in the seven variables describing dispersal and boldness were analyzed with ANOVA's using species as a grouping factor. I looked at the effect of day as a blocking factor and water temperature as a covariate, but these were not significant factors for any of the variables and were removed from final analyses. In order to better satisfy assumptions of parametric tests, all proportions (the proportion of fish dispersing, proportion of fish colonizing a pool, proportion of fish colonizing downstream versus upstream

pools, and the proportion of fish out of refuge) were arc-sin squared-root transformed, and dispersal rate scores and dispersal tendency measures were log-transformed (natural log) before being subjected to analyses. For dispersal distance, *G. hispaniolae* was omitted from the analysis since there were so few dispersing fish. Distances were left untransformed since data were normally distributed and sample variances were similar. For comparing whether dispersing fish that colonized a pool preferred the upstream versus downstream pool, I looked at the effect of direction and the interaction between direction and species on the proportion of dispersing fish that ended up in the pools. To compare species pairs once a species effect was detected, I used the Bonferroni procedure to perform multiple comparisons. The relationship between the dispersal variables and the proportion of fish in refuge was evaluated using general linear models on the transformed data. Regression coefficients were tested by t-tests with $\alpha = 0.05$. SYSTAT® Version 10 was used for all analyses.

RESULTS

Dispersal characteristics

Dispersal characteristics varied significantly among the four *Gambusia* species examined. Some aspects of dispersal fit my expectation of greater dispersal for invasive (as compared to non-invasive) species; however, other aspects of dispersal did not fit predictions. I found a strong effect of species on the proportion of fish that dispersed by the end of the 1-hour trials ($F_{3, 36} = 7.9$, $p < 0.0001$). *G. affinis* (invasive) and *G. geiseri* (non-invasive) showed the greatest dispersal, with 97 % and 85 % of fish dispersing out of the introductory pool respectively (Figure 4.2a). Surprisingly, *G. holbrooki*, the other invasive species, had significantly lower dispersal than *G. affinis* ($p < 0.0001$), with an average of 55 % of the fish tested actually dispersing. *G. hispaniolae* exhibited the lowest dispersal, only an average of 26 % dispersing, which was significantly different from *G. affinis* ($p < 0.0001$) and *G. geiseri* ($p = 0.007$).

For those fish that dispersed, species also significantly differed in their time to dispersal ($F_{3, 26} = 4.2$, $p = 0.02$). *G. affinis* not only dispersed the most but also dispersed first, leaving the introductory tank on average within the first 14 minutes of the 60 minute trial (Figure 4.2b). In contrast, *G. hispaniolae*, the species with the least dispersal, also took the longest to disperse, with females dispersing on average at minute 38 of the trial period. These two species differed

significantly from each other ($p = 0.01$), with no significant differences detected among the other species comparisons.

Distance traveled by dispersers (while in the river channel) showed species differences that better fit my predictions (although the analysis was unable to include *G. hispaniolae* because of too few dispersers). I found a strong effect of species on the mean distance traveled by females ($F_{2, 19} = 8.2$, $p = 0.003$). The invasives, *G. affinis* and *G. holbrooki* traveled significantly greater distances in the experimental streams than *G. geiseri* ($p = 0.006$ and $p = 0.007$ respectively). Invasive mosquitofish females covered on average 3.5 times more ground over a 1 min. period that did the non-invasive *G. geiseri* females (Figure 4.3a).

Species differed significantly in overall dispersal tendency (the product of the above three variables; $F_{3, 29} = 9.3$, $p = 0.0001$). The overall pattern was for *G. affinis* to exhibit the highest dispersal tendency, *G. hispaniolae* the lowest, and *G. holbrooki* and *G. geiseri* intermediate values (Figure 4.3b). Although dispersal tendencies appeared higher for *G. affinis* relative to *G. holbrooki*, this difference was not significant ($p = 0.11$). I found significantly higher dispersal tendencies for *G. affinis* compared to *G. hispaniolae* and *G. geiseri* ($p < 0.0001$ and $p = 0.02$). Although most *G. geiseri* left the introductory pool relatively quickly, because they moved relatively little while in the channel, their overall dispersal tendency score was much lower than that of *G. affinis*. For *G. holbrooki*, marginally significant differences were only found with *G. hispaniolae* ($p = 0.06$), not *G. geiseri*.

With regard to habitat choice after dispersal, I found no species differences in the locations that fish dispersed into after leaving the introductory pool. An analysis of variance performed on the proportion of dispersers moving into pools (either pool, as opposed to staying the channel) found no significant effect of species ($F_{3, 26} = 0.5$, $p = 0.70$). On average, 54 % of all fish in the experiment colonized either pool (Figure 4.4), while the remaining 46 % stayed in the channel. No fish ever returned to the introductory pool from the channel. For the individuals that colonized a new pool, I found that invasive and non-invasive species did not differ in their 'tendency to colonize' upstream versus downstream pools ($F_{3, 52} = 1.7$, $p = 0.17$). Instead, all species preferentially colonized the downstream pool rather than the upstream one ($F_{1, 52} = 27.8$, $p < 0.0001$) indicating similarities among the species in either their preference for moving with flow (as opposed to against it) or their ability to overcome flow. On average, 47 % of dispersing fish colonized the downstream pool, while only 7 % made it to the upstream pool (i.e., the other

46 % stayed in the channel). *G. affinis* and *G. geiseri* were the only species to have any fish move into the upstream pool, while no *G. holbrooki* or *G. hispaniolae* fish ever did (Figure 4.4).

Boldness and its relationship to dispersal

I detected a strong effect of species on boldness, the proportion of females outside of refuge while still in the introductory pool ($F_{3, 28} = 5.4$, $p = 0.005$). Mean boldness was lowest for *G. hispaniolae*, the species with the least dispersal, and highest for *G. affinis* and *G. geiseri*, the species with the most dispersal (Figure 4.5a). On average, 86 % of females of the two high dispersal species, *G. affinis* and *G. geiseri*, were observed out of refuge in the introductory pool, while only 38 % of *G. hispaniolae* females were observed out of refuge ($p = 0.03$ for *G. affinis* versus *G. hispaniolae*, and $p = 0.007$ for *G. geiseri* versus *G. hispaniolae*). Refuge use by *G. holbrooki* was intermediate (69 % out of refuge) and did not differ significantly from the three other *Gambusia* species. As predicted, I found a positive correlation between boldness and the proportion of fish dispersing out of the introductory pool for all species combined ($R^2 = 0.34$, $p = 0.001$) (Figure 4.5b). Boldness was also found to be a significant predictor of the overall dispersal tendency of the *Gambusia* species ($R^2 = 0.26$, $p = 0.009$) (Figure 4.5c). I found no relationship between boldness and dispersal rate ($R^2 = 0.10$, $p = 0.16$) or between boldness and dispersal distance ($R^2 = 0.01$, $p = 0.72$).

DISCUSSION

The transition between a species being non-indigenous and becoming invasive is often characterized by a lag phase followed by a period of rapid population growth and range expansion (Mack et al. 2000). This is presumably a consequence of not only advantageous demographic traits that allow the species to quickly increase in abundance, but also a result of the species' high dispersal rate. Thus, successful invasive species are expected to exhibit greater dispersal ability and/or tendency when compared to closely related species of low invasive potential. The results provide partial support for this hypothesis. I found strong evidence that *G. affinis* (invasive) has superior dispersal, at least in an artificial arena, than *G. hispaniolae* (non-invasive). *G. affinis* females were more likely to disperse out of the introductory pool, dispersed sooner, and showed an overall greater dispersal tendency score than *G. hispaniolae* females. The

dispersal performance of the other two species in my experiment, *G. holbrooki* (invasive) and *G. geiseri* (non-invasive), was less clear. While *G. affinis* had greater dispersal distances and dispersal tendency scores than *G. geiseri*, both species had similar proportions of fish dispersing and dispersal rates. For *G. holbrooki* (invasive), dispersal tendency scores were intermediate to those of *G. affinis* and *G. geiseri*. *G. holbrooki* females also dispersed less than *G. affinis* females, while their dispersal distances were equal.

Overall my results showed that *G. holbrooki* and *G. geiseri* have intermediate levels of dispersal compared to *G. affinis* and *G. hispaniolae*. That is, dispersal ability or tendency appears to fall on a continuum rather than into distinct categories (high vs. low dispersal) for these four species. While invasive species might tend to disperse more than non-invasive ones, there might be substantial variation within each type and overlap between the types. Indeed, the classification of species into 'invasive' versus 'non-invasive' might also be misleading. Invasiveness might not have two distinct classes, but might fall on a continuous gradient. If this is true, quantitative measures of relative invasiveness as a function of species traits would be useful information to policy makers and natural area managers faced with prioritizing the control of many invasive species.

The study found no differences among species in habitat choice. Overall, about half of the fish in the experiment moved into the side pools where flow was absent and refugia was greater, while the other half remained in the river portion of the setup. For those fish that moved into the side pools, I found a strong preference across all species for the downstream pool. This might be an overall reflection of the poor swimming ability of fish in this genus compared to other fishes. *Gambusia* are deep-bodied fish that prefer slow-moving waters and are not traditionally fast swimmers (Casterlin and Reynolds 1977). Flow has been found to be a significant barrier to dispersal for invasive *Gambusia* (Congdon 1995). Invasive *Gambusia* are also known to get flushed out of invaded streams during floods, while native fishes are able to persist (Arthington and Lloyd 1989).

The differences in dispersal characteristics found between the invasive sister species were surprising. *G. affinis* and *G. holbrooki* are very similar in their ecology, physiology, and morphology such that they are distinguished from each other only by slight differences in the structure of the gonopodium (Rosen and Bailey 1963) and by counts of rays in their dorsal and anal fins (Hubbs and Lagler 1964). Introductions of these species in the U.S. and the rest of the

world have not discriminated between the species since until recently mosquitofish were considered to be a single species (Wooten et al. 1988). For example, *G. holbrooki* was introduced to Australia and Mediterranean Europe, while *G. affinis* was introduced in the western U.S., Hawaii, and parts of Africa (Lever 1996, Fuller et al. 1999). While the relative degree of success of the two species in their invaded ranges is unknown, my dispersal data suggest that these sister taxa might differ in their mechanisms for invasion success. The dispersal results from this experiment suggest that *G. affinis* might be a better invader than *G. holbrooki*, while data on other potentially important traits such as fecundity, and maximum population growth rates suggest that *G. holbrooki* is a superior invader (Scribner 1993, Chapter 5). The relative invasiveness of the species may thus vary depending on the relative ecological importance of dispersal versus rapid population growth in invaded habitats.

Some of the dispersal characteristics and refuge use (boldness) in *G. geiseri* were comparable to those of the highly invasive *G. affinis*. These results along with other data on the foraging response to competition of these species (Chapter 3) seem to indicate that behaviorally speaking *G. geiseri* might fit the profile of a successful invader. *G. geiseri* seems to be as bold as invasive *Gambusia*. However, *G. geiseri* is extremely restricted in its native range, found in only two springs in central Texas. *G. geiseri* has also been unsuccessful at spreading outside points of introduction in western Texas (Hubbs and Springer 1957). I suspect that other traits such as demographic parameters and abiotic tolerances (Chapter 5) might be limiting and are important in explaining both its native distribution and its lack of invasiveness. This result argues for a need to carefully analyze a range of potential traits involved in order to fully understand the role of species traits in influencing invasion success.

Wilson et al. (1993) argued for the existence of a shy-bold continuum in natural animal populations similar to that found in humans. Previous studies have related variation in whether organisms are bold or shy to their learning ability and their antipredator response (Greenberg 1989, Coleman and Wilson 1998, Seferta et al. 2001). Fraser et al. (2001) linked behavioral assays of boldness to dispersal distance in both experimental and natural streams. My study found a similar positive relationship between dispersal (in an experimental stream) and boldness. The proportion of females dispersing and their overall dispersal tendency score were positively, although not strongly, correlated with the proportion of females out of refuge. I also found species differences in boldness that provided mixed support for my hypothesis of how boldness

should relate to invasive ability. *G. affinis* (invasive) was significantly bolder than *G. hispaniolae* (non-invasive) but not *G. geiseri* (non-invasive). I expected that how organisms behaviorally respond to completely unfamiliar situations (e.g., novel habitat, prey, predators, competitors) might be an important determinant of whether a species fails or succeeds to establish and spread in a newly invaded community. Whether a species' invasive ability can thus be predicted from its response to novel stimuli is an issue deserving further exploration.

The study of biological invasions has been largely descriptive in nature (Kolar and Lodge 2001), especially for vertebrate invaders. Researchers have expressed a need for more experimental research in invasion biology (Parker and Reichard 1998, Williamson 1999). Researchers have also pointed the need for a better understanding of the role of behavioral mechanisms as key predictors of invasion success (Holway and Suarez 1999, e.g. Sol et al. 2002). My study uses an experimental assay to characterized dispersal of invasive and non-invasive species and its relationship to a behavioral trait (boldness). Future work will address whether dispersal measured in experimental streams closely predicts movement in the field as found by Fraser et al. (2001). While experimental setups like the one used in this study have been useful tools in ecology (Gelwick and Matthews 1993), they have been rarely used to test predictions in invasion biology. Future studies on species invasions would benefit by more experimental manipulations and by investigating the role of behavioral tendencies as potential mechanisms underlying invasiveness.

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Figure 4.1: Diagram of one of the artificial streams used in the experiment. Single-pointed arrows indicate direction of water flow, while double pointed arrows show setup dimensions. Shaded areas indicate placement and relative size of fish refugia. The 'x' shows the location where fish were released at the start of each trial.

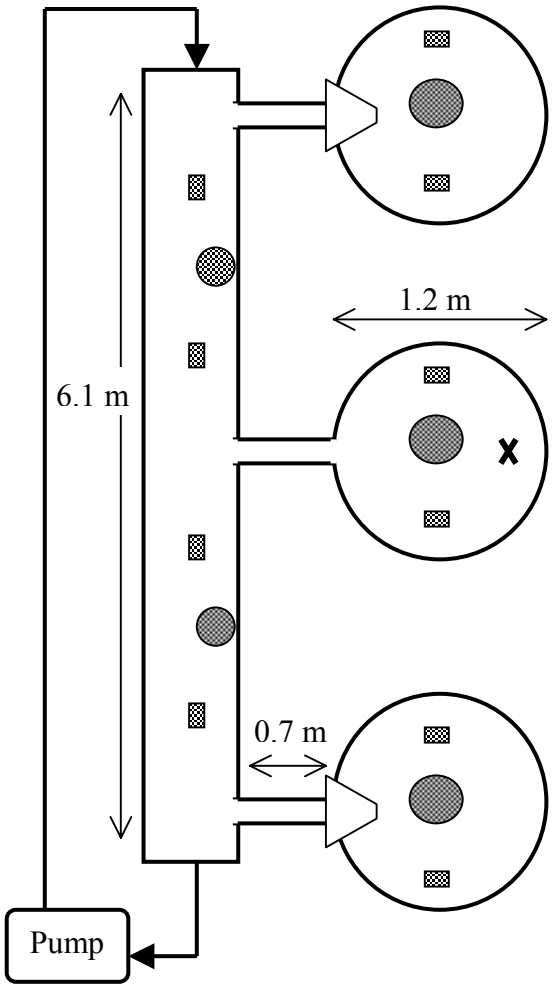


Figure 4.2: (a) Proportion of fish (out of 3 females) that dispersed out of the introductory pool by the end of the 1-hour trial for each *Gambusia* species. (b) Ranking of dispersal event for the dispersers (the higher the ranking the sooner in the trial the dispersal event occurred). Dark bars represent the two invasive species, *G. affinis* and *G. holbrooki*, and light bars the non-invasive, *G. geiseri* and *G. hispaniolae*. Bars represent mean values ± 1 standard error (SE).

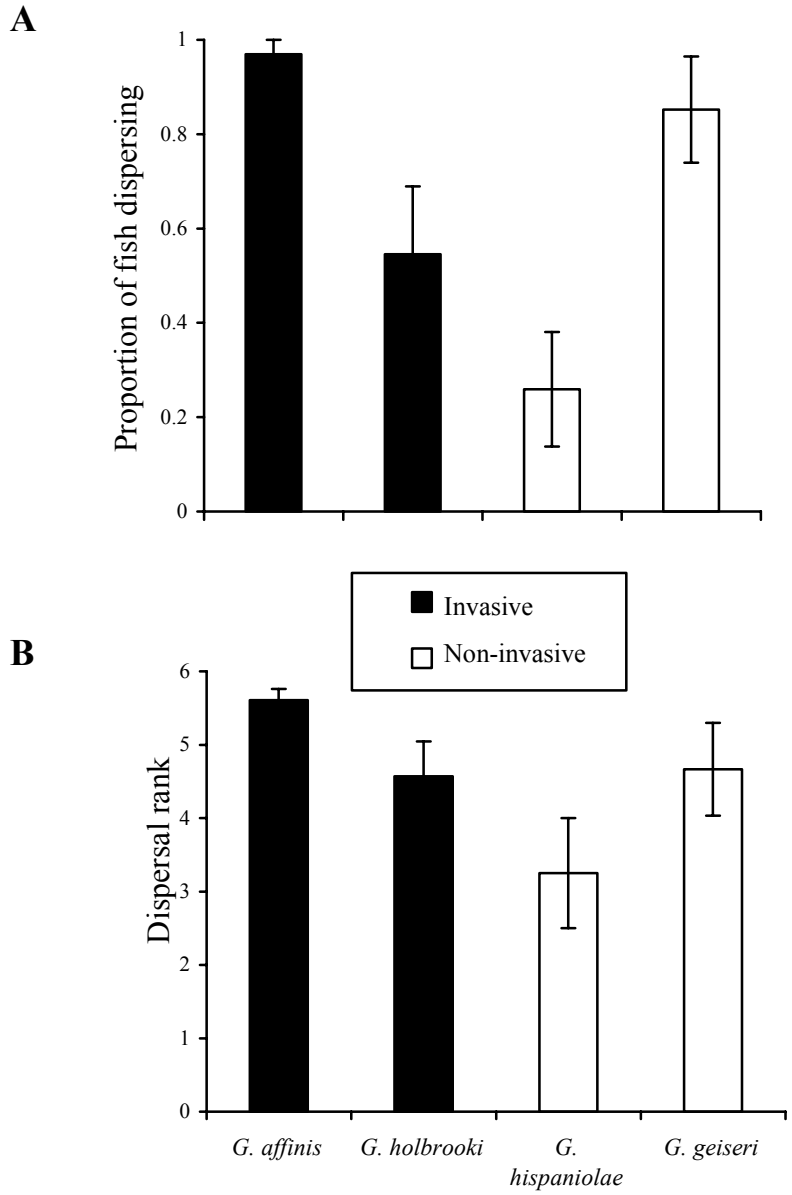


Figure 4.3: (a) Distance traveled (mean ± 1 SE) by dispersing females in the river portion of the setup calculated in meters per minute. Distance traveled is not shown for *G. hispaniolae* because numbers of dispersers were too small. (b) Dispersal tendency (mean ± 1 SE) (product of proportion of fish dispersing, dispersal rate and distance) for each *Gambusia* species. Mean dispersal tendency is zero for *G. hispaniolae*.

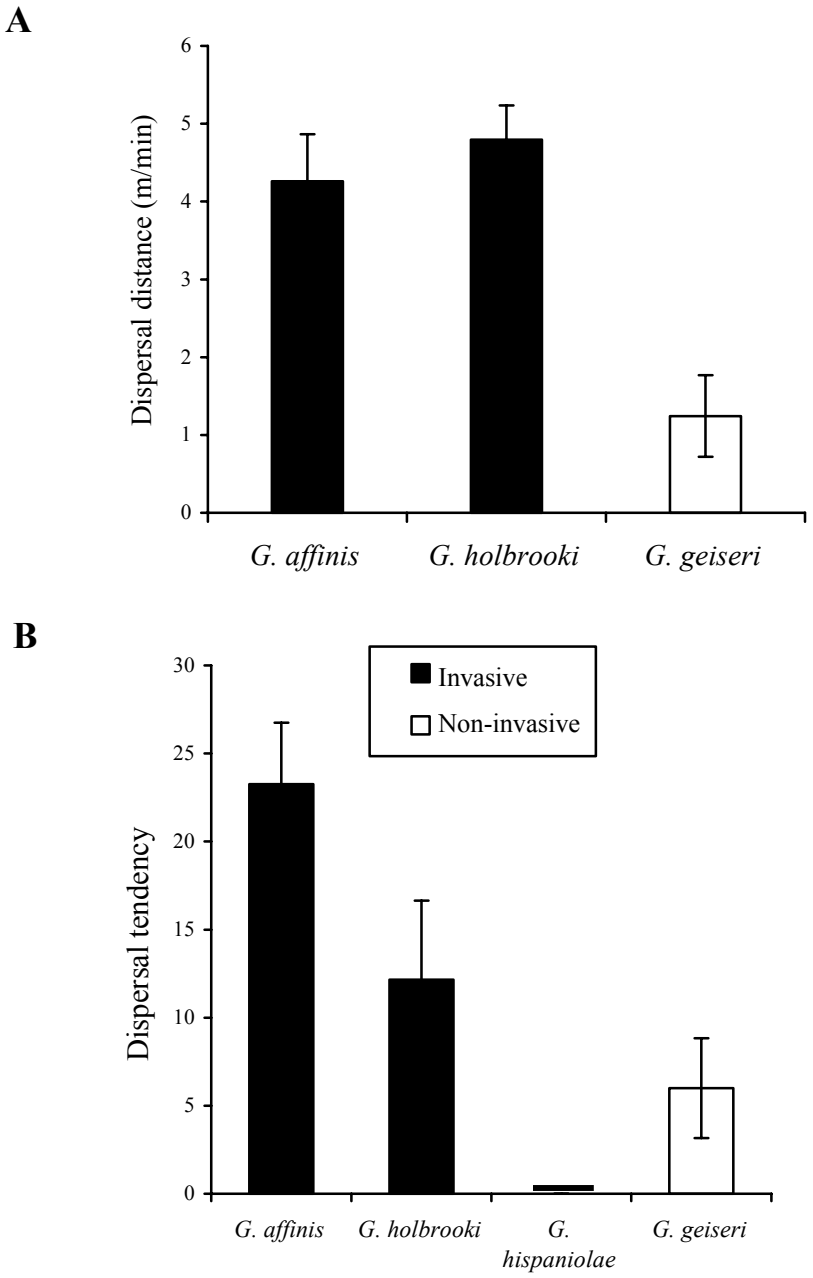


Figure 4.4: Proportion of dispersing females that arrived to suitable habitat in pools. Mean proportions are partitioned by whether fish dispersed to the downstream (shaded) or the upstream pool (no shading). Error bars correspond to the mean proportion of fish dispersing into pools before partitioning.

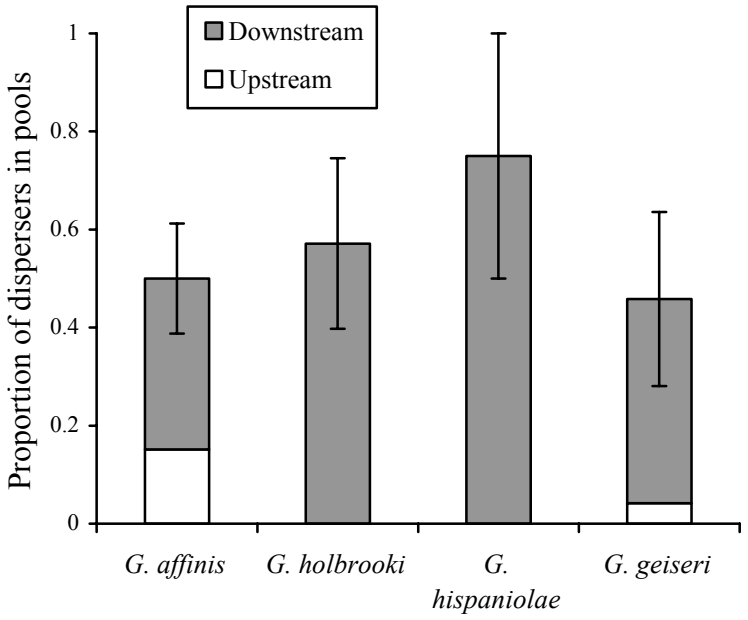


Figure 4.5: (a) Proportion of fish out refuge (mean ± 1 SE) in the introduction tank for each *Gambusia* species. (b) Proportion of fish dispersing out of the introduction tank as a function of the proportion of fish out of refuge for all species combined. Symbol size approximates number of data points overlaid (number is shown in parenthesis below corresponding data points). Best-fit line is shown ($R^2 = 0.33$).

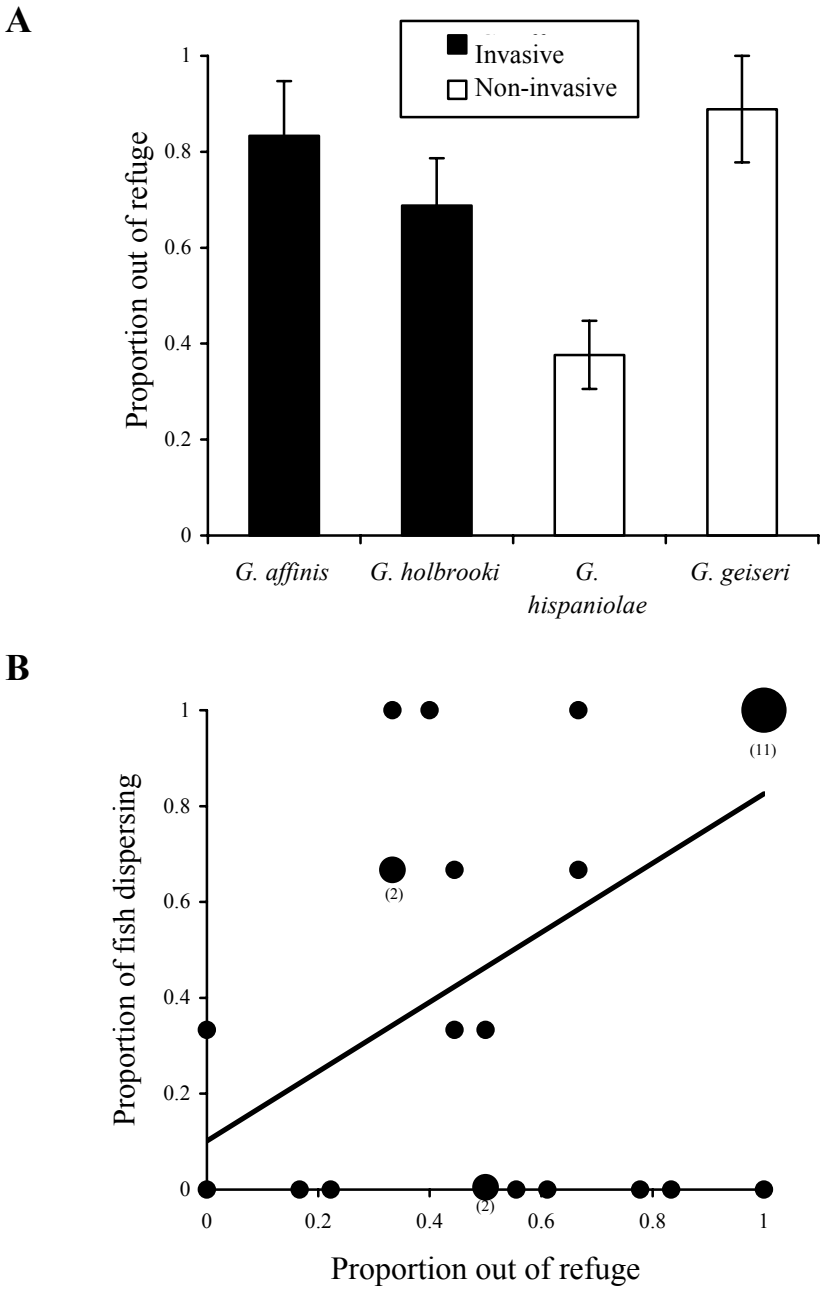
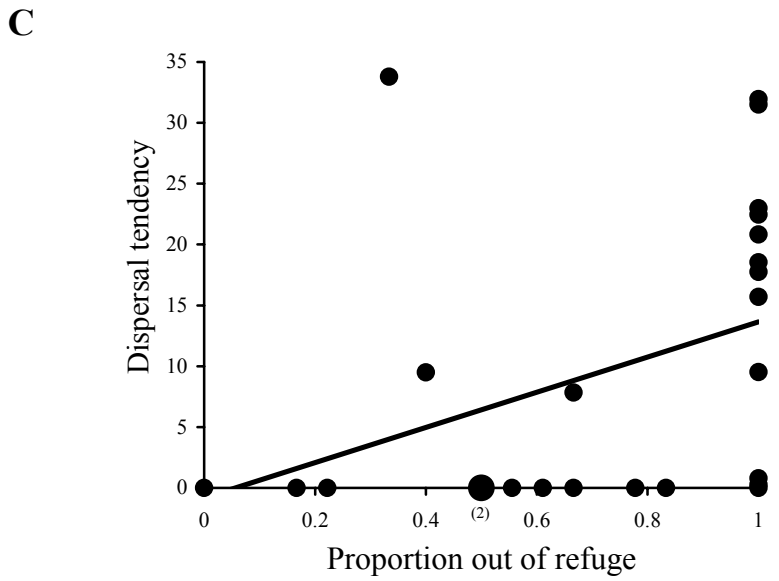


Figure 4.5: (c) Dispersal tendency score as a function of the proportion of fish out of refuge for all species combined. Symbol size approximates number of data points overlaid (number is shown in parenthesis below corresponding data points). Best-fit line is shown ($R^2 = 0.26$).



Chapter Five

Life histories, temperature tolerances, and success in a simulated invasion: A comparison of invasive and non-invasive *Gambusia* species

SUMMARY

For establishment to occur, non-indigenous species must be able to survive and reproduce in the novel community. A major reason for establishment failure is the inability of arriving organisms to overcome the novel abiotic conditions of the community. Organisms with broad abiotic tolerances are expected to have higher survival and be more likely to become established. Successful establishment is also dependent on the ability of invaders to become abundant from low densities. Life history traits are known to influence a species' intrinsic growth rate. In this study, I examined the role of abiotic tolerances and life histories as key determinants of establishment success. To gain insight into whether these traits are related to invasiveness, I compared traits among closely related invasive and non-invasive species. I investigated the low temperature tolerance of invasive and non-invasive *Gambusia* by mimicking overwintering conditions in laboratory incubators and quantifying survivorship. I compared fecundity (brood size), offspring size, juvenile growth rates, and age and size at sexual maturity among the two invasive and two non-invasive species. To test whether these traits had consequences for establishment success, I then mimicked the invasion of simplified temperate pond communities by each of the four species and monitored population performance over 10 months. Invasive *Gambusia* were more tolerant of low temperatures than non-invasives. Invasives exhibited higher fecundity, higher juvenile growth rates, and consequently reached sexual maturity sooner and at smaller sizes than non-invasives. As may be predicted from these trait differences, I found only invasive *Gambusia* populations to successfully establish in the experimental ponds by becoming more abundant (higher *r* and K) and persisting in spite of overwintering conditions.

INTRODUCTION

Recent research has shown that species-specific traits are key elements to the predictive understanding of invasion success by non-indigenous species (Kolar and Lodge 2001). Although efforts at finding trait generalities that convey invasiveness to all species in all invaded communities have largely failed, great strides have been made when the scope of prediction has been more limited (Kolar and Lodge 2002). For example, efforts at understanding the traits involved in allowing invaders to colonize a particular ecosystem or geographic region have been particularly useful. Moyle and Light (1996b) argued that the invasion success of fish species in California watersheds is primarily determined by the species' ability to cope with the fluctuating hydrologic regime of Mediterranean-type streams. Another important approach has been the comparison of closely related species of varying invasiveness (Mack 1996a). Rejmanek and Richardson (1996) reported that for *Pinus* species, earlier and more consistent reproduction (a shorter interval between large seed crops) and small seeds are the key traits distinguishing invasive pine species from non-invasives.

One of the key insights in the study of invasive species is the idea that invasions occur as a sequence of steps (arrival, establishment, spread, and impact) (Ashton and Mitchell 1989, Williamson 1996, Mack et al. 2000). Processes and factors affecting invasion dynamics are likely unique to each stage (Moyle and Light 1996b). In particular, different species traits are expected to help invaders succeed at each stage of the sequence (Carlton 1996, Vermeij 1996). Traits beneficial in one stage might be detrimental or not important in another stage (Duncan et al. 2001). For instance, for 52 species of birds introduced to Australia, an omnivorous (or carnivorous) diet was a good predictor of establishment success, but was unrelated to the amount of spread attained by the invaders. Native range size was a good predictor of spread, but a weak predictor of establishment. Surprisingly, body size, while a weak predictor in both stages, was positively correlated with establishment and negatively correlated with spread. As illustrated by this example, a clear understanding of the relationship between species traits and invasion patterns requires careful examination of how traits affect invasion dynamics separately along each stage of the invasion sequence (Kolar and Lodge 2001).

In this study, I focused on the establishment phase of an invasion and asked, what are the key species traits responsible for a species' recruitment and local persistence in the newly

invaded habitat? In order for a non-indigenous species to successfully transition between arrival and establishment, organisms must be able to (a) survive and (b) reproduce in the novel community. Mortality of newly arrived organisms is thought to be enormous (Mack et al. 2000), and only 10 % of species released or dispersed are expected to become established (di Castri 1989, Kolar and Lodge 2001). A primary reason for their failure to establish is their inability to overcome the novel abiotic conditions they encounter (Lodge 1993a, Mack et al. 2000, Richardson et al. 2000). In fact, invaders are often more successful if there is a high degree of climatic matching between their native and invaded ranges (Newsome and Noble 1986, Welcomme 1992, Green 1997, Moyle and Light 1996a, Williamson and Fitter 1996b, Carlton 1999, Wonham et al. 2000, Duncan et al. 2001). In the absence of climate matching, organisms with broad abiotic tolerances usually have higher survival and are more likely to become established in newly invaded habitats (Moyle and Light 1996b, Brown and Moyle 1997). For example, fishes that established successfully in the Great Lakes region are (beyond growing faster) tolerant of a wider range of temperatures and salinity conditions than species that did not establish (Kolar and Lodge 2002). Similarly, Marchetti et al. (2003), in a more detailed analysis of fish invasions in California streams, also found environmental tolerance to be an important predictor of establishment.

In some instances, invaders survive, but do not establish because they fail to reproduce successfully (Crivelli 1995, Williamson and Fitter 1996a, Wonham et al. 2000). Establishment in a new range is dependent on the ability of arriving organisms to find habitat and abiotic conditions favorable to reproduction. Successful establishment is also dependent on the reproductive potential of arriving organisms and their ability to become abundant from low densities (Reichard and Hamilton 1997, Shea and Chesson 2002). Life history traits that result in a high maximum reproductive output and a high intrinsic growth rate in the invaded habitat (e.g., high fecundity, short juvenile period, short interbrood interval) should increase the probability of successful establishment (Baker 1965, Ehlrich 1989, Newsome and Noble 1986, Rejmanek and Richardson 1996, Sakai et al. 2001). Few studies, however, have quantitatively tested this idea by comparing intrinsic growth rates of similar invasive and non-invasive species (Williamson 1996, Kolar and Lodge 2001). This is especially true in aquatic systems (Lodge 1993b, Lodge et al. 1998).

The major objective of this study is to examine the role of abiotic tolerances and life histories as key traits determining whether a non-indigenous species becomes established in a newly invaded habitat. To address this issue, I first compared the temperature tolerances and life history traits of four *Gambusia* species, two invasive and two non-invasive. I investigated temperature tolerances by mimicking overwintering conditions in laboratory incubators and quantifying adult and juvenile survivorship. I examined life history variation by comparing fecundity (brood size), offspring size, juvenile growth rates, and age and size at sexual maturity among the four species. If life histories and abiotic tolerances are in fact important to allowing a species to invade, I expected the two invasive *Gambusia* species to have greater tolerances and a larger number of smaller offspring, higher growth rates, and reach sexual maturity sooner at smaller sizes. To test if trait differences had consequences for establishment success, I then mimicked the invasion of simplified temperate pond communities by both invasive and non-invasive *Gambusia* and compared whether the species were able to 'establish' in the novel community and the degree of establishment success (i.e., population size) achieved and maintained by each species over a period of 10 months.

The poeciliid fish *Gambusia* is an ideal system for investigating traits that might explain relative invasiveness in closely related taxa. Species in this genus show great variation in their geographic distribution, abundance, and apparent invasiveness. At one end of the spectrum, the sister species *G. affinis* and *G. holbrooki* (western and eastern mosquitofish) have widespread distributions in North America and have been successfully introduced for mosquito control in over 40 countries (Welcomme 1992, Lever 1996). Introduced mosquitofish have successfully spread from points of introduction worldwide (Arthington and Lloyd 1989) and have significantly impacted invaded communities and native biota (Schoenherr 1981, Meffe et al. 1983, Lloyd 1989, Gamradt and Kats 1996, Howe et al. 1997, Webb and Joss 1997, Goodsell and Kats 1999) to the extent that they have been designated among the 100 worst invasive species worldwide (ISSG 2000).

In contrast, despite strong similarities in their ecology, morphology, and body size, the rest of the species in the genus have restricted geographic distributions, have rarely been introduced, and when they have been introduced have failed to spread. For this study, I focused on two such non-invasive species, *G. geiseri* and *G. hispaniolae*. *G. geiseri* is endemic to spring habitats of the Southwestern U.S. (Hubbs and Springer 1957) and appears to be a sister taxon to the invasive

species pair (partial phylogeny by Lydeard et al. 1995). Outside its native range, the success of *G. geiseri* has been very limited. While this species has been introduced to several habitats similar to its own (Fuller et al. 1999), *G. geiseri* has not spread and remains localized to points of introduction (C. Hubbs, personal communication). *G. hispaniolae* is a Caribbean species native to the central region of Hispaniola. Although it is the most widespread of the three endemic *Gambusia* species in the island (Burgess and Franz 1989), it is still rather restricted in its range and has never been translocated. Even presently, when irrigation canals have increased the connectivity of the region, *G. hispaniolae* does not seem to be spreading out of its native range, which suggests that its potential invasiveness is low; therefore, I treat it as a non-invasive.

METHODS

To examine whether invasive and non-invasive *Gambusia* differed in their low temperature tolerances, I exposed F1 and F2 generation fish from each species and from collections made in the summer and fall of 1999 to decreasing temperatures over a period of 6 weeks. To examine *Gambusia* life histories, I compared fecundities of F1 fish and then raised their offspring under standardized favorable conditions to sexual maturity and measured offspring size, growth rates, and age and size at sexual maturity. For the simulated invasion, 10 F1 *Gambusia* were added to experimental mesocosms after inoculation with pond fauna. Each *Gambusia* species invaded a community on its own, in which it was the only predatory species. Population trajectories were monitored over 42 weeks, which included overwintering conditions and their effect on population performance. Source populations for the three experiments were from within each species' native range. *G. geiseri* and *G. affinis* were descendents of fish collected from Comal Springs and the Comal River respectively in Comal County, TX. All *G. holbrooki* were progeny of fish collected from Leon Hines Lake, Escambia County, AL. *G. hispaniolae* were the progeny of collections made in La Azufrada, Lake Enriquillo, Dominican Republic. The only exception was the non-invasive F1 adults in the temperature tolerance experiment. *G. geiseri* adults were descendents of collections made in San Marcos Springs, Hays County, TX, and *G. hispaniolae* adults were descendents of collections made in Los Borbollones, Lake Enriquillo, Dominican Republic.

Temperature tolerance

I investigated whether *Gambusia* species differed in their temperature tolerance by measuring adult and juvenile survivorship when exposed to decreasing temperatures, 23° C to 4° C, over a period of 45 days. I used three incubators as replicates, and I compared fish survival in incubators to one group of each species held at constant 23° C for the length of the experiment (referred to as the control treatment). Fish were selected from stock tanks and randomly assigned to either the overwintering treatment or the control treatment. In each incubator (and the control treatment), four 38 L tanks held fish of each of the four *Gambusia* species separately. Each tank held 8 adults (4 males and 4 females) and 8 juveniles, resulting in 64 fish per species, and a total sample size of 256 individuals. Because adult *Gambusia* are cannibalistic, adults and juveniles were separated by a mesh partition within each tank. Each tank was provided with sand substrate, aeration, and refuge in the form of a clump of java moss, *Vesicularia dubyana.* Fish were fed *ad libitum* a combination of flakes, a liver and spinach paste enriched with vitamins and minerals, and newly hatched *Artemia* every other day. At feedings, I also checked for mortality (dead fish were counted and removed) and verified temperature settings.

The experiment was started on April 8, 2001 and was terminated on May 22, 2001. Prior to the experiment, fish were held at 21-25° C in stock tanks. The temperature regime in the incubators was as follows: on day 1 temperatures were lowered from 23° C to 20° C, on day 5 from 20° C to 16° C, and subsequently temperatures were lowered by 4° C every 10 days (to a minimum of 4° C starting on day 35 of the experiment). In order to consistently simulate temperate overwintering conditions, I also shortened the photoperiod in both the control and overwintering treatments (from 14 h L: 10 h D to 8 h L: 16 D).

Life histories

I quantified brood size, offspring size, juvenile growth rates, and age and size at sexual maturity for the four *Gambusia* species under laboratory conditions in the summer of 2001. *Gambusia* are ideal organisms for life history studies because of their small size (2-5 cm long), short generation times, livebearing reproduction, and ease of determining reproductive status. *Gambusia* exhibit internal fertilization (sperm is transferred via a modified anal fin, the gonopodium), and females are able to fertilize their eggs with sperm from multiple males stored

for several months, thus reducing the negative impacts of founder effects (Chesser et al. 1984, Scribner et al. 1992, Zane et al. 1999). Interbrood intervals generally range from 28 to 40 days (Krumholz 1948, Hubbs 1996).

Gravid F1 females of each species were selected at random from stock tanks, isolated in 6 L aquaria overnight, and provided with refuge for newborns. A total of 20 *G. affinis*, 20 *G. holbrooki*, 23 *G. hispaniolae*, and 18 *G. geiseri* were selected. Prior to this experiment, females of all species were reared along with males under similar densities in 76 L aquaria and fed *ad. libitum* a combination of flakes, *Artemia*, and liver paste. At parturition, offspring number was quantified and female standard length (SL, tip of the upper lip to beginning of caudal fin) was measured to the nearest 0.5 mm. Females remained isolated for an additional 24 h after parturition to ensure that all offspring were accounted for.

For each species, a subset of the broods was randomly selected to quantify offspring size, juvenile growth rates, and age and size at sexual maturity. For large broods 10 offspring were selected at random, and for smaller broods all offspring were used. Within 24 h of birth, offspring were measured (SL to 0.5 mm), and for each brood, offspring were randomly assigned to one of 10 38-L aquaria. This was done separately for all four species, for a total of 40 38-L aquaria (10 per species). Offspring were individually raised in 0.9 L plastic containers floated in these tanks. Containers were outfitted with sand substrate, refuge (java moss), constant aeration, and water circulation (via several mesh-covered holes in containers). A maximum of 8 containers were placed in each aquarium, each containing an individual from a different sibship. Because of species differences in brood size, the number of sibships used varied (9 for *G. holbrooki*, 10 for *G. affinis* and *G. hispaniolae*, and 13 for *G. geiseri*). Offspring were fed brine shrimp *ad libitum* on a daily basis. Freshly hatched brine shrimp feedings were alternated with feedings of refrigerated brine shrimp hatched 24 h prior. Water temperature in aquaria was maintained between 24.5-26.5 °C, and photoperiod was maintained at 13 h L: 11 h D for the duration of the experiment.

Offspring SL was measured at 20 days after birth and at sexual maturity. Sexual maturity in males of all four species was determined by the full elongation of the gonopodium as described by Turner (1941) and Rosen and Bailey (1963). Sexual maturity of females was determined by the appearance of pigmented lateral brood spots (Constanz 1989) and enlarged abdomens (a sign of fully yolked eggs) (Krumholz 1948) in all species except *G. affinis*. For *G.*

affinis females, brood spots were generally absent in our study population and sexual maturity was scored by their enlarged abdomens, usually acquiring a yellow coloration at maturity. I do not think this difference in markings biased the scoring because either if brood spots were absent or present, enlarged abdomens were the key indicator of sexual maturity and were scored in the same manner for all four species. When fish were sexually matured, their SL was measured and the day of maturity recorded. The experiment ran from June 24, 2001 to September 1, 2001. Because of time constraints, the experiment was terminated before all of the *G. hispaniolae* fish had reached sexual maturity. Fifty-one of the 80 *G. hispaniolae* offspring that started the experiment matured by September 1. Because of *G. geiseri*'s lower fecundity, only 46 offspring were included in the life history analyses (sample sizes for *G. affinis* and *G. holbrooki* were 75 and 74 individuals respectively).

Establishment success

I conducted this experiment in large outdoor tanks at the University of Kentucky Ecological Research Facility in Lexington, KY. I constructed replicate pond communities in 550-L plastic tanks where the only difference was the identity of the *Gambusia* species present. I intended for these pools to simulate a simplified community under invasion by *Gambusia*. Each species' community was replicated three times for a total of 12 experimental units. Tanks were inoculated equally with nutrients (rabbit feed), phytoplankton, zooplankton, and macrophytes on July 17, 2001. Invertebrates and plankton (mostly cladocerans and chironomids) were introduced to each pool by the addition of 5 L of unfiltered water from local ponds at the Ecological Research Facility. Tanks were also left uncovered for the duration of the experiment to allow further colonization by aquatic invertebrates. Brazilian elodea, *Egeria densa*, a common exotic weed species in Kentucky ponds, was added in equal amounts to all pools to provide structural complexity and cover for newborns. Because I wanted to simulate a natural invasion and since *Gambusia* did not appear to be food limited, tanks were not supplemented with fish food. *Gambusia* populations were started with five adult F1 males and five gravid F1 females 10 days after community inoculation. I monitored population trajectories over a period of 42 weeks, ending on May 21, 2002. I measured each tank's population size through surveys where all fish were seined, counted, and replaced. Sampling effort was standardized by seining until three empty seine hauls were obtained in each pool. Surveys were conducted at weeks 7, 11, and 16

(September 13, October 13, and November 18, 2001) after fish inoculation. A final survey was conducted early the following spring at week 42 to determine overwintering survival.

Statistical analyses

Temperature tolerances for adult and juvenile *Gambusia* were calculated as the proportion of surviving fish per tank. To detect whether or not *Gambusia* species were affected by cooling conditions, I compared confidence intervals of survival proportions (of the three replicate tanks) in the overwintering treatment to the proportion of fish surviving in the control treatment (one replicate) separately for each species and on day 45 of the experiment. Because variances were zero for some of the species overwintering survival estimates, I was unable to compare these survivorships using t-tests.

To determine how species differed in their sensitivity to decreasing temperatures, I compared survivorship of fish in the overwintering treatment only in a repeated measures ANOVA with species and time as grouping factors and a species by time interaction. For the time factor, I compared survivorships at days 5, 15, 25, 35, and 45 of the experiment. These corresponded to exposure to the following temperatures: 20, 16, 12, 8, and 4° C. Proportions of surviving fish were transformed (arc-sin squared-root transformation) in order to reduce variance heterogeneity. Analyses were done separately for juveniles and adults.

Fecundity differences among *Gambusia* were analyzed with a one-way ANCOVA using female size as a covariate. Brood sizes were log transformed (natural log) in order to reduce heterogeneity of treatment variances and non-normality. MANOVA's were used to test for species and sex effects, and a species by sex interaction on the following five life history parameters measured on the F2 offspring: size at birth, growth rates to 20 days and sexual maturity, and age and size at sexual maturity. I then performed statistical analyses on the individual variables using ANCOVA's with female size and brood size (i.e., the number of siblings) as covariates. All five variables were left untransformed for analysis since they were approximately normally distributed, sample sizes were large, and variances were similar among treatments. Juvenile growth rates were calculated over a fixed interval (growth to day 20) and a variable interval (growth to sexual maturity). Both growth rate measures were calculated as instantaneous rates where growth = [Ln (SL at time 2) - Ln (SL at time 1)]/ (number of days between times 1 and 2) with units 1/d (Ricker 1979).

For the life histories analysis, individuals were treated as independent experimental units. Individuals were raised in individual containers and in separate tanks, and no significant tank effects were detected in preliminary analyses. To ensure that the species differences were not overestimated by potentially low within sibship variation, I performed a second analysis where only two individuals (a male and a female) chosen randomly from each sibship were included and the same effects were tested. The results of this analysis were virtually identical to the analysis with the entire dataset (the only difference is that the sex by species interactions were lost). Thus, I feel confident that the analyses reported here including all individuals are valid.

Differences in population sizes were analyzed as a repeated measures ANOVA with species and time effects, and the species by time interaction. Population sizes were log transformed (Ln +1) prior to analysis to reduce variance heterogeneity. Intrinsic rates of increase (r) and carrying capacities (K) were estimated for each replicate of each species based on linear regression using the logistic equation: $dN/dt = rN\,[(K-N)/K]$ following Scribner (1993). R estimates were based on population sizes at week 7, while K estimates were calculated from values at weeks 7, 11, and 15, except for *G. hispaniolae* where only population sizes from weeks 7 and 11 were used because of significant winter mortality at week 15. Comparisons of r and K among species were conducted with a one-way analysis of variance on untransformed values.

For all analyses, once a species (or interaction) effect was detected, orthogonal contrasts were used to test three hypotheses of interest: whether the two invasive species differed from the two non-invasive species, whether the two invasive species differed from each other, and whether the two species categorized as non-invasive differed from each other. P-values for comparisons of the two invasive against the two non-invasive species correspond to one-tailed tests. All other reported p-values reflect two-tailed tests. LSD pairwise comparisons were used look at the species by sex comparisons. All analyses were performed using Proc GLM (and type III sums of squares) in SAS (SAS Institute, Cary, NC, USA) and SYSTAT® Version 10.

RESULTS

Temperature tolerance

In general, cold temperatures affected only the survival of the non-invasive *Gambusia* species, *G. geiseri* and *G. hispaniolae*. For the two invasive species, juvenile survivorship in the

overwintering treatment did not differ significantly from survivorships in the control treatment (100 % survival), but did differ for the non-invasives (95 % confidence intervals are 0-0 for both species). The pattern was slightly different for the adults. Adult survival differed significantly between the overwintering and the control treatments for three of the four species tested (Confidence intervals are: *G. affinis*, 0.75-0.75; *G. geiseri*, 0-0, and *G. hispaniolae*, 0-0, and do not overlap the 1.0 survival in the control). Only adult *G. holbrooki* (invasive) exhibited survivorships in the overwintering treatment that were indistinguishable from survivorship at room temperature (Confidence interval is 0.6-2.0). Thus, invasive *G. affinis* adults seemed to be somewhat affected by cold temperatures, although their survivorships were much greater than the survivorships of the non-invasive species, 75 % compared to 0 % survival for both non-invasives.

Gambusia species differed in how temperature affected their survival in the overwintering treatment. The effects were similar for juveniles and adults. I found a significant species by time interaction on both adult and juvenile survivorships (Table 5.1). Invasive juvenile and adult *Gambusia* had higher survival in response to decreasing temperatures than the two non-invasive species, *G. hispaniolae* and *G. geiseri* (Figure 5.1). Orthogonal contrasts revealed that after exposure to 12, 8, and 4° C, invasive survivorships (both age classes) were significantly greater than non-invasive survivorships ($p < 0.03$ for all comparisons). For instance, after exposure to 4° C (the lowest temperature of the experiment), survivorships for the two invasive species averaged 69 % for adults and 77 % for juveniles, while non-invasives had experienced 100 % mortality in both age classes.

Invasive survivorships were identical for adults but differed for juveniles. *G. holbrooki* juveniles tended to have lower survival than *G. affinis* after exposure to 4° C (58 % compared to 96 %) ($F_{1, 8} = 4.7$, $p = 0.06$) (Figure 5.1a). In contrast, non-invasive survivorships were similar for juveniles but differed for the adults. *G. geiseri* adults tended to be more tolerant of low temperatures than *G. hispaniolae* adults. The pattern of sensitivity was also different between the non-invasive adults. While survivorship decreased gradually for *G. geiseri* adults, the decrease was sharp for *G. hispaniolae* adults. After exposure to 12° C, *G. hispaniolae* exhibited 100 % survival compared to 77 % survival for *G. geiseri* ($F_{1, 8} = 4.6$, $p = 0.06$). As temperatures decreased to 8° C, the effect changed dramatically and survivorships were 8 % for *G.*

hispaniolae and 58 % for *G. geiseri* ($F_{1, 8} = 6.7$, $p = 0.03$). After exposure to 4° C, *G. geiseri* survivorships still tended to be higher than *G. hispaniolae*'s (38 % compared to 0 %) ($F_{1, 8} = 4.2$, $p = 0.08$).

Life histories

Species had a highly significant effect on brood sizes of the lab-reared F1 females ($F_{3, 76} = 11.3$, $p < 0.0001$). Mean brood sizes of the two invasive *Gambusia* species were significantly larger than brood sizes of the two non-invasive species ($F_{1, 76} = 13.4$, $p = 0.0003$). Fecundities of invasive *Gambusia* averaged 14 offspring compared to only 8 offspring for the non-invasive species (Figure 5.2). Female size was a highly significant covariate; larger *Gambusia* had larger broods ($F_{1,76} = 21.1$, $p < 0.0001$). Of the invasives, *G. holbrooki* has a significantly greater fecundity than *G. affinis* ($F_{1, 76} = 4.6$, $p = 0.04$). For the non-invasives, the analysis of covariance showed that, *G. geiseri*, the smallest of the four species, had a greater fecundity than *G. hispaniolae* ($F_{1, 76} = 4.1$, $p = 0.05$).

The MANOVA's comparing size at birth, juvenile growth rates, and age and size at sexual maturity showed strong species effects, sex effects, and species by sex interactions (Wilks' Lambda, all p-values < 0.0001). For size at birth, an ANCOVA showed a highly significant species effect (Table 5.2). When paired, the two invasive species had significantly smaller offspring than the two non-invasive species ($F_{1, 268} = 620.8$, $p < 0.0001$), but I also found significant differences between the species in each category. *G. affinis* newborns were significantly larger than *G. holbrooki* newborns ($F_{1, 268} = 19.8$, $p < 0.0001$), while *G. hispaniolae* newborns were significantly larger than *G. geiseri* newborns ($F_{1, 268} = 639.2$, $p < 0.0001$) (Figure 5.3). The largest disparity in size at birth was observed between *G. hispaniolae* offspring and the three other species combined, 11.4 mm compared to an average of 7.6 mm. This result makes sense since *G. hispaniolae* is the species with the largest mean adult size. However, accounting for female size as a covariate did not explain any of the variation in offspring size. I did detect a strong effect of a second covariate, brood size (Table 5.2). As expected from tradeoffs between offspring size and offspring number (Smith and Fretwell 1974), the overall relationship between brood size and offspring size was negative; though, on a species by species basis, the relationship existed only for *G. affinis* ($F_{1,73} = 29.3$, $p < 0.0001$).

I found a highly significant species effect on both growth rates measured (Table 5.2). Invasive *Gambusia* had significantly higher growth rates to day 20 and also to sexual maturity than non-invasive *Gambusia* ($F_{1, 264} = 173.8$, $p < 0.0001$ and $F_{1, 229} = 258.0$, $p < 0.0001$ respectively). On average, growth rates of invasive fish were 29 % higher than growth rates of non-invasives (Figure 5.4). Differences between invasives and non-invasives were greater for growth rates to sexual maturity than for growth rates over the first 20 days of development (34 % compared to 23 %). For the invasives, I found no differences in either growth measure between *G. affinis* and *G. holbrooki*. In contrast, the non-invasive species exhibited marked differences in their growth ($F_{1, 264} = 67.6$, $p < 0.0001$ and $F_{1, 229} = 31.9$, $p < 0.0001$). *G. hispaniolae* juveniles grew on average 30 % slower than *G. geiseri* juveniles both in the first 20 days of development and over the entire juvenile period (Figure 5.4).

The effect of gender on growth rates to maturity depended on the fish species (species x sex interaction, Table 5.2). In general, females grew faster than males (Figure 5.4). This may be expected since poeciliid females have indeterminate growth, while males usually decrease growth significantly at sexual maturity (Constanz 1989). The magnitude of the difference seemed to vary whether species were invasive or not. For the two invasive species, female growth rates were on average 25 % greater than those of males, while for the non-invasive species, females grew only 16 % faster than males. For growth rates to day 20, I detected only a trend of a species by sex interaction (Table 5.2).

Parental female size had a significant effect on both growth rate measures. Interestingly, regressing growth rates on female size across all four species showed the relationship to be negative (Growth rate to 20 d: $F = 44.9$, $p < 0.0001$; growth rate to sexual maturity: $F = 5.4$, $p = 0.021$). Larger females seemed to have slower growing offspring. On a species by species basis, the relationship between size of the mother and growth rates of the offspring did not hold, except for *G. holbrooki* where the relationship was significant and positive (Growth rate to 20 d: $F = 8.1$, $p = 0.006$; growth rate to sexual maturity: $F = 6.8$, $p = 0.011$).

Gambusia species differed significantly on the age and size at which they became sexually mature (Table 5.2). As expected from the growth data, the two invasive species reached sexual maturity at significantly younger ages than the non-invasives species ($F_{1, 229} = 115.7$, $p < 0.0001$). Invaders reached sexual maturity at about 34 days, while non-invaders matured on average 10 days later (Figure 5.3). Within the invasives, *G. holbrooki* fish became mature sooner

than *G. affinis* ($F_{1, 229} = 4.0$, $p = 0.05$). For the non-invasives, *G. geiseri* juveniles became reproductive sooner than *G. hispaniolae* ($F_{1, 229} = 10.5$, $p = 0.01$). The effect of gender on age at sexual maturity differed between species. *G. affinis* females reached maturity sooner than males (LSD pairwise comparisons, $p = 0.05$), while for *G. geiseri* the opposite was true, males became reproductive before females ($p = 0.002$). I found no differences in the ages *G. holbrooki* and *G. hispaniolae* males and females matured.

Invasive species not only became mature at younger ages than the non-invasives, but also at smaller sizes ($F_{1, 230} = 40.2$, $p < 0.0001$) (Figure 5.3). On average, invasive fish matured at 20.2 mm of SL, while non-invasives matured at 21.7 mm SL. I found significant differences in size at sexual maturity between the two invasive sister species ($F_{1, 230} = 39.9$, $p < 0.0001$). *G. affinis* juveniles became reproductive at 21.4 mm SL, while *G. holbrooki* juveniles did so at 19.2 mm. The greatest disparity in size at sexual maturity was found between the two non-invasive species. *G. hispaniolae* juveniles reached sexual maturity at the largest size, 24.2 mm SL, while *G. geiseri* reached maturity at the smallest size, 17.5 mm SL ($F_{1, 230} = 58.9$, $p < 0.0001$) (Figure 5.3). The species by sex interaction was marginally significant (Table 5.2). Females of both invasive species reached sexual maturity at sizes that were approximately 20 % greater than males. *G. hispaniolae* females became reproductive at 15 % larger sizes than males, while *G. geiseri* sexually mature females and males differed in size by over 32 %. Brood size did not have an effect on either size or age at sexual maturity. I found a trend for parental female size to be a significant factor in size at maturity, but this was not the case for age at maturity (Table 5.2). Offspring of larger females tended to become reproductive at larger sizes.

Establishment Success

I found differences in the ability of the four *Gambusia* species to establish self-sustaining populations that were generally consistent with species differences in temperature tolerances and life histories. Population trajectories in our simulated pond invasion varied significantly among the species over the four sampling periods (Table 5.3). The invasive species, *G. holbrooki* and *G. affinis* grew to and maintained significantly larger population sizes than *G. geiseri* and *G. hispaniolae* (all sampling periods, $p < 0.0001$) (Figure 5.5a). The largest difference in population sizes between the invasives and non-invasives was observed at week 7 of the experiment (103 versus 42 total individuals). Accordingly, the invasives showed higher intrinsic rates of increase

(r) ($F_{1, 8} = 41.9$, $p = 0.0001$) and higher carrying capacities ($F_{1, 8} = 35.3$, $p = 0.0002$) than the non-invasives. Rates of increase and carrying capacities for invasives more than double those of non-invasives (Figure 5.5b).

Throughout this experiment, I found no differences in population sizes, r's or K's for the invasive species. There were also no differences between the r and K estimates of the non-invasive species, but population trajectories of non-invasive *Gambusia* differed significantly from each other at weeks 11 and 16 of the experiment ($F_{1, 8} = 15.7$, $p = 0.004$ and $F_{1, 8} = 311.3$, $p < 0.0001$). By week 11, *G. hispaniolae* populations had began to decrease and were more than 50 % smaller than *G. geiseri* populations (Figure 5.5a). By week 16, cold autumn temperatures resulted in 100 % mortality of *G. hispaniolae* in all three replicate populations. Minimum water temperatures probably fell below the 8° C shown to result in 0 % survival for *G. hispaniolae* in the laboratory experiment. In fact, field notes showed that nighttime temperatures in the experimental pools often fell below 5 ° C by week 16 (mid November). *G. geiseri* also suffered 100 % overwintering mortality in all replicates, but it was not detected until week 42. In contrast, *G. affinis* and *G. holbrooki* populations survived overwintering conditions despite significant mortality (84 % averaged for both species). Furthermore, by week 42, invasive populations averaged 15 individuals, a 50 % increase relative to the initial 'invasion' population size of 10 individuals.

DISCUSSION

Establishment is a key phase of the invasion process characterized by the ability of a non-indigenous species to maintain a localized, viable, and self-sustaining population in an invaded community. In some sense, success at establishment means that whatever 'ecological resistance' the community had (Elton 1958) was broken down by the invader (Vermeij 1996). Moyle and Light (1996b) considered this resistance to consist of three interacting factors: environmental, biotic, and demographic. Thus, invading species that possess greater abiotic tolerances, are better at coping with novel biotic interactions (i.e., predation and competition), and/or exhibit greater reproductive potential, should be better equipped to overcome this resistance and become established. In this study, I examined the influence of two of these types of factors, abiotic and demographic, on establishment success by four *Gambusia* species. The species were known to

differ in their invasive ability and were expected to differ in certain key traits (abiotic tolerances and life histories). By simulating the invasion of a common novel environment, I examined the relative importance of these trait differences in explaining variation in establishment success.

The results showed that invasive *Gambusia* species had broader temperature tolerances than non-invasive species. When exposed to low temperatures (4-8° C), *G. geiseri* and *G. hispaniolae* suffered 100 % mortality compared to only 25 % mortality for *G. affinis* and *G. holbrooki*. Invasive also possessed a suite of life history traits that convey greater reproductive potential relative to non-invasives. *G. affinis* and *G. holbrooki* had greater fecundities (of on average smaller offspring), higher growth rates, and reached sexual maturity sooner and at smaller sizes than *G. geiseri* and *G. hispaniolae* (Table 5.4). Based on these differences, one may predict that if introduced to a temperate community, the establishment success of *G. geiseri* and *G. hispaniolae* might be severely limited by their inability to cope with overwintering conditions. I would also predict that the degree of establishment success (i.e., abundance) would be greater for the invasive species than the non-invasives. In fact, that is exactly what was seen in the simulated invasion. When equal numbers of individuals of each species were introduced on their own to replicate, simple aquatic communities (without *Gambusia* enemies), invasives outperformed non-invasives. Invasives exhibited greater intrinsic growth rates and became more abundant than non-invasive *Gambusia. G. holbrooki and G. affinis* were ultimately the only species to successfully 'establish' since *G. geiseri* and *G. hispaniolae* populations suffered 100 % mortality after experiencing overwintering conditions.

Broad temperature tolerances have been documented to be a key factor in the establishment success of non-indigenous fishes worldwide (Meffe 1991, Welcomme 1992, Crivelli 1995, Brown and Moyle 1997). Invasive mosquitofish are known to be extremely tolerant of an array of physical conditions (e.g., temperature, salinity, dissolved oxygen, human disturbance) (Krumholz 1948, Courtenay and Meffe 1989, Ehrlich 1989, Lloyd 1989). This physiological tolerance is thought to be one of the key elements responsible for their success in their invaded range (Arthington and Mitchell 1986, Courtenay and Meffe 1989). In our experiment, their greater tolerance of low temperatures allowed the invasives species, but not the non-invasive ones, to make the transition between arrival and establishment. This result indicates that abiotic tolerances are, as suspected, a key limiting factor to arriving species trying to establish. In particular, abiotic tolerances appear to be an absolute limit to establishment (i.e.,

they determine whether or not establishment occurs) and not just a factor affecting the degree of establishment success achieved. This result concurs with previous research by P. Moyle and colleagues on the significance of abiotic tolerances to establishment success. Their work showed that non-indigenous fishes with a broad range of physiological tolerances were more likely to establish in California streams than less tolerant species (Moyle 1986, Moyle and Light 1996a, Brown and Moyle 1997, Marchetti et al. 2003), while other factors (i.e., biotic tolerances and demographic factors) were secondary in importance (Moyle and Light 1996b).

Demographic factors also play a role in this system. Life history traits have been of particular interest in the study of invasion patterns, presumably because of their seemingly strong predictive power relative to other species-specific traits (Sakai et al. 2001; e.g., Rejmanek and Richardson 1996, Reichard and Hamilton 1997, Williamson and Fitter 1996a). The general expectation is that successful invasive species have *r*-selected life histories or are able to shift easily between *r*- and K-selected strategies (Ehrlich 1989, Lodge 1993b). Differences between the life histories of invasive and non-invasive *Gambusia* measured under constant favorable conditions (high temperature and unlimited resources) appear to fit these predictions.

The life history results indicate that even if all four *Gambusia* species were introduced or invaded more tropical areas where overwintering mortality would not be a concern, *G. affinis* and *G. holbrooki* species would still exhibit greater establishment success than *G. geiseri* and *G. hispaniolae* because of their greater ability to become abundant. In particular, their greater abundance should allow invasive *Gambusia* to better cope with demographic or environmental stochasticity, which often causes extinctions among localized invader populations (Crawley 1989). Invasive *Gambusia* may also have a greater potential for spread and impact because of their greater abundances. Species that are able to quickly become abundant from a few propagules are generally expected to have greater post-establishment spread (i.e., larger invaded range) (Rejmanek and Richardson 1996, Reichard and Hamilton 1997). Beyond their per capita effect, how abundant invaders become is also a fundamental component of their community impact (Parker et al. 1999). Thus, *G. affinis* and *G. holbrooki* are likely to outperform *G. geiseri* and *G. hispaniolae* as invaders in multiple stages of the invasion sequence, even if environmental conditions (i.e., temperature) were more favorable and *G. geiseri* and *G. hispaniolae* were able to survive through the establishment phase.

Previous comparative work on invasive *Gambusia* suggests that *G. holbrooki* might be a superior competitor (when in sympatry in its native range) and perhaps a superior invader than its sister species, *G. affinis,* when introduced outsider its native range. Scribner (1993) while finding no difference in mosquitofish fecundities, reported larger offspring, shorter juvenile periods, and larger sizes at sexual maturity for *G. holbrooki* compared to *G. affinis*. Scribner and Avise (1994b) also reported higher K, greater population sizes, and lower overwinter mortality for *G. holbrooki* relative to *G. affinis*. Scribner and Avise (1994a) found evidence of directional introgression by *G. holbrooki* genotypes along the species' contact zone and in experiments. Based on these demographic and genetic differences, these investigators hypothesized that *G. holbrooki* has been actively displacing *G. affinis* from a larger historical native range and may be a superior invader when introduced outside its native range (Scribner 1993, Angus and Howell 1996). Data on the number of invasions by each species seems to support this hypothesis; most invasions have in fact been by *G. holbrooki* (Lever 1996). However, this may only reflect greater introductions by *G. holbrooki*, especially if we consider the stepping stone nature of most introduction efforts (i.e., lots of secondary introductions), and not necessarily be a reflection of greater invasive ability. This study found some differences between *G. affinis* and *G. holbrooki* in fecundity, offspring size, and age and size at sexual maturity (Table 5.4). *G. holbrooki* had larger numbers of smaller offspring which became sexually mature at younger ages and smaller sizes. However, these differences did not translate to differences in population performance. Both species were equally successful in the invasion simulation. Contrary to Scribner and Avise (1994b), I also found both mosquitofish species to be equally tolerant of temperature stress in the laboratory and under semi-field conditions. Thus, my data do not provide strong support for their hypothesis.

Important species differences were also found within the category 'non-invasive'. *G. geiseri* showed a life history strategy more similar to the invasives' than *G. hispaniolae*'s (Table 5.4) and consistent with its greater population performance in the simulated invasion (before winter mortality). *G. geiseri* was also more tolerant of low temperatures than *G. hispaniolae*. These results suggest that these two species differ in their relative 'non-invasiveness'. *G. geiseri*, although lacking the traits of successful invasive species, appears to have greater invasion potential than *G. hispaniolae*, at least in the contexts I compared them. This makes sense in light of phylogenetic relationships. *G. geiseri* is a sister species to the invasive species pair (partial

phylogeny by Lydeard et al. 1995). Invasiveness might then be better described as a continuum rather than a dichotomy, where a species has more or less invasion potential relative to another. Quantitative measures of relative invasion potential would provide useful information to natural resource managers faced with prioritizing prevention and eradication efforts for many non-indigenous species.

The species chosen for this study (four congeneric species) allowed me to test the idea that there are taxonomic patterns to invasiveness (Daehler and Strong 1993). A few studies have found certain taxonomic groups to be more prone to invasion than others, so that relatedness to a known successful invader may confer high invasion potential (Daehler 1998, McKinney and Lockwood 1999). This comparative study found only the known invasive species to possess the traits needed for establishment and invasion success (broad temperature tolerance and life history traits that yield high reproductive potential and contribute to high intrinsic rates of increase). This suggests that the rest of the genus lacks the traits of successful invasive species, and invasiveness is, in fact, not a trait of the entire taxonomic group. Thus, for at least this genus, invasive potential must be evaluated on a species by species basis.

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Table 5.1: Summary of repeated measures ANOVA's for adult and juvenile survivorships in the temperature tolerance experiment.

Survivorship	*Effect*	*df*	*F*	*p-value*
Adult	Species	3, 8	3.9	0.06
	Time	4, 32	83.1	0.0001
	Species x time	12, 32	13.5	0.0001
Juvenile	Species	3, 8	2.4	0.15
	Time	4, 32	54.4	0.0001
	Species x time	12, 32	13.3	0.0001

Table 5.2: Summary of analyses of covariance for: size at birth, growth rates to day 20 of development and to sexual maturity, and size and age at sexual maturity. Shown are effects tested (species, sex, and the interaction of species by sex) and covariates (brood size and female size).

Effects	*Traits*								
	Size at birth			GR to 20 d			GR to SM		
	df	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*
Species	3	465.8	0.0001	3	100.6	0.0001	3	104.7	0.0001
Sex				1	45.7	0.0001	1	80.1	0.0001
Species x sex				3	2.1	0.17	3	4.5	0.004
Female size	1	0.2	0.69	1	4.5	0.04	1	3.8	0.05
Brood size	1	7.7	0.006	1	0.01	0.91	1	1.3	0.26
Error	273			264			238		

Effects	Size at SM			Age at SM		
	df	*F*	*p*	*df*	*F*	*p*
Species	3	35.5	0.0001	3	43.0	0.0001
Sex	1	412.9	0.0001	1	4.9	0.03
Species x sex	3	2.6	0.05	3	4.9	0.003
Female size	1	3.0	0.08	1	0.3	0.58
Brood size	1	0.1	0.76	1	1.6	0.21
Error	230			229		

Table 5.3: Summary of analyses of variance for population sizes, intrinsic rate of increase '*r*', and carrying capacity 'K' for the four *Gambusia* species in the simulated invasion. Shown are effects tested (species, time, and the interaction of species by time).

Effects	Population Size			*r*			K[1]		
	df	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*
Species	3, 8	272.5	0.0001	3, 8	14.3	0.001	3, 8	13.1	0.002
Time	3, 24	351.9	0.0001						
Species x time	9, 24	46.3	0.0001						

[1]Species was the only effect tested for *r* and K.

Table 5.4: Summary of results of orthogonal contrasts performed on all variables tested in the three experiments. Contrasts tested whether the two invasive species differed from the two non-invasives, and whether there were species differences within the categories 'invasive' and 'non-invasive'. Abbreviations are as follows: Inv = invasive, Non = non-invasive, G. aff = *G. affinis*, G. hol = *G. holbrooki*, G. gei = *G. geiseri*, G. his = *G. hispaniolae*, GR = growth rate, and SM = sexual maturity.

Variable	Invasive vs. Non-invasive	Within Invasive	Within Non-invasive	Covariates and Other Effects
Temperature tolerance				
Adult survival	Inv > Non	G. aff = G. hol	G. gei > G. his	-
Juvenile survival	Inv > Non	G. aff = G. hol	G. gei = G. his	-
Life histories				
Brood size	Inv > Non	G. aff < G. hol	G. gei > G. his	Female size
Size at birth	Inv < Non	G. aff > G. hol	G. gei < G. his	Brood size
Juvenile GR (to 20 d)	Inv > Non	G. aff = G. hol	G. gei > G. his	Sex dependent, female size
Juvenile GR (to SM)	Inv > Non	G. aff = G. hol	G. gei > G. his	Sex dependent, female size
Age at SM	Inv < Non	G. aff > G. hol	G. gei < G. his	Sex dependent
Size at SM	Inv < Non	G. aff > G. hol	G. gei < G. his	Sex dependent
Establishment Success				
Population persistence	Inv > Non	G. aff = G. hol	G. gei > G. his	-
Intrinsic growth rate	Inv > Non	G. aff = G. hol	G. gei = G. his	-
Carrying capacity	Inv > Non	G. aff = G. hol	G. gei = G. his	-

Figure 5.1: (a) Percent survival (means ± 1 SE) of juveniles and (b) adults of the four *Gambusia* species exposed to decreasing temperatures. Filled symbols represent the two invasive species, and open symbols the non-invasive species.

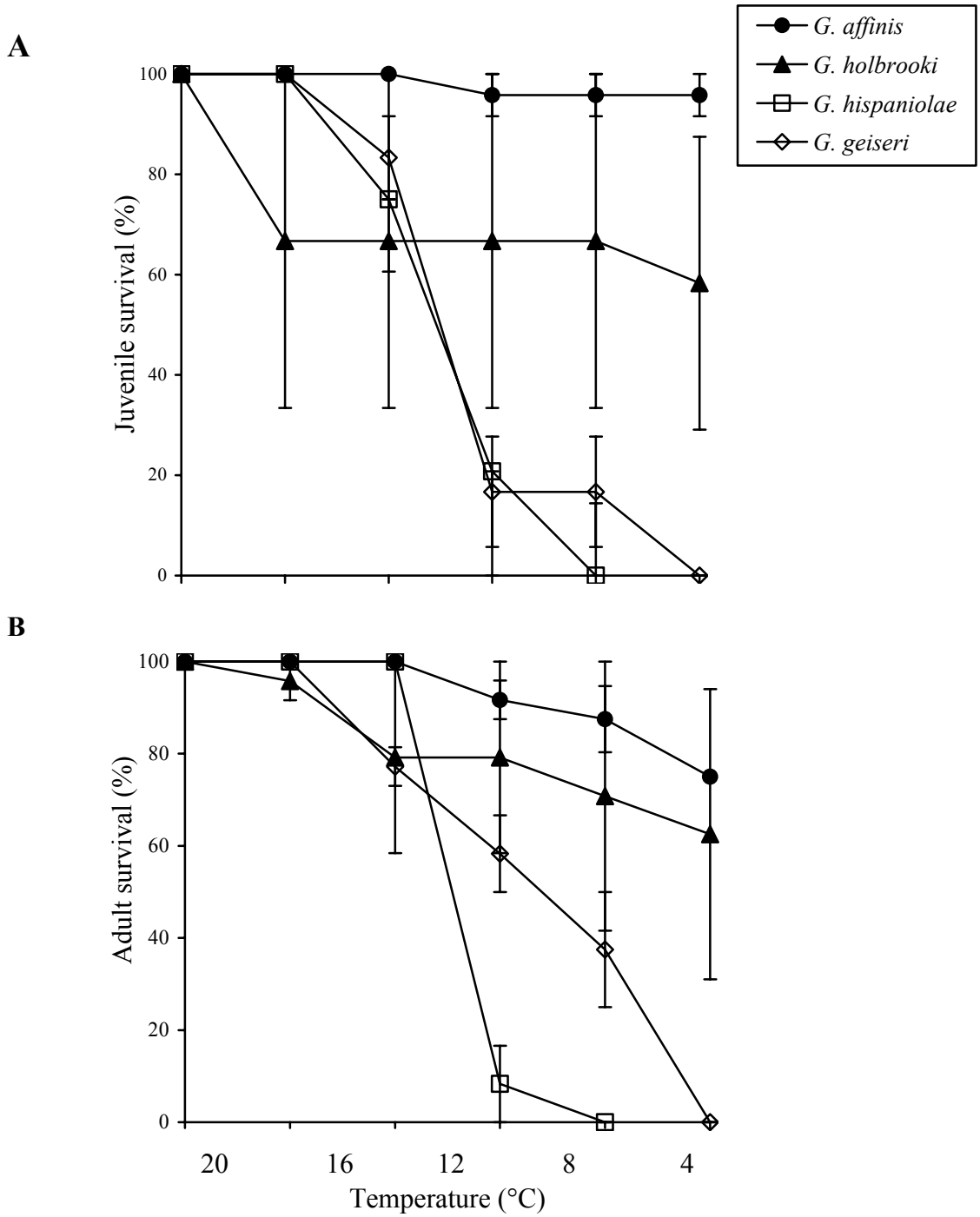


Figure 5.2: Plot of brood sizes as a function of female size (SL in mm) for the four *Gambusia* species. Filled symbols represent the two invasive species, and open symbols the non-invasive species. A separate least-squares regression line is shown for each species.

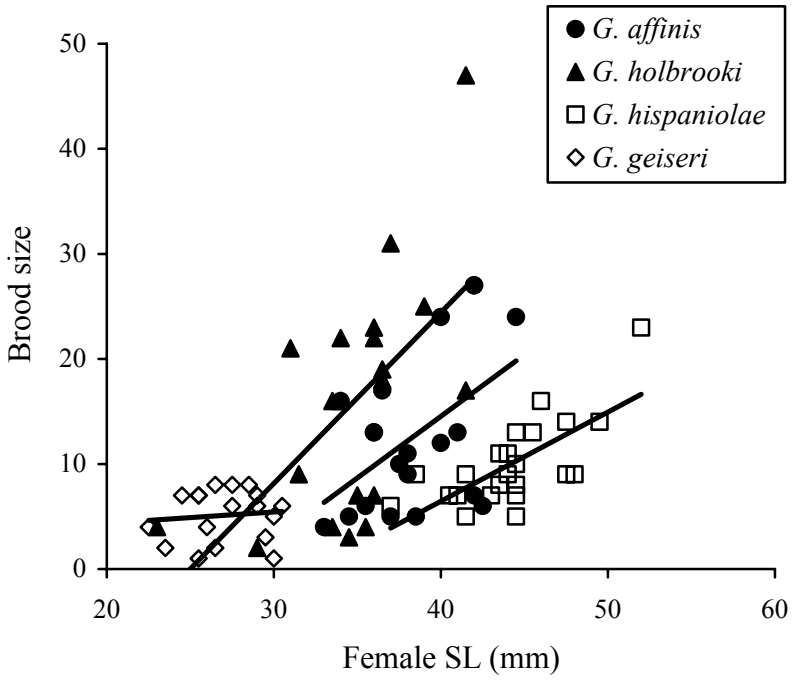


Figure 5.3: Offspring size as a function of days from birth to sexual maturity for each *Gambusia* species. Filled symbols represent the two invasive species, and open symbols the two non-invasive species. Shown are means (± 1 SE).

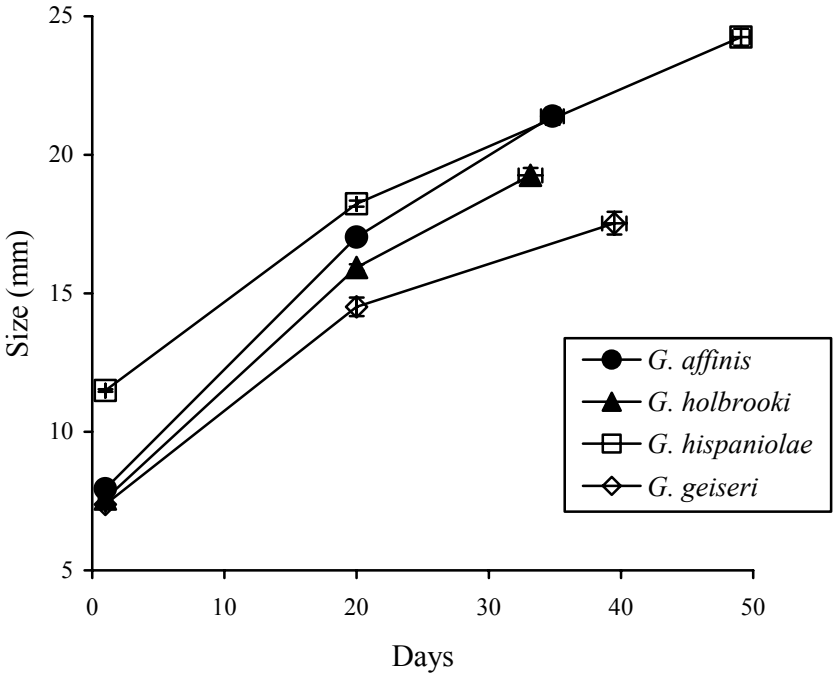


Figure 5.4: (a) Juvenile growth rates (1/d) from birth to 20 days and (b) to sexual maturity by *Gambusia* species and gender. Filled bars represent females and open bars males. Shown are means ± 1 SE.

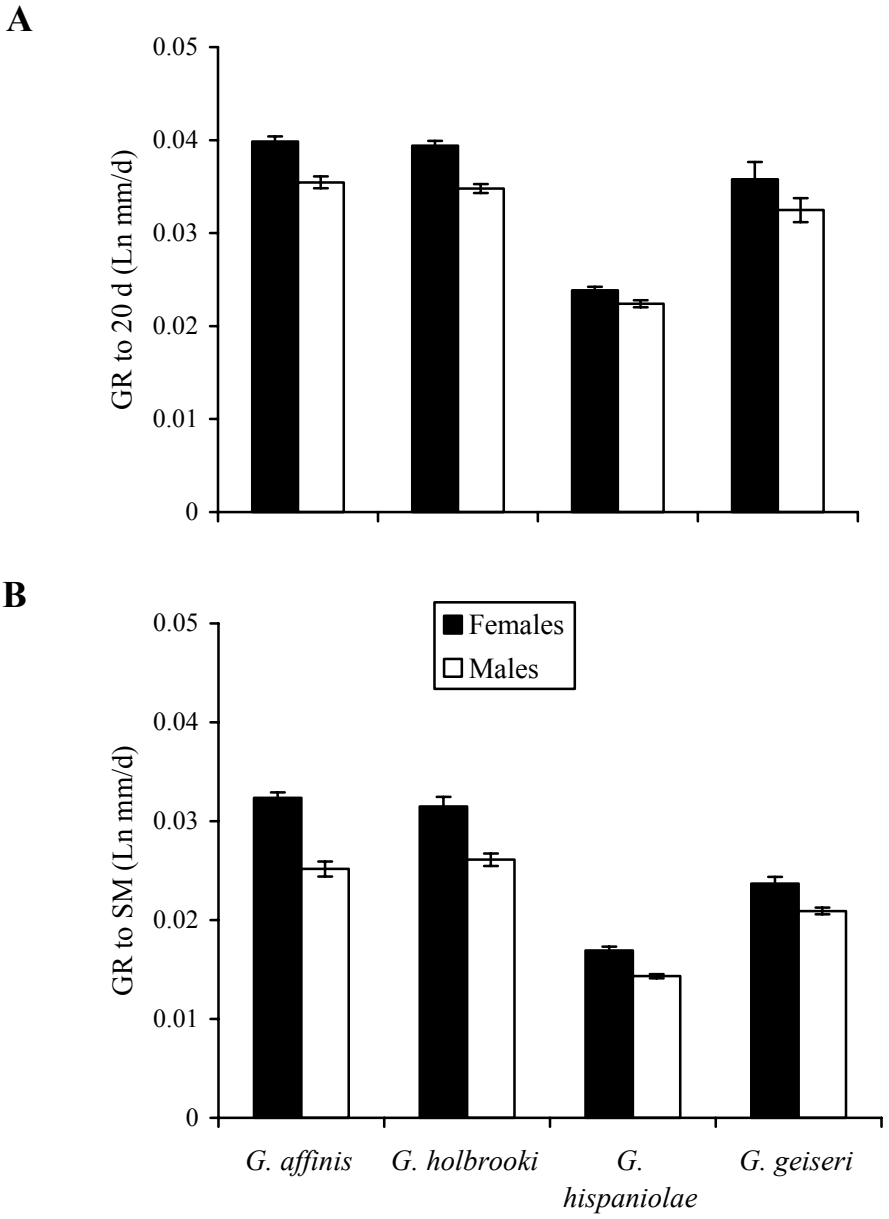
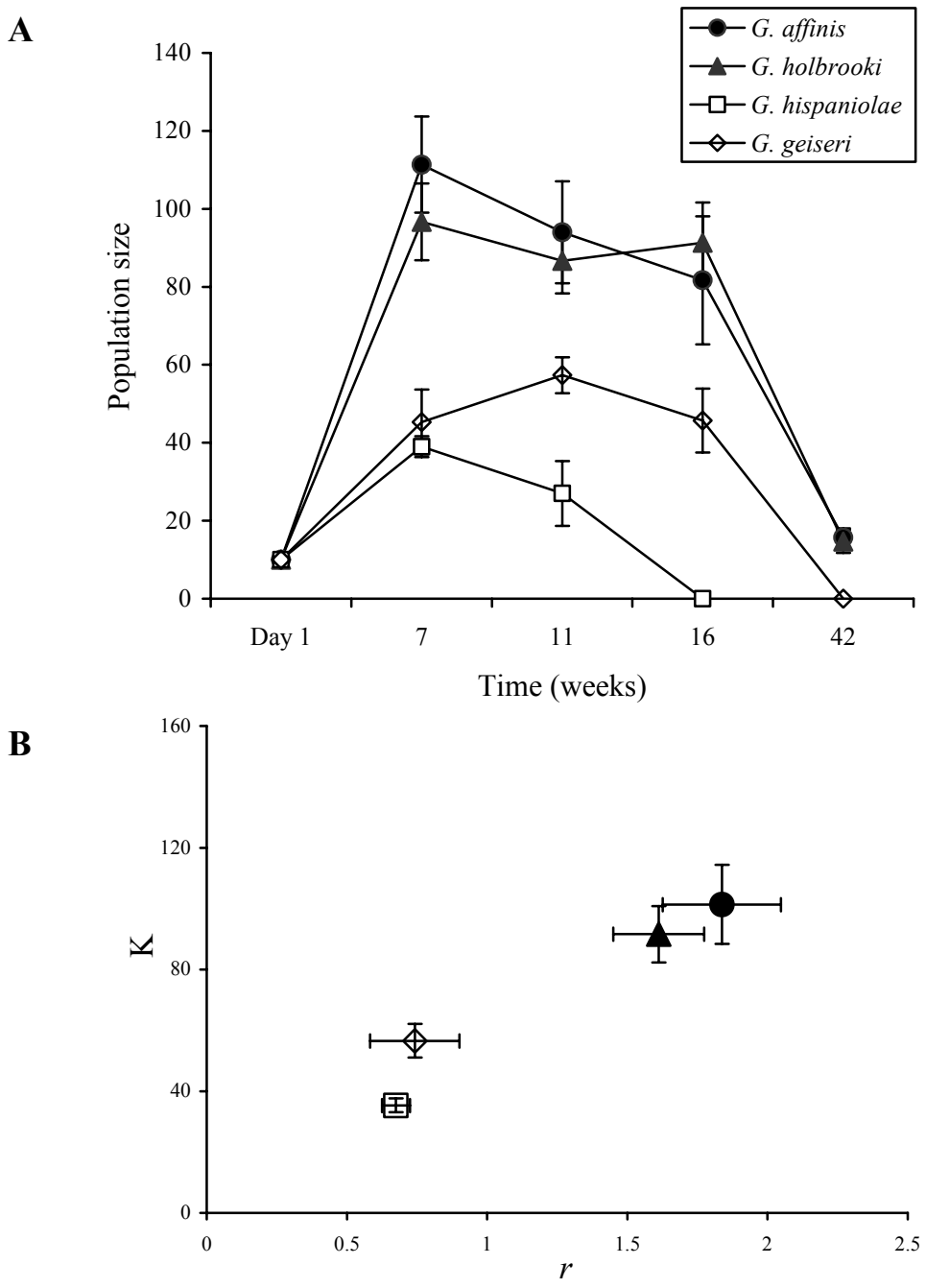


Figure 5.5: (a) Population trajectories (mean population sizes ± 1 SE) of *Gambusia* species in the simulated invasion as a function of time (sampling weeks). (b) Mean carrying capacity K as a function of mean intrinsic growth rate *r* for each species (± 1 SE). Filled symbols represent the two invasive species, and open symbols the two non-invasive species.



Chapter Six

Comparing the establishment success, response to competition, and community impact of invasive and non-invasive *Gambusia*

SUMMARY

How an invader responds to the novel biotic elements of a new community (e.g., novel competition) will affect its ability to invade. In a new community, invaders will likely experience a variety of novel generalist competitors to which they might lack adaptive responses. Species that are able to cope well with these novel competitors might be expected to achieve greater establishment success. To test this, I compared the population-level responses of two *Gambusia* species of differing invasion success to novel competition and competition from each other. I simulated the invasion of a simplified pond community by each *Gambusia* species on its own, by both species together, and into communities already inhabited by a novel competitor to the *Gambusia* species, the red shiner *Cyprenella lutrensis*. I measured the effect of competition on establishment success by comparing population abundances achieved by each species in competition and no competition treatments. I also examined whether the invasive and non-invasive *Gambusia* differed in their community impact by comparing their effects on the abundances of pond fauna. I used N and C stable isotope analysis to investigate potential differences between the species in trophic roles and trophic responses to competition. I found both novel and intrageneric competition to negatively affect both invaders' abundances, but the invasive *G. affinis* managed to remain more abundant than *G. geiseri* in the presence and absence of competition. Stable isotope analysis revealed the *Gambusia* species to have similar trophic placement and showed competition to cause significant trophic shifts only in *G. geiseri*. Comparison of abundances of the pond fauna and phytoplankton revealed that their impacts also differed. *G. affinis* had a greater impact on the zooplankton community, while *G. geiseri* impacted benthic invertebrate abundances more strongly. A cascading effect (reduced phytoplankton abundances) was detected only when *G. affinis* was the top predator in the experimental communities.

INTRODUCTION

A species can successfully invade a new community if the community provides open niche opportunities (Chesson 2000). For the invading species, how it responds to this novel niche space will determine whether or not it becomes established (Shea and Chesson 2002). More specifically, how the invader responds to the novel abiotic and biotic (i.e., resources, natural enemies) elements of the community will affect its ability to invade. Among the biotic factors, interspecific competition may be a particularly important limiting factor (Pimm 1989). Competition is generally expected to be particularly important (and thus a stronger force in shaping community composition) in recent invasions where species have not yet coevolved or displaced each other (MacArthur and Levins 1967, Schoener 1975). While invaders might lose specialized enemies when invading a new community (Keane and Crowley 2002), invaders might also gain new generalist enemies (Hänfling and Kollman 2002). These enemies may have particularly strong negative effects, since invaders and resident species have no evolutionary history and no adaptive responses to each other (Mack 1996b). Species or organisms that are able to cope well with these novel enemies, including novel competitors, may be expected to achieve greater establishment success.

Another key aspect in the community ecology of invasions is the impact that a successful invader has on the invaded community (Shea and Chesson 2002). A non-indigenous species that establishes and becomes abundant can interact with and impact numerous resident species. The overall impact is a function of the range occupied by the invader, their abundance, and their local (per capita or per biomass) effect (Parker et al. 1999). In some cases, high impact is due to the invader becoming very abundant and a dominant feature of the community (e.g., the zebra mussel, Lodge 1993a), while in other cases impact is due to a key per capita effect (e.g., nitrogen fixation in a nitrogen-deficient habitat, Vitousek and Walker 1989). It is estimated that anywhere from 2 to 40 % of invasive species have impacts that are large enough to be detected in ecological studies (Lodge 1993a), and less than 10 % have severe effects that result in species extinctions (Simberloff 1981, Williamson 1996). These serious impacts often involve predation (Lodge 1993a Moyle and Light 1996a, Mooney and Cleland 2001).

A major objective of this study was to examine whether competition is a key limiting factor to invasion success, particularly establishment success. To address this issue, I compared

the population-level response of two congeneric species of differing invasion success to novel competition and competition with each other. In outdoor mesocosms, I simulated the invasion of a standardized pond community by a highly invasive and high impact species, the mosquitofish, *Gambusia affinis*, and by a closely related species of lower invasion success (and impact), *G. geiseri.*

Because of their ability to spread widely and their negative impacts on aquatic communities, invasive *Gambusia* (*G. affinis* and its sister species *G. holbrooki*) have been designated among the 100 worst invasive species worldwide (ISSG 2000). Both species have been widely introduced to over 40 countries as mosquito control agents (Krumholz 1948, Welcomme 1992, Lever 1996). Their impact is primarily through predation, usually of the eggs, fry, and larvae of native fishes and amphibians (Schoenherr 1981, Meffe 1985, Lloyd 1989, Courtenay and Meffe 1989, Gamradt and Kats 1996, Goodsell and Kats 1999). Their predatory impact is also known to result in significant changes in community composition (Hurlbert et al. 1972, Hurlbert and Mulla 1981, Harris 1995).

G. geiseri is native to two springs in central Texas, which are characterized by low environmental variation (Hubbs 1995). *G. geiseri* is limited to the springheads, while *G. affinis* is native in the downstream rivers. Hubbs and Springer (1957) suggested that the ancestor of *G. geiseri* and other spring *Gambusia* were probably widespread, but became restricted to springheads either due to drought-caused isolation or competition with the ancestor of *G. affinis*. In the 1930's, *G. geiseri* was introduced to at least seven springs habitats in western Texas (Fuller et al. 1999) with slightly more variable environmental conditions than their native springs (C. Hubbs, unpublished data). However, *G. geiseri* remains localized to the points of introduction and has never spread (C. Hubbs, personal communication), which indicates that this species is non-invasive. This research should shed light on whether competition is an important mechanism for habitat partitioning between *G. geiseri* and *G. affinis* in their native habitat and whether it is also an important factor limiting the spread of *G. geiseri* in introduced habitats.

Simple replicate pond communities were created by inoculating tanks with standardized amounts of pond biota (phytoplankton, periphyton, zooplankton, and benthic invertebrates). Each species invaded communities where they were the only predatory species present, as well as communities that were already inhabited by another fish species. In competition treatments, either *Cyprenella lutrensis* (a novel competitor) was already present or both *Gambusia* species

‘invaded’ at once. I quantified the establishment success of both *Gambusia* by measuring their population trajectories (with and without competition) over a period of 12 weeks. I quantified their N and C stable isotope signatures to test whether competition (either novel or from each other) resulted in shifts in their trophic placement. Relative to *G. geiseri*, I expected *G. affinis* to be less affected by competition and exhibit greater population growth rates, both when it was the only fish species in the community and when *C. lutrensis* or *G. geiseri* also inhabited the community.

The experiment included a treatment where no fish were present, which allowed me to test whether *Gambusia* had significant top-down effects in the experimental communities and whether the effects differed between the invasive and non-invasive species. I assessed community impact by measuring the abundances of phytoplankton, periphyton, zooplankton, and invertebrates in communities with either *G. affinis* or G. *geiseri* as the top predators and communities with no fish present. I used stable isotope signatures to assess whether *G. affinis* and *G. geiseri* had equal trophic roles in the community. I hypothesized *G. affinis* and *G. geiseri* to have functionally non-equivalent community roles and expected *G. affinis* to have more pronounced impacts on lower trophic levels than *G. geiseri*. Previous work on the feeding behavior of these species (Chapter 2) suggests that *Gambusia,* because of strong feeding preferences for planktonic prey, may have a particularly strong impact on zooplankton. Invasive and non-invasive *Gambusia* were also found to have similar diet breadths but feeding rates across all prey were higher for the invasive *Gambusia,* suggesting a potential for greater impact.

METHODS

The experiment was conducted in outdoor mesocosms at the Putah Creek Aquatic Facility at the University of California, Davis. Replicate aquatic communities were established in 18 745-L tanks inoculated with local pond biota. Six and a half liters of unfiltered pond water was added to each tank providing phytoplankton, zooplankton, invertebrates, and detritus to create representative and identical pond communities. Tanks were also inoculated with 50 g of rabbit pellets for nutrients and 80 g of *Elodea canadensis* (wet weight) for structural complexity. Inoculation of tanks was done over an 8-week period prior to the addition of experimental fish. Once fish were added, tanks were covered with bird netting (2.0 cm mesh size), which prevented

predation of fish and reduced oviposition by insects. To avoid fish mortality due to stressful conditions, all tanks were partially shaded (1/3 of surface area was covered with shade cloth) and provided with gentle aeration. A few rocks and tiles were placed in the bottom of tanks to act as spawning substrate for the red shiners.

Each tank was randomly assigned one of the following six treatments (replicated three times): (1) No fish, (2) *G. affinis*, (3) *G. geiseri*, (4) *G. geiseri* and *G. affinis*, (5) *G. affinis* and *C. lutrensis*, (6) *G. geiseri* and *C. lutrensis*. I selected *C. lutrensis* as a novel competitor because like fathead minnows (Chapter 3) they are a widespread and an invasive species that spreading *Gambusia* are likely to encounter and compete with, but which do not co-occur with *Gambusia* study populations in their native range. As a result of bait introductions, red shiners have become a common species in North American streams and a serious threat to native fishes, particularly in the western United States (Fuller et al. 1999). They are tolerant of a wide range of environmental conditions, including highly disturbed habitats, and like *Gambusia* they prefer slow moving waters and are trophic opportunists (Moyle 2002).

As in Chapter 5, tanks were intended to simulate simplified communities (with and without another fish species already present) under invasion by *Gambusia*. Because successful invasion may often occur by a small number of individuals, fish populations in tanks were started with only four individuals. In competition treatments (4, 5 and 6), densities were doubled and populations were started with four fish of each species. Fish used in the experiment were collected from within each species native range in early May 2002 and were added to tanks on June 29, 2002. *G. geiseri* and *G. affinis* were collected from the spring-fed headwaters and downstream portions of Comal River, Comal County, TX, respectively. *C. lutrensis* were collected at Beaver Pond in the Rio Grande floodplain, Big Bend National Park, TX. Due to the high mortality of *Gambusia* males during transport to the research facility, sex ratios varied between *Cyprenella* and *Gambusia*. For both *Gambusia* species, populations were initiated with 3 females and 1 male, while for *Cyprenella*, initial populations consisted of 2 males and 2 females. Population trajectories of the two *Gambusia* species and the red shiners were monitored over a period of 12 weeks. We measured each tank's population size and each species' abundance in two surveys conducted at 6 and 12 wks. For the 6 wk. survey, all fish were seined, counted, and placed back in tanks. In both surveys, sampling effort was standardized by seining until three consecutive seine hauls yielded no fish.

Differences in community impact between invasive and non-invasive *Gambusia* were assessed by quantifying effects on primary productivity (phytoplankton and periphyton) and zooplankton and invertebrate abundance. Comparison of these community parameters were only done among treatments 1, 2 and 3 and tested whether the invasive and non-invasive *Gambusia* differed in their top-down direct and indirect impacts on the community. To measure effects on primary productivity, samples were taken before fish addition and at 12 wks. (experiment endpoint). Phytoplankton abundance was assessed by extracting chlorophyll a from 300 mL water column samples taken at both sampling times. Extractions were done with methanol, and chlorophyll concentrations were read using a Turner 10-A Fluorometer. Periphyton growth was measured by scraping tank sides using a small fine-mesh dipnet (10 x 8 cm). The net had a bent rim that matched the curvature of tanks and was slowly drawn up for half the tank's depth (30 cm). Samples were dried overnight at 60° C and later weighed.

For effects on the aquatic fauna, samples were collected only at 12 wks. Because of careful inoculation efforts, faunal abundances prior to fish addition were assumed to be equal in all tanks. Samples were taken by sweeping a D-frame dipnet (243 µm mesh, 29 cm width, 51 cm depth) in a diagonal fashion across tanks (from bottom on one side to top of opposite side of tank). Before sweeping, all contents of the tank were brought into suspension and homogenized by stirring the tank in a standardized manner. Samples were preserved in 10 % formaldehyde dosed with Rose Bengal to aid in later sorting and identification. Macroinvertebrates (> 2 mm) were handpicked from samples and counted. For the smaller zooplankton and benthic invertebrates, two subsamples were taken using a 10 mL Hansen-stempel pipette. The entire sample was first placed in a beaker and brought to a 1 L volume by adding water. Sample contents were suspended and homogenized by creating a vortex, and then subsamples were immediately drawn. Zooplankton and benthic invertebrates in subsamples were counted using a Wildco® Counting Wheel under a microscope.

To assess whether *G. geiseri* and *G. affinis* differed in trophic placement (and thus their potential impact) or whether competition caused trophic shifts in either species, we analyzed stable carbon and nitrogen isotopic ratios for each *Gambusia* species. Three F1 fish of each species (all late juveniles, likely females) were randomly collected at the end of the experiment from each tank and frozen for analysis. Fish were then dried at 60° C for 96 hours, and ground into a fine powder using a mortar and pestle. Stable isotope analyses were performed using a

Europa Hydra 20/20 isotope ratio mass spectrometer at the Stable Isotope Facility, University of California, Davis, CA. Stable isotope ratios are shown in delta notation, $\delta^{13}C$ and $\delta^{15}N$, and represent deviations (in parts per thousand or $^{0}/_{00}$) of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios from set standards (Pee Dee belemnite limestone and atmospheric nitrogen respectively). Larger isotopic values (more positive for $\delta^{15}N$ and less negative for $\delta^{13}C$) indicate that the sample is 'enriched' meaning that it contains a greater proportion of the heavier isotope (^{13}C or ^{15}N). Enrichment in ^{15}N occurs in a stepwise fashion with trophic level (i.e., higher trophic levels exhibit higher values), so that $\delta^{15}N$ signatures are indicative of trophic position (Fry 1988). In contrast, $\delta^{13}C$ signatures vary at the bottom of food webs and tell us about the energy sources for higher trophic levels (Vander Zanden and Rasmussen 1999).

Statistical analyses

Differences in population sizes achieved by the two *Gambusia* species in the five fish treatments were examined by running repeated measures ANOVA's with the species-treatment combination as the between subject effect and time and the time by species-treatment interaction as the within subject effects. The time factor in this analysis consisted of the 6 wk. and the 12 wk. population surveys. Orthogonal contrasts were used to test for species differences once a significant species-treatment effect was found. Comparison of population sizes in treatments 2 and 3 tested whether the invasive and non-invasive *Gambusia* differed in maximum population growth rates. Comparison of each species' population size in the presence and absence of each other (treatments 2 vs. 4 and 3 vs. 4 separately by species) tested for the population level effect of intrageneric competition. Comparison of treatments 2 vs. 5 and 3 vs. 6 tested for population level responses to novel competition from the red shiners, and comparison of treatments 4 vs. 5 and 4 vs. 6 (by species) tested whether the effect of competition varied between competition from a congener and from a more distantly related, novel competitor.

I compared effects of invasive and non-invasive *Gambusia* (relative to the no fish control) on primary productivity by running a repeated measures ANOVA on periphyton dry weights (g) and chlorophyll concentrations (µg/L) (treatments 1-3). For these two variables, the time factor comprised of the before and after fish addition measures. For significant treatment by time interactions, mean comparisons were done using the Bonferroni procedure once a dummy variable was created with all the species by treatment combinations.

Because of subsampling, treatment differences in zooplankton, small benthic invertebrate abundance, and stable isotope ratios were tested with a nested analysis of variance with species and tanks nested within species as the grouping factors. Analyses of impacts on the zooplankton and small benthic invertebrate community were done on transformed abundances (Ln of observed value + 1). Impacts on macroinvertebrates were tested with a simple one-way ANOVA with species as the main effect since there was no subsampling. As with the other measures of impact, only treatments 1-3 were included in faunal analyses, but all *Gambusia* treatments (and treatment-species combinations) were included in the analysis of isotopic signatures. Multiple treatment comparisons were done using the Bonferroni procedure.

Finally, to test whether species differences in impact were related to differences in their abundance and not to greater per capita effect per se, I compared impact measures between *G. affinis* and *G. geiseri* (only treatments 2 and 3) with an analysis of covariance with *Gambusia* population sizes as a covariate. In accordance with predictions on species differences (greater population performance and impact by *G. affinis* relative to *G. geiseri*), p-values reported for treatment effects (on impact variables) and species effects correspond to one-tailed significance tests. All statistical tests were conducted using SYSTAT® Version 10.

RESULTS

Population growth and competition

Both *Gambusia* species were successful at establishing self-sustaining populations in the experimental communities. *Gambusia* populations grew significantly from four founding individuals at the start of the experiment to an average of 40 individuals at 6 wks. and 49 individuals at 12 wks. Population trajectories did not differ significantly between the species (no time by species-treatment interaction), but a strong species effect was found on overall population sizes (Table 6.1). In the absence of competition, invasive *G. affinis* populations achieved significantly greater population sizes than non-invasive *G. geiseri* (At 6 wks.: $F_{1, 12} = 31.3$, $p < 0.0001$ and at 12 wks.: $F_{1, 12} = 8.4$, $p = 0.006$). On average, populations in *G. affinis* tanks doubled those of *G. geiseri* (Figure 6.1). The largest population size in the experiment (85 individuals) was observed in one of the *G. affinis* tanks.

With competition, *G. affinis* populations remained larger than *G. geiseri* populations. In the mixed treatment where both *Gambusia* species were present, *G. affinis* abundances surpassed *G. geiseri* by two-fold by week 6 and three-fold by week 12 ($F_{1, 12} = 10.2$, $p = 0.004$ and $F_{1, 12} = 16.3$, $p = 0.001$). When in competition with *C. lutrensis*, invasive *Gambusia* populations were twice as high as non-invasive populations by week 6 and five times as large by week 12 ($F_{1, 12} = 6.8$, $p = 0.01$ and $F_{1, 12} = 13.3$, $p = 0.002$).

Competition negatively affected both *Gambusia* species (i.e., populations with competitors were usually significantly smaller than populations without competitors) (Figure 6.1), but there were some differences between the two species. For *G. affinis*, competition with *G. geiseri* resulted in 33 % smaller populations at 6 wks., and a trend for a difference at 12 wks. ($F_{1, 12} = 9.7$, $p = 0.005$ and $F_{1, 12} = 2.1$, $p = 0.09$). In contrast, *G. geiseri* populations in competition with *G. affinis* were identical to the *G. geiseri* alone treatment at 6 wks., but were half as large by week 12 ($F_{1, 12} = 0.5$, $p = 0.25$ and $F_{1, 12} = 6.6$, $p = 0.01$).

The effect of competition by *Cyprenella* on *Gambusia* population performance was similar to the effect of intrageneric competition. For both *Gambusia* species, there was no difference between *Gambusia* population sizes when in competition with each other versus when in competition with the novel *Cyprenella*. *G. affinis* populations were reduced by 47 % by week 6 and 38 % by week 12 when *Cyprenella* were present ($F_{1, 12} = 19.9$, $p = 0.0005$ and $F_{1, 12} = 8.0$, $p = 0.01$) (Figure 6.1). For *G. geiseri*, competition with red shiners resulted in smaller populations (79 % smaller) only at the 12-week census ($F_{1, 12} = 12.9$, $p = 0.002$). Overall, by the end of the experiment, competition averaged over both competitors resulted in a 67 % reduction in *G. geiseri* populations compared to only a 29 % reduction in *G. affinis* populations.

In contrast to *Gambusia*, *C. lutrensis* failed to successfully recruit and increase population sizes in experimental mesocosms. In spite of evidence of mating activity and nest guarding, and even evidence of spawning (two larval fish were seen in two different tanks during the experiment- both were *G. geiseri* competition tanks), red shiner populations remained at four individuals for the entire experiment.

Stable isotopes, trophic roles and trophic shifts

$\delta^{13}C$ and $\delta^{15}N$ ratios differed significantly between the two *Gambusia* species (Table 6.1). Pairwise Bonferroni comparisons, however, revealed that there were no differences in $\delta^{15}N$ ratios

when the *Gambusia* species were alone (treatments 2 and 3), indicating that in the absence of competition, *G. affinis* and *G. geiseri* had equal trophic placement (Figure 6.2). In contrast, $\delta^{13}C$ ratios in the same two treatments were significantly more enriched for *G. affinis* than *G. geiseri*, indicating that although the species had identical trophic roles, the identity of the primary producers at the bottom of their feeding links differed ($p < 0.0001$).

I found that for both isotopic ratios, only *G. geiseri* treatments differed significantly from each other, while no differences were found among signatures in *G. affinis* treatments. $\delta^{13}C$ and $\delta^{15}N$ were identical for *G. affinis* fish whether they were the single fish species in the community or whether *G. geiseri* or *C. lutrensis* were present (Figure 6.2). For $\delta^{13}C$ values, *G. geiseri* signatures were significantly enriched (relative to the *G. geiseri* alone treatment) when the competitor was *G. affinis* ($p = 0.004$). For $\delta^{15}N$, enrichment was detected for *G. geiseri* in competition with red shiners ($p = 0.002$).

Community impacts

The effect of the two *Gambusia* species on the pond fauna of experimental communities varied by taxon. Odonate nymphs, plus a few ephemeropteran nymphs, and notonectids dominated the macroinvertebrate fauna in pools and their abundances were not affected by the presence of *Gambusia*. The abundances of these macroinvertebrates were similar among the no fish control, the *G. affinis*, and the *G. geiseri* treatments (Figure 6.3). In contrast, I found strong treatment effects on the abundances of zooplankton and small benthic invertebrates (Table 6.2). The zooplankton community was dominated by ostracods and to a lesser extent cladocerans and copepods. While both *Gambusia* species significantly reduced zooplankton abundances (Bonferroni pairwise comparisons, *G. affinis* vs. no fish, $p < 0.0001$, *G. geiseri* vs. no fish, $p = 0.005$), the impact was greater by *G. affinis* (*G. affinis* vs. *G. geiseri*, $p = 0.0005$). Zooplankton abundances were reduced by 33 % in *G. geiseri* communities and 76 % in *G. affinis* communities (Figure 6.3). The impact on the small benthic invertebrate community (chironomid larvae, oligochaetes, and nematodes) was markedly different from the zooplankton impact. Impact on the small benthic invertebrate community was greater by *G. geiseri* than *G. affinis* (Figure 6.3). *G. affinis* reduced benthic invertebrate abundances by only 24 %, and this was not statistically different from abundances in control communities. In *G. geiseri* tanks, however, abundances

were reduced by 75 % (p = 0.0005), which was also significantly lower than abundances in communities with *G. affinis* as the top predator (p = 0.002).

I found the addition of *Gambusia* to significantly impact the phytoplankton abundance of pond communities (Table 6.2). This effect was entirely due to greater chlorophyll a concentrations in communities where *G. affinis* was the predatory fish species present (Figure 6.4a). Levels of chlorophyll in the water column tended to be 50 % higher in tanks 12 weeks after the addition of *G. affinis* relative to before and also relative to tanks where *Gambusia* were absent or where *G. geiseri* was the fish species present. In contrast, no effect of treatment was detected on periphyton growth (Table 6.2). Dry mass of periphyton scrapes from tank sides was similar among all treatments before and after fish addition (Figure 6.4b). In analyses of covariance, I found no indication that the community impacts detected were due (solely or partially) to differences in abundances between *G. affinis* and *G. geiseri*. *Gambusia* population sizes did not explain any of the variation in chlorophyll a values, faunal abundances, or periphyton weights.

DISCUSSION

Novel competition may be an important mechanism affecting the invasion success of non-indigenous species. In this study, competition negatively affected the degree of establishment success achieved by two invading fish species. *Gambusia* populations were significantly smaller in experimental communities where red shiners were already present relative to communities without them. Populations were also smaller when both *Gambusia* invaded together. However, the effect of competition was smaller on the highly invasive *G. affinis* than on its non-invasive relative, *G. geiseri*. In competition (as in the absence of competition), *G. affinis* populations were on average two to three times larger *G. geiseri* populations. By the end of the experiment, competition from *C. lutrensis* and the congener resulted in a 67 % reduction in *G. geiseri* populations compared to only a 29 % reduction in *G. affinis* populations.

How abundant invaders become is often thought to be a key factor determining their impact (Parker et al. 1999). Aside from abundance, the level of per capita effect can also determine impact. Results from this experiment showed that not only do *G. affinis* and *G. geiseri*

differ in their abundance, but they also differ in their top-down predatory impact despite similar trophic roles. *G. affinis* strongly reduced zooplankton abundances while *G. geiseri* had a greater impact on small benthic invertebrates. A cascading effect was also only detected in communities where *G. affinis* was the fish species present. Data analyses suggest that these effects were not due to the greater population sizes of *G. affinis*.

As documented in Chapter 5, invasive *G. affinis* exhibited greater inherent population growth than *G. geiseri* under the relatively favorable conditions of the experimental communities and in the absence of competition. Population sizes of the invasive species were on average twice as large as those of the non-invasive species. These results agree with life history data from Chapter 5 indicating the *G. affinis* has higher individual growth rates, shorter juvenile periods, and greater fecundity (more offspring per brood). *G. affinis* seems to also have shorter interbrood intervals than *G. geiseri* (Hubbs 1996). Overall, the invasive *Gambusia* has a life history that allows it to have higher maximum population growth rates than *G. geiseri*. In its native springs, however, *G. geiseri* reproduces all year around (*G. affinis* does not reproduce in the winter) (Hubbs 1998), which might allow it to compensate for its smaller broods. It is also possible that under the constant environmental conditions of the springs, *G. geiseri* might also be able to grow as fast or faster than *G. affinis*, become mature as soon as *G. affinis,* and have interbrood intervals of greater or equal length. If this is true, *G. geiseri* might be able to outperform *G. affinis* (thus explaining the absence of *G. affinis* in the springheads), but only under the precise environmental conditions of the springs. However, *G. geiseri* does not appear to exhibit the high reproductive output, under a variety of conditions (including ones it has never experienced), required to be a good invasive species.

Although interspecific competition did not prevent the establishment of the *Gambusia* species in the experimental pond communities, it did reduce their abundances (anywhere from 30 to 80 %). In spite of this intense effect of competition, G. *affinis* still remained more abundant that *G. geiseri*. *G. affinis* populations were larger than *G. geiseri* populations when *C. lutrensis* was the competitor and when the species were competing with each other. This result suggests that *G. affinis* not only has a strong capacity to invade because of its ability to become abundant (i.e., it is a 'weedy' species), but also because it has a strong competitive ability, even when encountering novel species. Because of evolutionary tradeoffs, we typically do not expect to find

species that are both good colonizers and good competitors (Huston and Smith 1987); however, successful invasive species might be the exceptions to this conventional expectation.

Interspecific competition is known to cause species to change their resource use when together relative to their resource use when apart (Werner 1986). This niche divergence is more likely to occur if species have similar niche requirements (Giller 1984). Analyses of stable isotope signatures are a useful tool in determining species' resource utilization patterns and trophic relationships (Vander Zanden et al. 1999). Stable isotope ratios in *Gambusia* tissues showed that both species had similar $\delta^{15}N$ ratios indicating that they shared the same trophic placement (Vander Zanden and Rasmussen 1999), and may then be expected to compete if found together. For *G. affinis*, $\delta^{13}C$ and $\delta^{15}N$ ratios in both competition treatments (with *C. lutrensis* and *G. geiseri*) were identical to ratios without competition, signifying no shift in resource use as a result of the presence of competitors. In contrast, *G. geiseri* $\delta^{13}C$ and $\delta^{15}N$ ratios in the competition treatments differed from levels when *G. geiseri* invaded alone, indicating that competition resulted in a change in *G. geiseri*'s resource use. This suggests that competitive interactions were largely asymmetrical between the *Gambusia* species and when both competed with *C. lutrensis*, perhaps indicating that *G. affinis* is a superior competitor.

Surprisingly, the effect of competition from *C. lutrensis* was similar to the effect of competition from congeners. Competition has traditionally been expected to be stronger between species that are more closely related, more similar, and thus more likely to exhibit overlapping resource use (e.g., Hairston 1949, Werner and Hall 1976). However, competition may also be expected to be strongest with novel species, to whom the invader lacks appropriate responses. In the experimental tanks, per capita competition was apparently much stronger with red shiners than with congeners if one considers that just four *Cyprenella* individuals resulted in significant *Gambusia* reductions in population size that were comparable to the effect of approximately 20 to 70 *Gambusia*.

While the mechanism underlying the difference in competitive response between *G. geiseri* and *G. affinis* documented here is unknown, insights may, in theory, be gained by looking at short-term behavioral responses to competition (Chapter 3). However, in competition trials, *G. affinis* and *G. geiseri* showed identical responses to another novel competitor (*P. promelas*). Both species responded appropriately to novel competition by increasing their foraging efficiency (prey consumed per unit time spent foraging) when competitors were present, which

allowed them to secure half of the prey consumed during trials. This suggests that both species should be equally capable of coping with exploitative competition. Aggression often allows organisms to better secure resources in competition (MacArthur 1972). Invasive and non-invasive *Gambusia*, however, were equally aggressive toward *P. promelas* (Chapter 3). This implies that *G. affinis* and *G. geiseri* should also not differ in their ability to cope with interference competition. Thus, behavioral and foraging mechanisms do not seem to account for the variation in competitive ability seen in this experiment. Further research on other potential mechanisms is needed to understand why competitive interactions differed between invasive *G. affinis* and non-invasive *G. geiseri* populations. In particular, whether the species differ in long-term resource-use efficiency should be explored, since it is a common mechanism for negative competitive interactions in other invaders (Williamson 1996, Holway 1999, Byers 2000).

Overall, pond communities were altered by the presence of *Gambusia.* Impact by *Gambusia* was detected on zooplankton, small benthic invertebrate, and phytoplankton abundances. There were no detectable effects on larger macroinvertebrate or periphyton abundances. In general, impact differed and appeared stronger when the invasive *Gambusia* was the top predator species, in spite of having the same trophic placement and equal diet breadth (Chapter 2). Impact was also unrelated to the greater abundance of *G. affinis*, demonstrating that *G. affinis* had in fact a greater per capita impact. Direct consumption by *Gambusia* best explains the effects on the zooplankton and benthic invertebrates (Harris 1995). In *G. affinis* communities, zooplankton abundances were greatly reduced relative to *G. geiseri* and no fish communities. In contrast, *G. geiseri* significantly impacted the benthic community.

I found evidence of a cascading effect on phytoplankton abundance only when *G. affinis* was the fish species in the communities. Water chlorophyll a concentrations were greater in *G. affinis* tanks relative to no fish tanks and relative to *G. affinis* tanks before fish addition. This result agrees with previous experimental research showing that invasive *Gambusia* can have important indirect effects by predating on primary consumers (but see Harris 1995). Hurlbert et al. (1972) documented that the presence of *G. affinis* in mesocosms similar to the ones used in this study resulted in increased phytoplankton, lower water clarity, higher water temperatures, and higher nutrient levels (phosphorous). Similar effects were noted by Hurlbert and Mulla (1981); besides dramatically reducing (or completely eliminating) zooplankton, *G. affinis*

significantly increased phytoplankton abundances and caused changes in water chemistry (increased pH and oxygen levels).

Aquatic communities are typically impacted more heavily if the introduced species is a top predator and a zooplanktivore (Moyle and Light 1996b). In this experiment, *Gambusia* were introduced to pond communities where they (and *Cyprenella*) were the top predators. While these might have represented overly simplified communities, and it may be argued that the observed impacts might not occur in more realistic communities (i.e., ones including piscivores and more competitors), *Gambusia* are often introduced to ephemeral or highly disturbed habitats (Arthington and Lloyd 1989) that lack other fish species entirely (e.g., Gamradt and Kats 1996).

Impact is often thought to be a function of the trophic role of the invader (Vitousek 1990). Yet, *G. affinis* and *G. geiseri* had identical $\delta^{15}N$ signatures and thus equivalent trophic roles, but different impacts. $\delta^{13}C$ signatures, however, differed between the species, indicating that the energy sources at the bottom of their feeding links were not the same. $\delta^{13}C$ values were more enriched in *G. affinis* tissues relative to *G. geiseri* tissues. These differences provide additional evidence that these species, while both secondary consumers, were consuming different primary consumers. $\delta^{13}C$ ratios are typically enriched for benthic algae relative to phytoplankton (Hecky and Hesslein 1995), indicating that *G. affinis* were likely consuming benthic grazers and *G. geiseri* consuming filter-feeding zooplankton. However, this contradicts the abundance results (*G. affinis* impacted the zooplankton while *G. geiseri* impacted the benthic invertebrates). Without having analyzed isotopic signatures for the zooplankton and invertebrates also, I cannot speculate further on how the *Gambusia* diets differed (beyond the faunal abundance differences). *G. affinis* is generally considered a trophic generalist (Farley 1980). Zooplankton are a major element of their diet (Crivelli and Boy 1987, Blaustein and Karban 1990, Garcia-Berthou 1999). Drifting and benthic invertebrates, terrestrial and neustonic prey are also major components (Lloyd et al. 1986, Garcia-Berthou 1999). Beyond the laboratory experiments reported in Chapter 2, nothing is known about the feeding habits of *G. geiseri*.

The results of this comparative study suggest that competition may in fact be an important limiting factor to both the native distribution of *G. geiseri* and its invasive ability. Since *G. affinis* is found in the rivers downstream from the springs where *G. geiseri* is native, spreading *G. geiseri* would necessarily encounter and have to cope with competition from *G. affinis*. My data suggest that they would be negatively impacted by such competition. Data from

Chapter 5, however, suggests that a major limitation to their spread might be their inability to cope with cold temperatures. Temperature tolerance has been found to be a key factor limiting a species' native distribution (Jenkins and Hoffmann 1999) and invasion success, especially in fishes (Meffe 1991, Welcomme 1992, Crivelli 1995, Brown and Moyle 1997). *G. geiseri* suffered 100 % mortality under overwintering conditions in both the laboratory and semi-field conditions (experimental ponds in Kentucky). The low temperatures experienced in that study were very different from the stenothermal conditions to which *G. geiseri* is adapted in its native springs (21-22° C all year around) (Hubbs 2001). Those results might explain why *G. geiseri* has not spread into colder northern areas, and even when introduced it has remained localized to other stenothermal springheads. However, overwintering conditions in the immediate streams to their springs (central Texas) might not be as severe as those *G. geiseri* experienced in the Chapter 5 experiments. If this is true, local spread may be limited by a combination of both temperature tolerances (to milder winters) and competition with *G. affinis* (and other competitors).

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Table 6.1: Summary of analysis of variance results for competition effects on *Gambusia* population sizes and stable isotope signatures.

Variable	*Effects*	*df*	*F*	*p-value*
Population size	Treatment-species combination	5, 12	20.7	0.0001
	Time	1, 12	5.0	0.05
	Treatment-species combination x time	5, 12	1.6	0.12
$\delta^{13}C$	Treatment-species combination	5, 33	25.1	0.0001
	Tank (Treatment-species)	12, 33	4.7	0.0001
$\delta^{15}N$	Treatment-species combination	5, 33	4.0	0.003
	Tank (Treatment-species)	12, 33	2.9	0.008

Table 6.2: Summary of community effects among no fish, *G. affinis*, and *G. geiseri* treatments. Statistics shown are for nested, repeated measures, and one-way analyses of variance on phytoplankton, periphyton, macroinvertebrate, zooplankton, and benthic invertebrate abundances.

Variable	*Effects*	*df*	*F*	*p-value*
Macroinvertebrates	Treatment	2, 6	1.5	0.15
Zooplankton	Treatment	2, 9	44.2	0.0001
	Tank (Treatment)	6, 9	7.0	0.005
Benthic invertebrates	Treatment	2, 9	17.0	0.0005
	Tank (Treatment)	6, 9	5.6	0.01
Chlorophyll a	Treatment	2, 6	3.4	0.05
	Time	1, 6	8.4	0.03
	Treatment x time	2, 6	4.7	0.03
Periphyton	Treatment	2, 6	0.5	0.31
	Time	1, 6	1.3	0.29
	Treatment x time	2, 6	0.004	0.50

Figure 6.1: Population trajectories of each *Gambusia* population over the duration of the experiment. Shown are the mean population sizes (the total number of individuals) at each sampling time ± 1 SE. Dark symbols represent *G. affinis* populations and light symbols *G. geiseri* populations. Treatments are as follows: (2) *G. affinis* alone, (3) *G. geiseri* alone, (4, G. aff) *G. affinis* in competition with *G. geiseri*, (4, G. gei) *G. geiseri* in competition with *G. affinis*, (5) *G. affinis* in competition with *C. lutrensis*, (6) *G. geiseri* in competition with *C. lutrensis*.

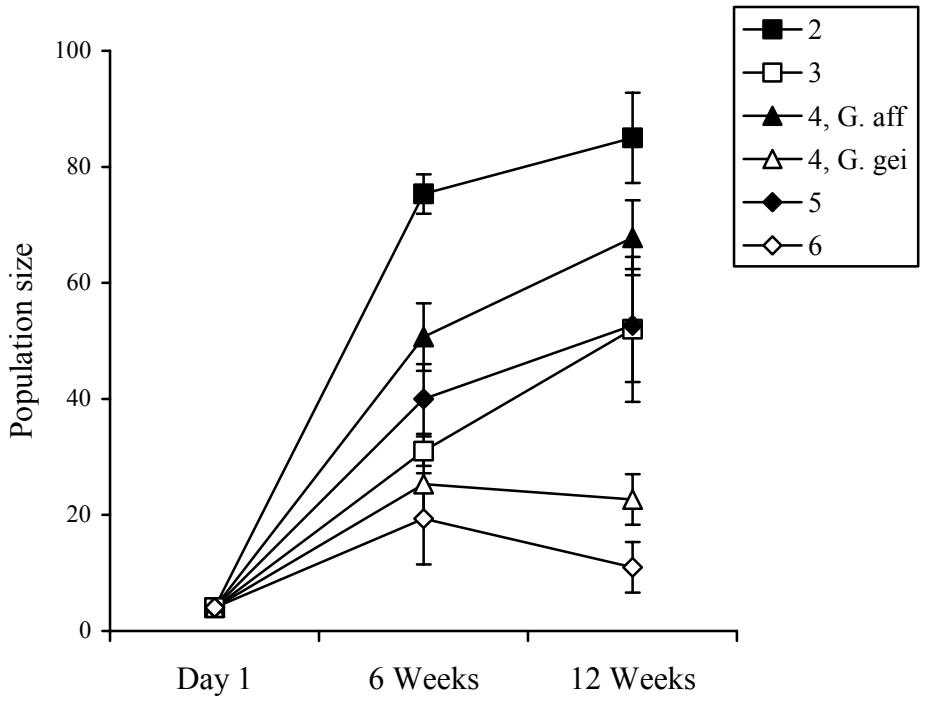


Figure 6.2: Mean $\delta^{15}N$ versus mean $\delta^{13}C$ (± 1 SE) signatures for *Gambusia* in competition and no competition treatments. Dark symbols represent *G. affinis* samples and light symbols *G. geiseri* samples. Treatments are as follows: (2) *G. affinis* alone, (3) *G. geiseri* alone, (4, G. aff) *G. affinis* in competition with *G. geiseri*, (4, G. gei) *G. geiseri* in competition with *G. affinis*, (5) *G. affinis* in competition with *C. lutrensis*, (6) *G. geiseri* in competition with *C. lutrensis*.

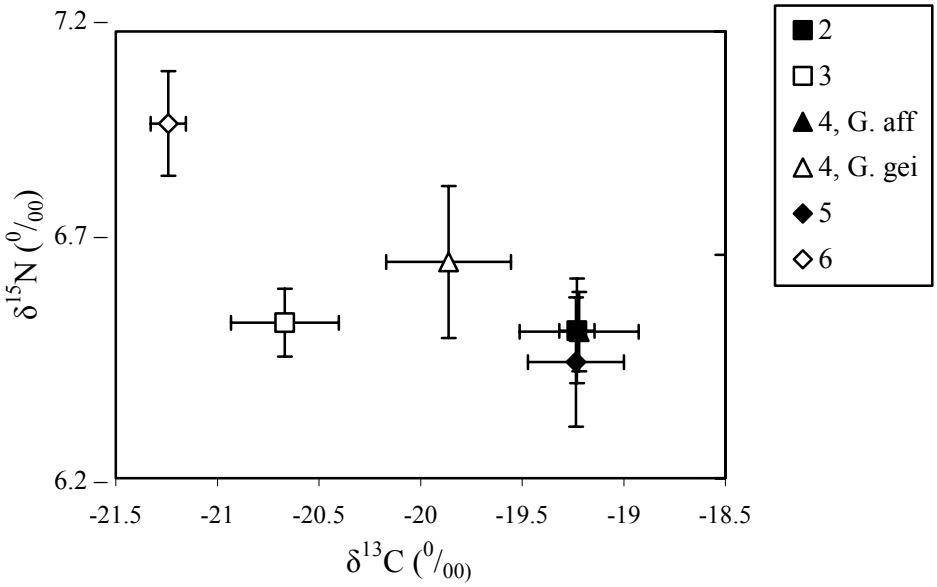


Figure 6.3: Mean total faunal abundances (± 1 SE) in sweep net samples for the macroinvertebrates and in 10 mL subsamples for the other two taxa at the end of the experiment (12 weeks) for the three community impact treatments of interest: no fish, *G. affinis* alone, and *G. geiseri* alone.

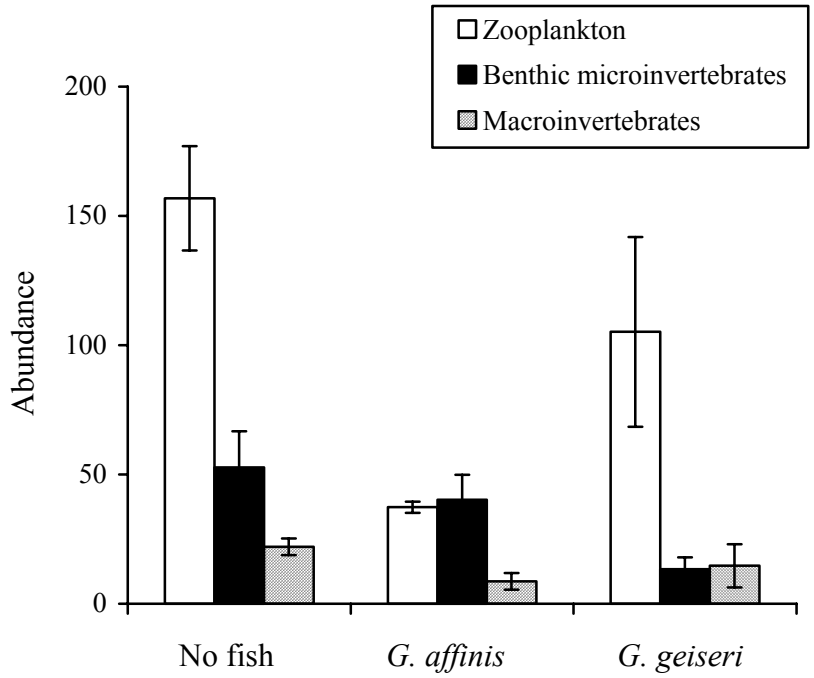
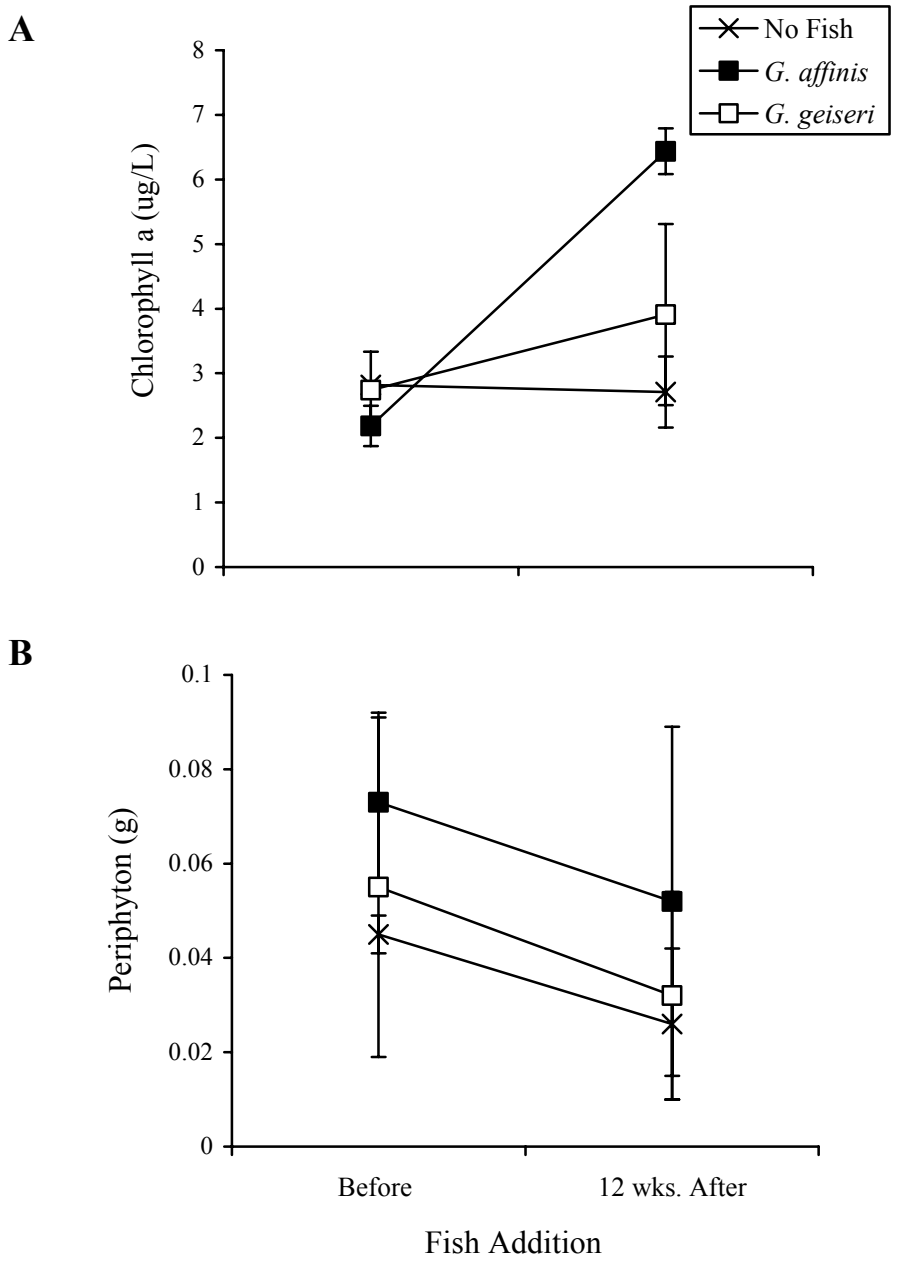


Figure 6.4: (a) Mean chlorophyll a concentrations and (b) mean dry weights (± 1 SE) of periphyton samples for the three community impact treatments of interest: no fish, *G. affinis* alone, and *G. geiseri* alone.



Chapter Seven

Conclusion

This study found that invasive and non-invasive *Gambusia* species differed in the majority of the traits tested (Table 7.1), suggesting that multiple traits affect the invasion success of non-indigenous species. I found the invasive *Gambusia* species to be more tolerant of low temperatures (Chapter 5) and to exhibit higher feeding rates (Chapter 2) and dispersal tendencies (Chapter 4) than non-invasives. Invasive species were more likely to respond appropriately to novel predation by reducing foraging and activity level and by increasing refuge use, and less likely to show lower foraging success when faced with novel competition (Chapter 3). Invasives exhibited higher fecundity and juvenile growth rates, and consequently reached maturity sooner than non-invasives (Chapter 5). The only traits where no differences were detected between invasives and non-invasives were feeding preferences, diet breadth (Chapter 2) and aggression levels (Chapter 3). Contrary to predictions, the invasive and non-invasive species exhibited similar feeding preferences, equally broad diets, and comparable aggressiveness.

By simulating invasions in experimental communities (Chapters 5 and 6), I was able to evaluate the relative significance of these trait differences to one of the stages of the invasion sequence, establishment. Recent studies point to a stage-based approach as a key element of research aimed at better understanding the relationship between species-specific traits and invasion dynamics (Carlton 1996, Vermeij 1996, Kolar and Lodge 2001; e.g., Duncan et al. 2001). In these simplified pond communities, successful 'establishment' only occurred if organisms were able to withstand the abiotic conditions they encountered (Chapter 5). In particular, low temperature tolerance was the key abiotic element that limited establishment. Non-invasive *Gambusia* (*G. hispaniolae* and *G. geiseri*) populations suffered 100 % overwintering mortality in these communities and thus these species were unable to 'establish'. In contrast, the invasives *G. affinis* and *G. holbrooki*, while suffering over 80 % overwintering mortality, managed to sustain populations in the experiment. This suggests that abiotic tolerances may constitute an absolute limit to establishment (i.e., they determine whether or not establishment occurs), while other traits seem to have secondary importance.

Many studies have reported the importance of abiotic elements to invasion success. Research consistently shows that invaders are often more successful if there is a high degree of climatic matching between their native and invaded ranges (Newsome and Noble 1986, Welcomme 1992, Green 1997, Moyle and Light 1996a, Williamson and Fitter 1996b, Carlton 1999, Wonham et al. 2000, Duncan et al. 2001). For fishes, broad temperature tolerances have been documented to be a key limiting factor to establishment and overall distribution in invaded areas (Meffe 1991, Welcomme 1992, Crivelli 1995, Brown and Moyle 1997). Work by P. Moyle and colleagues showed that whether or not non-indigenous fishes are able to cope with abiotic conditions (i.e., hydrologic regime) is the key determinant of successful establishment in California streams (Moyle and Light 1996a, Brown and Moyle 1997, Marchetti et al. 2003). Similarly, tolerance of a wide range of temperatures and salinity conditions (along with fast growth) are the key traits distinguishing establishment success from failure in fish species arriving to the Great Lakes region (Kolar and Lodge 2002).

While temperature tolerances determined whether or not establishment by *Gambusia* occurred, other traits became important in determining the degree of establishment success achieved (i.e., how abundant populations became if they survived the abiotic stress). Invasive *Gambusia* were able to achieve and maintain larger populations that non-invasive *Gambusia* even when temperature was not an issue (Chapter 6). Differences in life history traits (greater fecundity, shorter juvenile periods, and reaching sexual maturity at a younger age) are suspected to be the contributing factor to these differences in population performance (Chapter 5). Life history traits are among the most consistent correlates of invasiveness (Sakai et al. 2001). All major statistical analyses of large numbers of species used to discriminate and predict invasive from non-invasive species based on traits involve at least one life history trait (e.g., Rejmanek and Richardson 1996, Reichard and Hamilton 1997, Williamson and Fitter 1996, Marchetti et al. 2003). Life history traits are the key determinant of a species' ability to become abundant and therefore also affect a species' ability to overcome demographic and environmental stochasticity, which often extirpates newly established populations in invaded areas (Crawley 1989).

The other important secondary trait affecting the *Gambusia* species' abundances in the simulated invasion was their ability to cope with biotic interactions. Competition in experimental communities caused decreases in population sizes (relative to communities invaded by *Gambusia* alone) that were larger for the non-invasive species than the invasive species (67 %

compared to 29 %) (Chapter 6). Invasive *Gambusia* were better able to cope with competition (both novel and non-novel) and thus managed to maintain larger populations in the presence of competition, as they did in the absence of competition. Analysis of $\delta^{13}C$ and $\delta^{15}N$ isotope ratios provided further evidence that invasive *Gambusia* were able to better cope with competition. Trophic shifts as a result of competition were only observed in the non-invasive species; trophic placement for invasive *Gambusia* was the same whether or not competitors were present (Chapter 6).

Overall, this body of work provides strong evidence that species-specific traits can be good predictors of establishment success. Trait differences among invasive and non-invasive *Gambusia* corresponded nicely with their population-level performance in simulated invasions and with their known invasion success (or lack thereof) in natural communities. As predicted, *G. affinis* and *G. holbrooki* generally outperformed their non-invasive relatives (summarized in Table 7.1). Indeed, *G. hispaniolae* and *G. geiseri* seem to lack the traits of successful invasive species, suggesting that invasiveness is not a trait of the entire genus *Gambusia*.

Conservation implications

Results from this study and others suggest that if a non-indigenous species can cope with the abiotic conditions of a target community, establishment is likely to occur. Since efforts at control and eradication are usually most productive early in the invasion sequence (Mack et al. 2000), data on physiological tolerances of arriving species would help prioritize which arriving species should be targeted for eradication efforts. For example, invasive *Gambusia* are well-known to be extremely tolerant of an array of physical conditions (Krumholz 1948, Courtenay and Meffe 1989, Ehrlich 1989, Lloyd 1989), and establishment should be expected if introduced. In the absence of data on abiotic tolerances, information on the degree of climate matching between the native and invaded ranges of the non-indigenous species would help identify species with a high probability of establishment success. Data on abiotic factors should not only include the physiological limits for survival, but also the abiotic requirements for reproduction. Often organisms might be able to survive in the communities in which they arrive but will not be able to find suitable conditions for reproduction (Crivelli 1995).

As in other studies, life histories had a profound influence on how abundant invaders became in my experiments. A species' abundance is often linked to success at later stages of the

invasion sequence. Invaders that are able to increase in abundance rather quickly from a few propagules are likely to have greater post-establishment spread (i.e., larger invaded range) (Rejmanek and Richardson 1996, Reichard and Hamilton 1997). An invader's abundance is also a key determinant of the level of impact the invader may have in the invaded community (Parker et al. 1999). For policy makers and natural area managers, life history information would prove useful at identifying invaders that might spread if introduced and invaders that may also become a dominant feature of the invaded community and result in significant impact. Since conservation practitioners are often forced to allocate limited resources to only the control of the highest impact invaders, life history traits might be particularly important. Insights from my work on the foraging behavior of *Gambusia* also suggest that maximum feeding rates (not diet breadth) might be good indicators of the potential for direct predatory and indirect impacts on the community. Results from the response to novel competition data suggest that elements of the invaded community should be considered when evaluating the potential for invasion. As seen in this study, biotic interactions may act to limit the abundance and potential for spread and subsequent impact of an invader.

Lastly, my study found significant trait variation among species of seemingly equal invasiveness (Table 7.1). Trait differences were even detected between the sister invaders, *G. affinis* and *G. holbrooki*. These differences suggest that invasiveness might be best described as a continuum. If this is true, quantitative measures of relative invasiveness as a function of species traits would be useful information to conservation practitioners faced with prioritizing the control of many invasive species.

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Table 7.1: Summary of trait differences found among the four *Gambusia* species in all experiments conducted in this dissertation. Shown are the results of orthogonal contrasts testing whether the two invasive species differed from the two non-invasives, and whether there were species differences within the categories 'invasive' and 'non-invasive'. Checkmarks indicate whether invasives outperformed non-invasives in the manner expected and x's indicate that no differences were found.

Traits	*Invasive > Non-invasive*	*Between invasives*	*Between non-invasives*
Low temperature tolerance	✓	No	Yes
Diet breadth, 3 preference measures	×, ×, ×, ×	No	No
Competition - foraging behavior	✓	No	Yes
- aggression	×	No	No
Predation - foraging behavior	✓	Yes	No
- refuge use	✓	Yes	No
Feeding rates (3 prey items and all prey combined)	✓, ✓, ✓, ✓	No	No
Life history - Fecundity	✓	Yes	Yes
- Juvenile growth rates	✓	No	Yes
- Age/Size at sexual maturity	✓	Yes, yes	Yes, yes
Dispersal tendency	✓	Yes	Trend

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PROFESSIONAL PUBLICATIONS AND PRESENTATIONS

Rehage, J.S. and A. Sih. In review. Dispersal characteristics and boldness: a comparison of *Gambusia* species of varying invasiveness. *Biological Invasions*.

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