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Invasion Risk and Impacts of a Popular Aquarium Trade Fish and the Implications for Policy and Conservation Management

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Invasion Risk and Impacts of a Popular Aquarium Trade Fish and the Implications for Policy and Conservation Management

by

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Dedication

This dissertation is dedicated to my family, fellow pursuers of knowledge, who have always encouraged, motivated and supported me and my academic interests. I could not have come this far without you.

"The idea of wilderness needs no defense. It only needs more defenders."

Edward Abbey

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Invasion Risk and Impacts of a Popular Aquarium Trade Fish and the Implications for Policy and Conservation Management

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Invasive species, a top threat affecting global biodiversity, become invasive through a process including four stages: transport, establishment, spread, impact and integration. Species currently in this process provide opportunities to empirically derive the mechanisms driving each of these stages, make predictions based on these mechanisms and then to test these predictions. This research examines the current invaded distribution, potential invasion and community-level impacts of a popular aquarium trade fish (*Hemichromis guttatus* Günther, 1862) in an endemic hotspot, Cuatro Ciénegas, in Coahuila, México and discusses the policy and conservation management implications of these findings. In Chapter 1, the problem of invasive species, the study site and the focal species of this work are introduced. In Chapter 2, the critical thermal minimum and maximum temperature limits and temperature preference of *H. guttatus* are identified because temperature is hypothesized to be an important factor controlling this fish's distribution. The results indicate that *H. guttatus* has a wide temperature tolerance range (a characteristic of a 'good' invader), that preference is a more informative metric for predicting invasion than absolute tolerances, and that resource-poor environments may promote searching behaviors that cause an invasive fish to increase its range. In Chapter 3, the results of a field survey are analyzed and temperature, pH, depth and the presence of vegetation are all found to be related to *H. guttatus* presence. Invasion risk of several as-of-yet uninvaded sites in Cuatro Ciénegas is assessed. In Chapter 4, competitive and predatory interactions of *H. guttatus* on an endemic, threatened cichlid (*Herichthys minckleyi*) and a macroinvertebrate community respectively are investigated. The results suggest that while *H. guttatus* does not directly impact *H. minckleyi* through competition in these conditions, it may inhibit reproduction and alter *H. minckleyi*'s behavior through aggressive interactions. In Chapter 5, all results are synthesized and a determination of the invasive status of *H. guttatus* in Cuatro Ciénegas is made. The results presented here will be useful in identifying areas with a high risk of invasion by this popular ornamental fish, thus allowing the implementation of policy and management actions to prevent or at least ameliorate the impacts of an invasion and will add to the growing knowledge of how invasive species affect native systems.

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Chapter 1: Introduction

Invasive species, those species living outside their native range that are imposing negative impacts on the communities they have invaded, threaten global biodiversity at every level of biological organization. There are numerous mechanisms by which invasives accomplish this including hybridization with native species at the genome level (Rosenfield *et al.*, 2004), the introduction of pathogens to native conspecifics at the population level (Bartholomew & Reno, 2002), competitive and predatory interactions at the community level (Human and Gordon 1996, Ogutu-Ohwayo 1990), and via changes in nutrient cycles (Heath *et al.*, 1995) and natural fire regimes (Brooks *et al.*, 2004), and through direct habitat alteration (Anderson *et al.*, 2009) at the ecosystem level (Figure 1.1). Beyond the ecological impacts, there are also great economic impacts associated with invasive species. Globally, damages and control costs of invasives exceed \$1.4 trillion or 5% of the global GNP (Pimentel *et al.*, 2001). Approximately \$137 billion are spent on damages and control of invasive species in the United States alone (Pimentel *et al.*, 2000), and \$1 billion of this is lost to damages due to invasive fishes alone, and this value takes into account \$69 billion in revenue from the sport fishing industry (Pimentel, 2005).

The process of invasion occurs in a series of five stages (Colautti & MacIsaac, 2004). The first stage is transport where the species is taken from its native environment and transferred somewhere else. If survived, transport is followed by introduction (stage II) of the species into an exotic environment. If the species is able to survive the novel environment (pass through the abiotic filter), it becomes established or “localized and rare” (stage III), forming a localized population with few individuals. Some species may then be able to spread, passing through a dispersal filter to become “widespread but rare” (stage IVa), and others may overcome community interactions (passing through the biotic filter), increasing in abundance and becoming integrated or “localized but dominant” (stage IVb). Lastly, a species that passes through all three filters is both “widespread and dominant” (stage V) (Figure 1.2). In the context of this study, an ‘invasive species’ is defined as a species that has reached stage V of Colautti and MacIsaac’s (2004) terminology. Meinesz (2001) estimated that of the 50,000 introduced species in the United States, 7,000 have established and only 1,050 have become invasive. Thus, it is of great interest to ecologists both the characteristics of an environment that affect invasibility, and also the characteristics of species that make them good invaders.

Few generalities can be made about which environments are more easily invasible, though those habitats that are similar to the exotic’s native habitat, experience high human disturbance or that are species depauperate (Moyle & Marchetti, 2006) have a higher likelihood of being invaded. More generalities can be made about the characteristics of invading species themselves including that they often have wide physiological tolerances, a history of prior invasion elsewhere or are closely related to a known invader, have symbioses with or are desirable to humans, have a high propagule pressure (i.e., many individuals being introduced into an area) (Marchetti *et al.*, 2004b), and provide parental care (Marchetti *et al.*, 2004a).

One group of species that fits several of these generalities is the genus of cichlid fishes *Hemichromis*. It is a genus containing 11 species all native to western Africa (Froese

& Pauly, 2014). One of the species, *H. letourneuxi*, is a known invader in the Everglades in Florida (Schofield *et al.*, 2013) and this same species has been shown to have wide physiological tolerances to salinity (Langston *et al.*, 2010), cold (Schofield *et al.*, 2010) and hypoxia (Schofield *et al.*, 2007). All of the species are transported globally for the ornamental fish trade thus creating a high risk for potential introduction around the world (high propagule pressure), and in fact there are several documentations of introduced *Hemichromis* species worldwide including *H. bimaculatus* in Canada (Welcomme, 1986), *H. letourneuxi* in Florida, USA (Shafland, 1996, Eldredge, 2000), *H. elongatus* in Hawaii, USA (Eldredge, 2000), *H. guttatus* in Mexico (Contreras-Balderas & Ludlow, 2003) and Australia (Koehn & MacKenzie, 2004), *H. bimaculatus* (Olden *et al.*, 2008) in Australia, *H. lifalili* (Webb, 2008) also in Australia, and unidentified *Hemichromis* spp. in Tuscany, Italy (Piazzini *et al.*, 2010) and Warmbad, Austria (Petutschnig *et al.*, 2008).

One of these species, *H. guttatus* in northern Mexico is of interest because of the unique ecosystem into which it has been introduced. The Cuatro Ciénegas valley is a prehistorically isolated desert oasis in the Chihuahuan Desert. It is a 'W'-shaped valley, bisected by the Sierra San Marcos and nestled in the Sierra Madre Oriental. Due to its prehistoric isolation (canals were built starting in the late 1800s connecting surface waters here to those outside the valley (Minckley, 1969)), the highest density of endemic species in North America exists here (Stein *et al.*, 2000) thus motivating the region to be named a World Wildlife Foundation conservation priority (Abell *et al.*, 2000), a UNESCO biosphere reserve (UNESCO, 2010), a RAMSAR convention wetland of international importance (Instituto Nacional de Ecología (Mexico) & U.S. Fish and Wildlife Service, 2007), and a federally protected area for flora and fauna (Secretaría de Desarrollo Social, 1994). There are 21 species of fishes here, 10 of which are endemic. Eleven of the fishes in Cuatro Ciénegas are listed as endangered or threatened by the Mexican federal government (Secretaría de Desarrollo Social, 1994), and the presence of exotics is implicated as one of the contributing factors in all but one (Contreras-Balderas *et al.*, 2003).

Considering the potential risk of *H. guttatus* becoming invasive in this system, in this study I sought to determine characteristics of the environment associated with the presence of *H. guttatus* in Cuatro Ciénegas so as to assign invasion risk for as-of-yet uninvaded habitats in the valley through a series of laboratory experiments (Chapter 2) and field observations (Chapter 3). Further, I aimed to identify potential impacts of *H. guttatus* on one endemic, threatened cichlid, *Herichthys minckleyi*, and aquatic macroinvertebrate communities via a mesocosm experiment (Chapter 4). Lastly, I review the results and present my conclusions about the potential invasiveness of *H. guttatus* in local (Cuatro Ciénegas) and global contexts (Chapter 5).

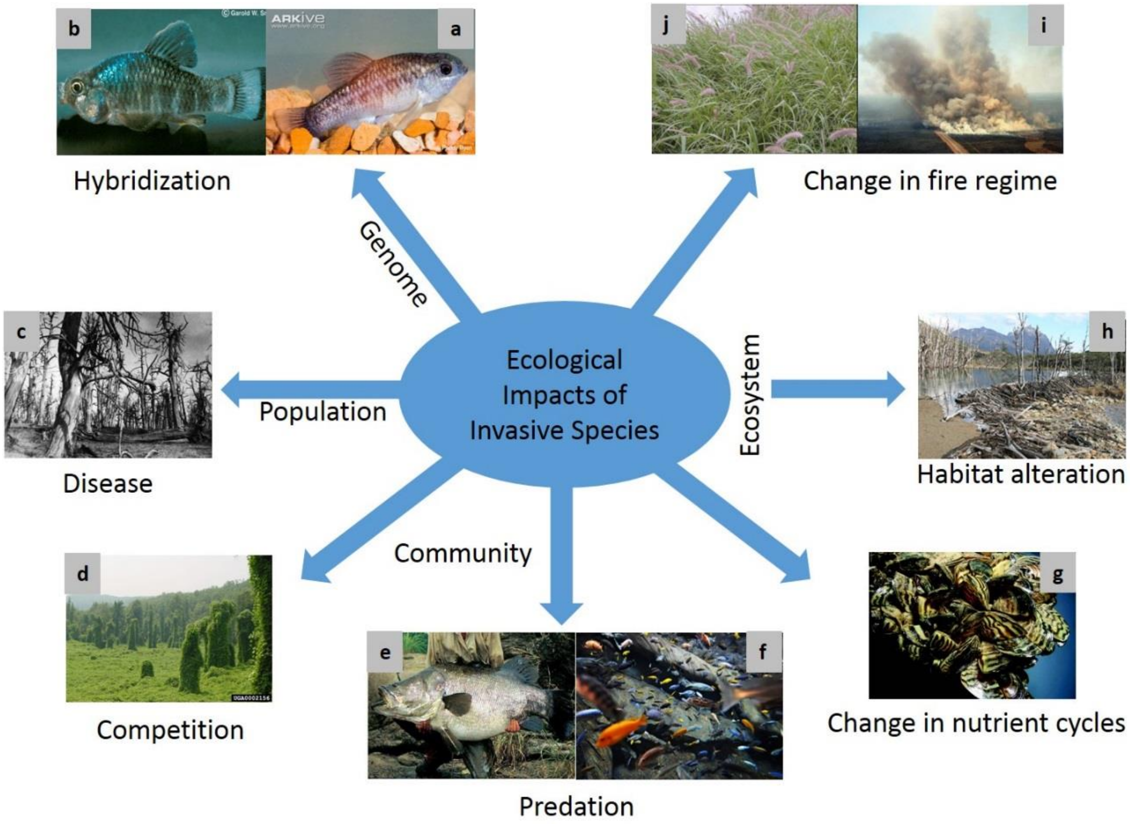


Figure 1.1: Various effects invasive species can have on native biota. Invasives can affect natives at every level of biological organization from the level of the genome to population to community to the ecosystem. Photo credits: a) Pecos pupfish (*Cyprinodon pecosensis*): Arkive; b) Sheepshead minnow (*Cyprinodon variegatus*): Fishbase; c) Chestnut blight (*Cryphonectria parasitica*) on Eastern chestnut trees (*Castanea dentata*): The American Chestnut Foundation ; d) Kudzoo (*Pueraria montana*): Illinois Department of Natural Resources; e) Nile perch (*Lates niloticus*): Livescience; f) East African lake cichlids (various spp.): Georgia Aquarium; g) Zebra mussels (*Dreissena polymorpha*): US Fish and Wildlife Service; h) Beaver damage (*Castor canadensis*): Invasive Species International; i) Chaparral Wildlife Management Area 2008 fire: Texas Parks and Wildlife; j) Buffel grass (*Pennisetum ciliare*): Texas A&M Uvalde Herbarium.

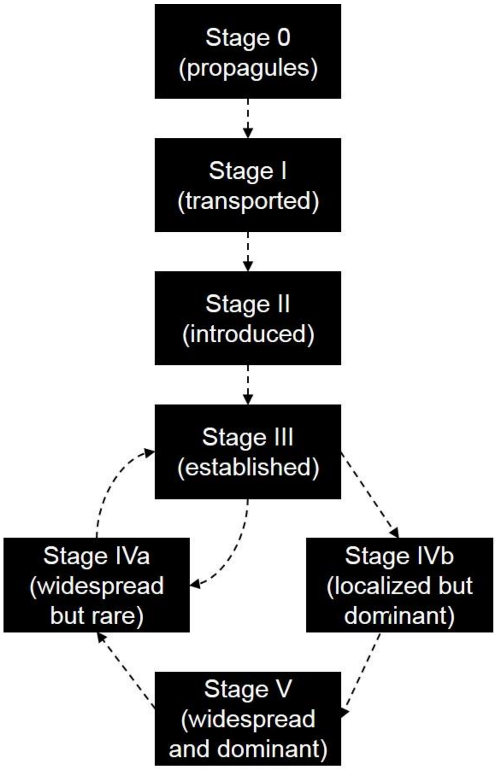


Figure 1.2: Framework depicting the five stages of the invasion process. At stage 0, propagules reside in a donor population, at stage I they are transported out of their native habitat and introduced elsewhere at stage II and, if able to survive, become established at stage III. From there, species may become widespread but rare by passing through a dispersal filter (stage IVa) or localized but dominant by passing through a biotic filter (stage IVb). If a species is able to pass through all filters, it becomes widespread and dominant (stage V). Adapted from Colautti and MacIsaac (2004).

Chapter 2: Motivational state affects temperature preference but not tolerance in a potentially invasive fish: implications for invasion risk

INTRODUCTION

Invasive species threaten biodiversity at every level of biological organization, and these effects can be found in every habitat and on every continent including Antarctica (Smith *et al.*, 2012). A query of the IUCN Red List database (IUCN, 2013) indicated that invasive species were contributing threats to 2,258 species that are extinct, extinct in the wild, critically endangered or endangered. Of these species, 292 are fishes (IUCN, 2013), and Clavero and García-Berthou (2006) report invasives as the second most important threat. Besides ecological impacts, annual economic impacts related to damages and control of invasive species are estimated at $120 billion in the United States (Pimentel *et al.*, 2005), and globally exceed $1.4 trillion, or nearly 5% of the global GNP (Pimentel *et al.* 2007).

The aquarium trade is one of five main pathways for the introduction of aquatic species (Chang *et al.*, 2009) with up to 1,000 aquatic species in 100 different families being traded globally and at least 150 species in 30-35 families traded in the USA (Chapman, 2000). As of 1990, there were established populations of 46 species of exotic fishes in the United States, nearly two-thirds of which originated from the aquarium trade (Courtenay & Stauffer, 1990). The west African jewel cichlids (genus *Hemichromis*) are popular ornamentals, with introductions of various species documented including *H. bimaculatus* in Canada (Welcomme, 1986), *H. letourneuxi* in Florida, USA (Shafland, 1996, Eldredge, 2000), *H. elongatus* in Hawaii, USA (Eldredge, 2000), *H. guttatus* in Mexico (Contreras-Balderas & Ludlow, 2003) and Australia (Koehn & MacKenzie, 2004), *H. bimaculatus*

(Olden *et al.*, 2008) in Australia, *H. lifalili* (Webb, 2008) also in Australia, and *Hemichromis* spp. in Tuscany, Italy (Piazzini *et al.*, 2010) and Warmbad, Austria (Petutschnig et al. 2008). In at least two sites, Canada (Hornung & Pacas, 2006) and Florida (Schofield *et al.*, 2010), they have become invasive and in another, Mexico (Marks *et al.*, 2011), they are established, spreading and potentially invasive.

This site in northern México, Cuatro Ciénegas (Figure 2.1) is an oasis in the Chihuahua Desert with diverse water bodies including marshes, rivers, streams, lakes and springs. The valley was prehistorically isolated from surface flow from outside the valley until recently (late 1800s to the present, Minckley, 1969), when canals were built to divert the spring water for agriculture creating aquatic habitats that are island-like and surrounded by a "sea of desert". This past isolation resulted in the evolution of many endemic species (Johnson, 2005). *Hemichromis guttatus* has become established here (Contreras-Balderas & Ludlow, 2003), and Marks *et al.* (2011) documented a negative effect of competition of this species on one of these endemic, threatened fishes, the Cuatro Ciénegas cichlid, *Herichthys minckleyi*, as well as potential competitive interactions with another endemic, threatened fish, the two-line pupfish, *Cyprinodon bifasciatus*, based on dietary overlap determined from stable isotope analyses. Blackburn *et al.* (2004) found that the effects of invasives can be stronger on islands, especially those with many endemic species, and so the presence of *H. guttatus*, a potential invader, is of great concern. It now exists in large numbers in certain areas of the valley, while in other parts, it is still absent.

Because most fishes are classic ectotherms, with their internal temperatures reliant on the temperature of the surrounding environment, temperature affects nearly all aspects of their biology, and in habitats that may be outside of tolerable or preferred temperature ranges, thermal refugia can be thought of as a resource for which they will compete (Beitinger *et al.*, 2000). Brett (1956) submitted a 'thermal primacy paradigm', which states that 'because of the all-pervading nature of environmental temperature, the fundamental requirement of fishes is an external environment most suitable for their internal temperature', and because temperature is so important to so many aspects of a fish's life history (Fry, 1947), it is perhaps of no surprise that certain aspects of temperature, such as absolute tolerances or preference, are good predictors of invasiveness (Kolar & Lodge, 2002). Kimball *et al.* (2004) found that the northern range limit of invasive lionfish in the Atlantic Ocean is at its minimum thermal temperature tolerance, and several other studies have used temperature tolerance as a predictor of invasion potential (e.g., Rixon *et al.*, 2005, Chang *et al.*, 2009) and to predict the potential invaded range (Green *et al.*, 2012) of certain aquarium trade fishes.

Knowledge of a species' environmental tolerances and preferences can aid in predicting whether it can become invasive in a particular place (Welk *et al.*, 2002). For species that are in the process of invasion, determination of these factors offers the rare opportunity to formulate hypotheses about how a species' environmental tolerances and preferences may direct establishment and enable spread into new areas. These hypotheses may then be tested directly in real time, ultimately allowing for more informed identification of high and low risk areas of invasion.

The distribution of organisms depends on a variety of often scale-dependent abiotic and biotic factors and their interactions (Hutchinson, 1957, Jackson *et al.*, 2001). The distribution of *H. guttatus* in Cuatro Ciénegas is also likely dependent upon many factors, though its presence in an intermediate-temperature spring (Mojarral Este) and absence in an adjacent and connected but warmer spring (Poza Azul) prompted the hypothesis that temperature may be one of the more important factors. Temperature tolerance is a physiological property of an organism (Fry, 1947), whereas temperature preference, as utilized here, refers to a behavioral tendency of individuals to select some temperatures from the broader overall total range of temperatures available to them. This definition is similar to Hassell and Southwood's (1978) definition of food preference of insects and Ivlev's (1961) and Singer's (2000) definition of electivity.

In this study, I performed a series of laboratory experiments that measure critical thermal minimum (CT_{min}) and maximum (CT_{max}) and temperature preference of *H. guttatus*. Also, because food availability can fluctuate seasonally in Cuatro Ciénegas (Dinger *et al.*, 2005), these values are determined for both hungry and satiated fish. My aim was to provide data on the thermal profile of *H. guttatus* that could be used at the local level to determine where to focus control efforts in Cuatro Ciénegas and at the global level to help identify regions of high invasion risk for this and similar species.

METHODS

Study species and acclimation housing environment

In March 2007, approximately 200 *Hemichromis guttatus* were collected from Poza Churince, the spring pool in Cuatro Ciénegas where the species was first detected and where the species now has the highest known densities in the valley. The stock was transported to the lab and bred. Individuals used in this study were from this stock, which included first and second laboratory generations. All experimental fish were acclimated for at least two weeks in 38 L holding tanks equipped with sponge filters and heaters set to maintain a water temperature of 26 °C, the average water temperature of Poza Churince based on measurements made during one week of field sampling in March 2007 (Dugan, *unpublished*) and that closely corresponded to mean annual temperature (27 ºC) as measured by a piezometer that collected hourly temperature readings in Poza Churince from November 1, 2006 – March 30, 2007 (INECC, 2013). For the CT_{min} and CT_{max} experiments, fish were housed 6 to a tank and for the preference trials, 7 or 8 to a tank. In all three experiments, a range of sizes (measured as standard length (SL)) was used.

Critical Thermal Maximum

Experimental housing environment

Fish were randomly assigned to one of two motivational state treatments: hungry or satiated. Hungry fish were fed 1.75 g of New Life Spectrum® cichlid pellets once every third day, and the satiated group received this same amount of food every day. Testing occurred on the third day before any feedings occurred. Food was withheld from the satiated group for the last 24 hours before a trial because Beitinger *et al.* (2000) suggest that a full stomach could affect the outcome of determination of the maximum tolerable temperature.

Testing apparatus

Trials were carried out in a 4.3 L capacity stainless steel test tank with 3.8 L of dechlorinated tap water. Visibility of the fish was from above. The tank was situated on a silicon heater controlled by a Fuji Electric® (Shinagawa-ku, Tokyo, Japan) PXG4 temperature controller that received information on water temperature from a Watlow® (St. Louis, MO, USA) thermocouple sensor and sent a signal to a Siemens Sirius® SC (Washington D. C., USA) semiconductor that acted as an on/off switch to the heater based on this information (Figure 2.2). An air stone in the test tank vigorously aerated and mixed the water to prevent temperature stratification. The system was programmed to heat the water at a rate of 0.3 ºC/min, a rate slow enough to prevent the internal temperature of the fish from significantly lagging behind external temperatures, but fast enough to prevent thermal acclimation (Beitinger *et al.*, 2000).

Test procedure

A fish randomly selected from one of the 26 ºC holding tanks was put into the test tank (also at 26 ºC) for a 20-minute acclimation period before heating at a rate of 0.3 ºC/min was initiated. Behavioral observations were noted as heating continued until the fish experienced loss of equilibrium, defined here as the point at which the fish could no longer right itself and would be unable to escape conditions that could cause its death, at which point the individual is "ecologically dead" (Cowles & Bogert, 1944) . Temperature at and time to LOE were noted, and the fish was moved from the test tank to a recovery tank held at 26 ˚C. After one hour of recovery, each test fish was anesthetized and fin-clipped to mark that it had been tested, measured (SL) and returned to its original holding tank. Each individual was tested only once.

Critical thermal minimum

The housing and feeding regimes were the same as for the CT_{max} experiments.

Test apparatus

Cold water from a 28 L Styrofoam ice water reservoir was pumped into the test tank, a 22 L capacity Styrofoam cooler containing 15 L of dechlorinated water in which temperature was controlled by the same temperature controller, semiconductor and thermocouple used for the CT_{max} trials (Figure 2.2). The system was programmed to pump chilled reservoir water into the test tank to cool it at a rate of 0.3 ºC/min. An airstone vigorously mixed and aerated the water to prevent stratification. Pumping rate varied depending on the differential temperature between the test tank and the reservoir, and increased as that difference decreased.

Test procedure

Trials and behavioral observation recording proceeded as for CT_{max} except the test tank water temperature was decreasing and some fish would stop moving before LOE was reached. In this case, the fish was gently prodded with a rod to initiate movement. The end point for CT_{min} trials was either LOE or lack of response to gentle prodding, whichever came first. At the endpoint (henceforth called LOE), the fish was treated in the same manner as in the CT_{max} experiments.

Critical thermal analyses

For both the CT_{max} and CT_{min} experiments, summary statistics (mean, standard deviation, range) were calculated for temperature at LOE, SL and rate of heating/cooling (°C/min) respectively. A t-test was performed to test whether SL or rate of temperature change differed between motivational groups. A multiple regression with temperature at LOE as the dependent variable was performed to determine whether SL, motivation, or the interaction of the two affected critical thermal temperatures.

Temperature preference

Experimental housing environment

Housing conditions were the same as for the $CT_{min/max}$ experiments except that there were 7 or 8 fish per holding tank and food was not withheld for 24 hours before the trials for the satiated group since the fish were presumably not being subjected to physiological stress that could interact with a full stomach as in the tolerance experiments.

Test apparatus

The experimental apparatus (Figure 2.3) consisted of a horizontal PVC pipe 10 cm in diameter, 3 m in length that was sectioned longitudinally to form a trough with 90° PVC elbows facing up at either end. Water from elevated cold and hot water reservoirs was continuously dripped into opposite ends of the apparatus (into the elbow openings). The cold reservoir was maintained at an average 6 °C via regular addition of refrigerated, dechlorinated water and ice. The hot reservoir was situated on a silicon heater controlled by the same temperature controller system used in the $CT_{min/max}$ experiments and was maintained at 60 °C via addition of dechlorinated water heated in an electric kettle. Airstones at four locations along the gradient prevented vertical stratification. Holes drilled along the length of the gradient near the top allowed excess water to drip out into an overflow pan below the pipe. A webcam (Creative Labs VF0050) above the gradient continuously recorded fish location while an infrared camera (MikroScan 7515 Thermal Imager) was used to monitor and record water temperature throughout the entire length of the gradient. Nine pieces of wet rope were placed equidistant along either side of the apparatus along its length in the overflow drip holes (eighteen pieces total) providing reference points that were visible in both the webcam and infrared images (Figure 2.4).

Preliminary trials were conducted with room temperature (24 °C) water to determine if there was a bias toward fish selecting a particular location in the experimental tank. Five individuals not a part of this study (and thus satiated) were placed into the apparatus one at a time for 20 minutes. Each individual ultimately settled in a particular location, but an ANOVA showed a significant difference between locations chosen by different individuals, indicating no bias toward a particular location or side within the homogenous temperature of the trough ($F=7.98$, $df=6$, $p<0.0001$).

Test trials were run to verify that a wide horizontal temperature gradient approximating the critical thermal range determined by the CT_{min} and CT_{max} experiments could be achieved with this design. Ten digital thermometers (accuracy to 0.1 °C) were placed one in each segment along the gradient and the cold and hot water was dripped in. Three trials were performed, each one lasting between 1 to 2 hours. Mean minimum temperature for all trials was 18.6 °C, and mean maximum temperature was 36.9 °C.

Test procedure

Once a gradient approximately spanning a range of 14 °C – 40 °C was established in the test apparatus (determined by viewing the gradient through the IR camera, which showed the gradient and a legend of the temperature value of each color seen and that closely matched the temperature range achieved in the preliminary tests), the temperature preference trial began by selecting an untested fish from a randomly chosen holding tank. To control for any bias that might exist related to entry point and orientation, test fish were placed into the gradient at one of three entry points (middle, toward the cold end or toward the hot end) and facing either the hot or cold end, alternating through the different positions/directions with each new fish (e.g., the first fish went in at the center of the gradient, facing the hot side, the second at the center facing the cold side, the third at the warm entry point facing the hot side, and so on). The fish was then allowed to acclimate to its new surroundings for 15 minutes with the webcam recording continuously. After 15 minutes, the clock was reset and the test period began with simultaneous webcam and IR pictures taken at 0, 3, 6, 9, 12, 15, 18 and 21 minutes (8 IR pictures total), and the selected location of the fish at each time point was termed its 'preference'. The fish was then removed from the gradient, lightly patted with a paper towel on both sides to remove excess water, weighed on a digital scale and measured (SL), anesthetized and lastly fin-clipped to mark it as tested. Tested fish were returned to their original holding tank once recovered. On any one day, 4-8 fish were tested and the gradient test tank was drained and cleaned. No individual was tested more than once.

Temperature preference analysis

Temperature preference determination

Webcam photographs corresponding to each time point were viewed in Image J V. 1.45s (Abràmoff *et al.*, 2004). The rope-delimited segment the fish was in was noted as was its position within the segment (right, left, center, etc.). The fish's position was then matched to, and the corresponding pixels selected from, its paired IR image using the

Mikrospec 4.0 software by LumaSense Technologies, Inc., and the mean pixel value within the selected area calculated (i.e., water temperature where the fish was located) (Figure 2.4). Preferred temperature for each fish was calculated as the mean of the mean pixel values for each of the 8 recorded locations. For each image, the minimum, maximum and average gradient temperatures (i.e., lowest, highest and mean pixel value in the gradient area) were also noted, and all 8 values of these measures per fish were averaged to produce a mean minimum, mean maximum and grand mean gradient temperature per individual tested, henceforth simply called the 'minimum and maximum and mean' gradient temperatures. A t-test was performed between motivational groups for each measure of gradient temperature to assess whether hungry and satiated fish experienced similar testing environments, and preferred temperature was regressed on each of these variables separately to determine if satiated and hungry fish responded differently to changes in the gradient temperature.

Comparison of motivational states

LeCren's condition factor (a common length-weight relationship used by fisheries managers) (LeCren, 1951) was determined for each motivational state group using the satiated group as the reference group for the predicted weights. A multiple regression was then performed with preferred temperature as the dependent variable and SL, condition and their interaction as the regressors. A log-likelihood ratio test was performed to compare the distributions of preferred temperatures of the two motivational state groups.

All analyses for all experiments were performed using SAS® software (Version 9.2 of the SAS system for Windows) except calculation of predicted masses for LeCren's condition, which were performed in R V 2.15.1 (R Development Core Team, 2011).

RESULTS

The critical limits for *Hemichromis guttatus* were determined to be 14.7 °C – 40.7 °C, and the preferred temperature was 26.5 °C ± 2.7 °C (Table 2.1). Motivational groups (hungry and satiated) were not significantly different from each other in terms of their mean thermal limits or preferences (Table 2.1), but the distribution of temperature preference was significantly different between groups (Figure 2.5). These results are explained in detail below.

Critical thermal maximum

Fifty-five fish with a mean SL of 5.76 cm ± 1.27 cm ranging from 3.2 cm – 8.0 cm were tested (Table 2.1). Mean heating rate was 0.276 ± 0.012 °C/min, and fish became increasingly active as temperatures increased, some even attempting to jump from the test tank. Mean temperature at the time of LOE was 40.7 ± 0.5 °C (Table 2.1), though initial signs of distress (swimming continuously) and jumping, occurred earlier at a mean temperature of 37.1 ± 1.0 ºC and 38.8 ± 0.6 °C respectively. There was no significant difference in SL, heating rate or temperature at LOE between motivational groups (Table 2.1). The multiple regression was significant, explaining 17% of the variation in temperature at LOE and with SL being positively significantly associated with temperature at LOE, while motivation and the interaction of it with SL were not (Table 2.2).

Critical thermal minimum

Fifty-eight fish with a mean SL of 5.78 cm ± 1.09 cm and ranging from 3.7 cm – 8.4 cm were tested (Table 2.1). Mean cooling rate was 0.285 ± 0.006 °C/min, and as trials progressed, the test fish became decreasingly active and eventually rested motionless but with their body vertically oriented on the bottom of the tank. Loss of equilibrium (laying on their side on the ground or being unresponsive to gentle prodding with a rod, whichever came first) occurred at 14.7 ± 0.8 °C (Table 2.1), though initial signs of distress (resting with body orientation other than vertical) occurred on average at 16.0 ± 0.6 °C. There was no significant difference in SL, heating rate or temperature at LOE between motivational groups (Table 2.1), and the multiple regression of temperature at LOE on motivation, SL and their interaction was not significant (Table 2.2).

Temperature Preference

Generally, during the acclimation period fish would move back-and-forth in the gradient when first released, with the distance swum continuously decreasing until finally stopping. Some fish would periodically move within the gradient during the trial period, although most showed great fidelity to a particular location throughout the trial. Fifty-four fish were tested, although, three observations were dropped from analyses as they experienced LOE in the cold end of the gradient during the trials.

A non-linear function best fit the length-weight relationship, and mean condition calculated from this function was 0.999 ± 0.107 for all fish. Mean preferred temperature for all fish was 26.5 ± 2.7 °C (Table 2.1). The multiple regression of temperature preference on motivational state, condition and their interaction was not significant (Table 2.2), however, a log-likelihood ratio test determined the distributions of preferred temperature to be significantly different between the two motivational state groups (Figure 2.5).

The temperature gradient in which fish were tested was consistent. Across all fish tested, the mean gradient temperature was 29.3 ± 1.0 ºC, the minimum gradient temperature was 16.9 ± 1.2 °C and maximum gradient temperature was 39.3 ± 1.5 ºC (Table 2.3), and t-tests showed that all three gradient temperature measures were the same between hungry and satiated groups (Table 2.3). Temperature preference was significantly positively associated with mean and minimum gradient temperatures in the satiated group (28% and 15% of variation explained respectively) and significantly positively associated with mean and maximum gradient temperatures for all fish combined (10% and 8% respectively), but was not significantly associated with any measure of gradient temperature in the hungry group (Figure 2.6).

DISCUSSION

While there is a large literature on species' critical thermal limits across diverse taxa, (Lutterschmidt & Hutchison, 1997, Sunday *et al.*, 2010), relatively few studies have included measures of thermal preference (e.g., Ferguson, 1958), and even fewer have estimated both thermal tolerances and preferences in controlled laboratory conditions (exceptions include Coutant, 1977, Richardson *et al.*, 1994, and Tsuchida, 1995). By confronting these questions experimentally, and by adding an additional level of complexity to our experimental designs - i.e., state of hunger, we were able to reveal more subtle responses to environmental variation than has been revealed by simpler study designs. Synthesizing these results greatly adds to our ability to estimate potential risk of invasion for this fish species, and hence be of more use to those organizations that regulate and manage for invasive species. The results are discussed in more detail below, along with their management and policy implications.

Critical thermal minimum and maximum

Wide physiological tolerance to temperature is a predictor of establishment (Marchetti *et al.*, 2004a) and spread (Kolar & Lodge, 2002, Marchetti *et al.*, 2004b) in invasive fishes. Further, Chapman (2000) noted that tropical fishes are rarely able to tolerate temperature below 18 °C and Kolar and Lodge (2002) found that in Great Lakes fishes, invasives' ability to survive low temperatures was related to their having a negative impact on other species (Kolar & Lodge, 2002). Thus, based on our finding that the CT_{min} for *H. guttatus* was low and the temperature tolerance range determined here was wide, i.e., from 14.7 °C - 40.7 ºC, *H. guttatus* should be considered a potentially dangerous invader.

Our finding of a significant positive association between temperature at LOE and SL for the satiated fish but not the hungry fish in the CT_{max} trials could be explained if the increase in length were associated with a greater relative increase in mass in satiated fish as compared to the hungry fish. Greater mass is accompanied by a longer lag time between when the external environment of the water is heated and the internal environment of the fish experiences this warming (Becker & Genoway, 1979). Peck *et al.* (2009) found the same effect of body size on maximum temperature tolerance in 14 species of marine invertebrates spanning 6 different phyla, suggesting that it may be smaller individuals that would be more likely to establish in the hottest areas in Cuatro Ciénegas.

Areas where water temperatures exceed the tolerable range should be considered unsuitable for this species. Within Cuatro Ciénegas, there are no known aquatic habitats where temperatures reach higher than 40.7 ºC except in a few fishless, hypersaline pools where occasional anomalous temperatures have been observed as high as 47 °C (Cole & Minckley, 1968). Fish in the CT_{max} trials, however, started showing signs of distress (at 37 °C) before losing equilibrium. In the field, the warmest springs with native (or exotic) fishes reach maximum temperatures of 34 °C - 35 °C, including Poza Azul, the warm spring on which our hypothesis is based that temperature is an important factor controlling jewel fish distribution. Less than ten individuals of *H. guttatus* have ever been caught here despite its surface connection to a slightly cooler spring, Mojarral Este, where there is an established population, suggesting that certain sites in the valley are near temperatures unsuitable for this species, thus excluding them.

The lower critical limit of 14.7 ºC does directly exclude *H. guttatus* from a few locations in Cuatro Ciénegas. For example, the National Institute of Ecology and Climate Change (INECC) datalogger on the Río Mesquites has recorded winter temperatures as low as 13.5 °C (INECC 2013), and in our experiments, fish started showing signs of distress at 16.0 ºC during CT_{min} trials, consequently excluding these downstream sites as potential habitat. Overall, however, the tolerable temperature range found in this study does not limit the potential distribution of *H. guttatus* in the valley to a great degree, indicating that temperature preference may be a more important factor than critical thermal limits in determining this species' distribution in Cuatro Ciénegas.

While pH was not measured during the trials, it is known that as temperature increases in neutral water, pH will decrease and vice-versa. Thus, at high temperatures, for example, the internal environment of a fish will become more acidic resulting in increased resting oxygen consumption and decreased maximum oxygen consumption (Munday *et al.*, 2009) and thus potentially decreasing the critical thermal limit breadth. Had pH been maintained at a constant level throughout the trials, the critical limits determined may have been more extreme in both directions. In Cuatro Ciénegas, however, the water is hard (Evans, 2005), which may buffer the pH effect on upper critical limits. Future studies examining the interaction between temperature and pH would illuminate how the two variables act together to affect critical limits and the related potential distribution of *H. guttatus* in the field.

Temperature preference

The mean preferred temperature for all tested fish was 26.5 °C ± 2.7 ºC. Data on water temperatures in West African (Sierra Leone to Cameroon) coastal rivers, where this species is native, are sparse, but mean temperature averaged from monthly data from 1991-1994 in the Pra River in Ghana, was 26.7 ºC and ranged between 21.2 °C – 32.0 ºC (UNEP 2006), well within the tolerated range and nearly equivalent to this study's laboratory-determined mean preferred temperature and preferred temperature range (20 °C – 32 °C). It thus appears that *H. guttatus* in Cuatro Ciénegas is preferring temperatures close to those that its ancestors were adapted to in their native rivers.

Acclimation temperature is known to have an effect on critical thermal temperatures (Beitinger *et al.*, 2000) and preferred temperatures in some fishes (e.g., Kelsch and Neill 1990, Hernandez and Barcenas 1995), though mixed results have been found in preferred temperature for other ectothermic taxa, e.g., a negative effect in fruit flies (i.e., *Drosophila simulans* and *D. melanogaster*) (Krstevska & Hoffmann, 1994), a slight effect in cockroaches (*Periplaneta americana)* (Murphy & Heath, 1983), and a positive effect in abalone (i.e., *Haliotis fulgens* and *H. corrugate*) (Díaz *et al.*, 2006). The acclimation temperature for all three experiments was 26 °C, which is very close to mean annual temperatures for three of four sites where *H. guttatus* exists in abundance in Cuatro Ciénegas: Poza Churince (27 °C), Río Mesquites (26 °C) and Poza Juan Santos (26 °C) (INECC 2013). These populations may thus be living at their final preferendum there, which Fry (1947) defined as the temperature at which the acclimation temperature equals the preferred temperature for an organism and noted that it is a “meaningful directive factor” that could motivate a fish to change location, or in this case, to stay where it is.

One site, Mojarral Este, with an established population of *H. guttatus* has a mean annual temperature that is just outside of the preferred range, 30 °C (INECC 2013). However, the population in this large spring with an approximate area of 15,150 m^2 is much less dense than in Poza Churince (34 caught in Mojarral Este vs. 356 in Poza Churince (approximately 1,000 m^2 (Aguilera González, 1998) on one day of sampling in the summer of 2010; Chapter 3) with a mean annual temperature nearly equivalent to the laboratory-determined mean preferred temperature. Further supporting the importance of temperature preference in *H. guttatus* establishment and spread is the absence of an established population in Poza Azul (less than ten individuals have been caught here), which is connected by surface flow to Mojarral Este, but which has a mean annual temperature of 32.2 °C (INECC 2013), well within the tolerated range, but outside of the preferred range determined in this study.

Although, there was no significant difference in mean preferred temperature between the two groups (hungry and satiated), the distributions of preferred temperatures did differ significantly. This suggests a more complicated response to temperature gradients than can be discerned by mean preference. The satiated group’s distribution was leptokurtic with temperature preferences of the majority of the individuals (81%) lying within one standard deviation of the mean (23.9 °C – 29.2 °C). In contrast, preferences of hungry individuals were spread more evenly across the whole range of preferred temperatures (Figure 2.5). One interpretation of this result is that the satiated fish were displaying their underlying preferences, responding strongly to the temperature gradient and choosing to rest at their preferred temperature, whereas, hungry fish were less motivated to express their temperature preferences and selected their position more randomly, or perhaps based on some other factor such as motivation to feed.

A second set of analyses supports this interpretation. While the mean, minimum and maximum gradient temperatures in each experimental trial were statistically the same for both the hungry and satiated groups, the two groups responded differently to small variations in these measures. Preferred temperatures of the satiated group were positively associated with mean and minimum gradient temperatures, accounting for nearly 28% and 15% of the variation, respectively. The hungry group's preferred temperatures, however, were not associated with any measure of temperature gradient (Figure 2.6). These results suggest that only satiated *H. guttatus* responded strongly to varying temperature gradients between trials.

Temperature preference as a limiting factor to distribution has been suggested in other introduced *Hemichromis* species. The distribution of an unidentified species of *Hemichromis* is apparently controlled by the interaction of critical limits and preferred temperature in Warmbad-Villach, Austria, where it is constrained in a 1 km reach of stream formed from the outflow of a hot spring. Temperatures in this reach remain 24 °C – 29 °C year round (equal to the high risk temperature range identified in our study) and drop sharply upon converging with a cold stream (12 °C) (Petutschnig *et al*. 2008). Likewise, Piazzini (2010) notes an established population of a *Hemichromis* species in Tuscany, Italy that is restricted to the middle section of the warm outflow stream of a hot spring (23.5 °C) with very few individuals (i.e., likely not an established population) downstream in a cooler section outside of this study's preferred temperature range but within critical limits (18.5 °C). Oddly, however, no individuals were found upstream at a site at 27.5 °C, though, two other non-native tropical fishes were documented there. Interactions between the *Hemichromis* species and other exotic biota and the thermal preferences of these fish could potentially be excluding it from this upper reach.

Policy implications

In the United States, importation regulations for live fishes and wildlife are governed by the Lacey Act. The original legislation was written in 1900, although a new bill (H.R. 996, 2013) was proposed in March of 2013 that would close loopholes in the listing process. The Lacey Act gives the US Fish and Wildlife Service the jurisdiction to prohibit import of those species determined to be 'injurious wildlife'. The result is a black list of species not allowed to be transported into the country. Inclusion on that list requires data on "the species' survival capabilities and ability to spread geographically; its impacts on habitats and ecosystems, threatened and endangered species, and human beings and resource-based industries; and resource managers' ability to control and eradicate the species" (USFWS 2007). Seventeen taxa (by species, genus or family) are listed as injurious, amounting to 239 species total. A little over half of these species (148 total) were already established in the United States at the time of their listing, and the prohibition of further importation has not reduced their establishment, spread or impact. However, of the remaining 91 species that had not been previously introduced or established at the time of their listing, none have become established, indicating that actions directed at the transport phase of invasion may be effective (Fowler *et al.*, 2007). The critical thermal limits and temperature preference determined in this study address the "species' survival capabilities and ability to spread" requirement and warrant consideration of this species for listing by the Lacey Act, at least for transport into regions where the water temperatures fall within the high risk range of preferred temperatures (within one standard deviation of mean preferred temperature).

While critical thermal and preferred temperatures are species-specific (Beitinger *et al.*, 2000), in the absence of data for a particular species, it is plausible to suggest that thermal characteristics determined for closely related species from similar native ranges could be used as a proxy when determining invasion risk and corresponding need for regulation in certain regions. As an example, Schofield *et al.* (2010) determined CT_{min} for *H. letourneuxi* (one of four *Hemichromis* species that overlaps with *H. guttatus* in its native range and that has become invasive in the Everglades in Florida) to be 10.8 °C – 12.5 °C (for acclimation temperatures of 24 °C and 28 °C respectively); roughly similar to that determined for *H. guttatus* in this study. Therefore, the results of this study are applicable to other *Hemichromis* species from the same native region and can be used as rough estimates of these other species' tolerable and preferred temperatures when these data are lacking.

Conservation management implications

Van Dijk *et al.* (2002) documented differences in how hungry and satiated individuals of the cyprinid fish *Rutilus rutilus* (common roach) responded to a temperature gradient. In this study, hungry fish preferred warmer temperatures during the day and cooler temperatures at night, presumably motivated by the sensation of hunger to search for higher food densities and conserve energy respectively, resulting in a bimodal distribution of preferred temperatures based on time of day. Conversely, the distribution of satiated fish's preferred temperatures was unimodal and concentrated around the mean. It is possible this response by the satiated fish was due to a preference for the optimal temperature for enzymatic digestion, which is species specific (Hidalgo *et al.*, 1999). In our study, we did not control for time of day, and the optimal temperature for enzymatic digestion is not known for *H. guttatus*, but it is possible that similar mechanisms were driving the pattern we saw of satiated fish having a greater fidelity to the preferred temperature than did the hungry fish. Further, Dill (1983) documented increased searching behavior in hungry coho salmon as well supporting the idea that hungry fish were responding more to the sensation of being hungry, which he describes as a response to a lack of stimulation of stretch receptors in the gut.

Regardless of the underlying mechanism, differences in feeding motivation among fish in the present study appear to have altered the hungry group's behavior in a manner which caused them to explore beyond their preferred temperature, presumably in search of food. Hence, in terms of dispersal of the potential invader into currently uninhabited sites, those individuals that are in poorer condition may be the ones more motivated to travel away from a preferred temperature and so are more likely to extend the range. If, however, there are abundant resources, temperature could then be the determining factor as to whether or not an invasive species were to spread. That is, potentially invasive *H. guttatus* may tend to remain near their preferred temperature of 26.5 º C under conditions of plentiful food availability (assuming all other factors constant), and this behavior may be present in other fishes as well.

Conclusions

Three main conclusions may be drawn from this study. Firstly, while it is highly likely that other factors (biotic and abiotic) contribute to its current and potential distribution, critical thermal limits and temperature preference do appear to play an important role in determining *Hemichromis* spp. distributions globally. These results indicate that water temperatures greater than 40.7 ºC and lower than 14.7 ºC will absolutely exclude establishment of *H. guttatus* and temperatures near 37.1 ºC and 16.0 ºC are highly unlikely to support populations of *H. guttatus*. Additionally, the results indicate that water bodies with temperatures within one standard deviation of mean preferred temperature (23.8 ºC - 29.2 ºC) will have the highest risk of establishment. Secondly, these data could be used in conjunction with the Lacey Act to prevent new introductions into high risk regions for *H. guttatus*, and in the absence of similar data, these values may be used as a proxy for closely related species. Lastly, I suggest that resource-poor environments may promote behaviors that would extend the range of an established population, while fish in environments with abundant resources will be less invasive, that is, more prone to seek out and remain in waters at their preferred temperature. The implications of how this phenomenon could relate to invasion risk warrants additional research to directly test this hypothesis.

Table 2.1: Summary statistics for critical thermal minimum (CT_{min}), critical thermal maximum (CT_{max}) and preferred (Tpref) temperature (ºC) for *Hemichromis guttatus* derived from laboratory experiments. For CT_{min} and CT_{max} experiments (Exp), the variables (Var) standard length (SL, measured in cm), temperature at loss of equilibrium (LOE) and cooling or heating rate (Rate, in °C/min) are shown, and for the temperature preference experiment, LeCren's condition factor (LC), preferred temperature (Pref) and three measures of gradient temperatures, mean (Mean grad), minimum (Min grad) and maximum (Max grad) are shown. In each experiment, fish were randomly assigned to one of two motivational state treatments: hungry or satiated. Comparisons between motivational state treatments were assessed with a t-test, except for non-normally distributed LOE in both the CT_{min} and CT_{max} trials, where a Wilcoxon signed rank test was used.

Exp	**Var**	**Group**	**N**	**Mean ± sd**	**Min**	**Max**	**Comparison by Motivation**		
							df	**t/Z-statistic**	**P > t/Z**
CT_{max}	SL	All	55	5.76 ± 1.27	3.2	8.0	53	0.18	0.875
		Hungry	26	5.79 ± 1.3	3.2	8.0			
		Satiated	29	5.73 ± 1.27	3.5	7.6			
	LOE	All	55	40.6 ± 0.5	38.1	41.4	N/A	-0.308	0.761
		Hungry	26	40.6 ± 0.6	38.1	41.3			
		Satiated	29	40.7 ± 0.5	39.8	41.4			
	Rate	All	55	0.276 ± 0.012	0.246	0.306	53	1.72	0.092
		Hungry	26	0.279 ± 0.010	0.260	0.298			
		Satiated	29	0.274 ± 0.013	0.246	0.306			
CT_{min}	SL	All	58	5.78 ± 1.09	3.7	8.4	56	-0.75	0.459

Table 2.1, continued

Exp	**Var**	**Group**	**N**	**Mean ± sd**	**Min**	**Max**	**Comparison by Motivation**		
							df	**t/Z-statistic**	**P > t/Z**
CT_{min}		Hungry	29	5.67 ± 1.05	3.7	7.2			
		Satiated	29	5.88 ± 1.13	4.2	8.4			
	LOE	All	58	14.7 ± 0.8	13.1	16.6	N/A	-1.114	0.265
		Hungry	29	14.8 ± 0.8	13.8	16.6			
		Satiated	29	14.5 ± 0.7	13.1	15.9			
	Rate	All	58	0.285 ± 0.006	0.263	0.303	56	0.110	0.912
		Hungry	29	0.285 ± 0.007	0.263	0.298			
		Satiated	29	0.285 ± 0.005	0.278	0.303			
Tpref	LC	All	51	0.999 ± 0.107	0.817	1.261	49	-0.93	0.360
		Hungry	24	0.985 ± 0.106	0.817	1.231			
		Satiated	27	1.101 ± 0.108	0.849	1.3			
	Pref	All	51	26.5 ± 2.7	20.4	32.7	49	-0.44	0.662
		Hungry	24	26.6 ± 3.0	21.1	30.6			
		Satiated	27	26.9 ± 2.5	20.4	32.7			
	Mean grad	All	51	29.3 ± 1.0	27.6	31.3	49	0.87	0.387
		Hungry	24	29.5 ± 0.8	27.9	31.1			

Table 2.1, continued

Exp	**Var**	**Group**	**N**	**Mean ± sd**	**Min**	**Max**	**Comparison by Motivation**		
							df	**t/Z-statistic**	**P > t/Z**
Tpref		Satiated	27	29.3 ± 1.1	27.6	31.3			
	Min grad	All	51	16.9 ± 1.2	14.1	19.3	49	-0.06	0.956
		Hungry	24	17.0 ± 1.2	14.1	19.0			
		Satiated	27	17.0 ± 1.2	14.8	19.3			
	Max grad	All	51	39.3 ± 1.5	35.5	41.9	49	1.6	0.115
		Hungry	24	39.6 ± 1.4	36.8	41.9			
		Satiated	27	39.0 ± 1.5	35.5	41.6			

Table 2.2**:** Multiple regression analyses for critical thermal minimum (CT_{min}), critical thermal maximum (CT_{max}) and preferred temperature (Tpref) for *Hemichromis guttatus* derived from laboratory experiments. Individual *H. guttatus* of a range of sizes (standard length, SL in cm) were randomly assigned to one of two motivational (Mot) states: hungry or satiated, and their minimum or maximum thermal tolerance or preferred temperature was determined. Minimum or maximum critical thermal temperature was regressed on SL and motivational state, and temperature preference was regressed on LeCren's condition factor (LC) and motivational state.

Experiment	Var	Coefficient	SE	df	t	p
CT_{min}	Intercept	14.696	0.751	1	19.92	<0.0001
	SL	-0.075	0.126	1	-0.60	0.553
	Motivation	-0.105	1.080	1	-0.10	0.923
	SL*Mot	0.069	0.184	1	0.37	0.710
			Model $R^2 = 0.047$, p = 0.451			
CT_{max}	Intercept	39.439	0.431	1	91.58	<0.0001
	SL	0.211	0.073	1	2.87	0.006*
	Motivation	0.505	0.621	1	0.81	0.420
	SL*Mot	-0.098	0.105	1	-0.93	0.355
			Model $R^2 = 0.173$, p = 0.021*			
Tpref	Intercept	22.355	5.096	1	4.39	<0.0001
	LC	4.495	5.005	1	0.90	0.374
	Motivation	2.990	7.401	1	0.40	0.688
	LC*Mot	-3.252	7.378	1	-0.44	0.661
			Model $R^2 = 0.022$, p = 0.790			

* Denotes a significant result at $\alpha = 0.05$.

Table 2.3: Linear regressions of preferred temperature on 3 measures of gradient temperatures: mean, minimum and maximum for each fish. Analyses were performed for all fish combined and for the subgroups hungry and satiated. Significant associations were found only for the satiated fish, not for the hungry fish suggesting that the satiated fish are responding more to their abiotic (thermal) environment, whereas hungry fish are responding to something else such as, perhaps, the sensation of being hungry.

Gradient Measure	Group	R^2	p
Mean	All	0.099	0.024*
	Hungry	0.097	0.624
	Satiated	0.278	0.005*
Minimum	All	0.021	0.314
	Hungry	0.008	0.672
	Satiated	0.150	0.046*
Maximum	All	0.077	0.049*
	Hungry	0.07	0.211
	Satiated	0.113	0.086

* Denotes a significant result.

Figure 2.1: Location of the Cuatro Ciénegas spring system (★) in the state of Coahuila in northern México.

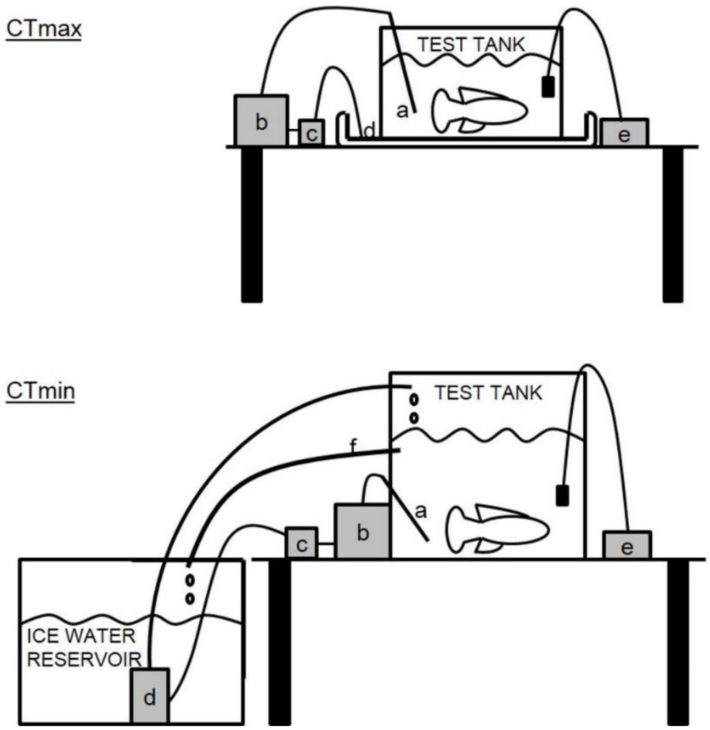


Figure 2.2: Critical thermal tolerance limits experimental design. Above: The experimental design for the critical thermal maximum trials. A (a) thermocouple sensor detected the water temperature in the stainless steel test tank and sent the information to the (b) temperature controller, which sent a signal to the (c) semiconductor that acted as a switch turning on or off the (d) silicon heater such that the water temperature was increased at a rate of 0.3 °C/min. An (e) air pump and stone were used to prevent stratification and aerate the test tank water. Below: The experimental design for the critical thermal minimum trials. The same (a) thermocouple, (b) temperature controller, (c) semiconductor and (e) air pump and stone were used, but in this case, the (b) temperature controller sent a signal to a (d) pump in an ice water reservoir that pumped cold water into the test tank to achieve a decrease in temperature of 0.3 °C/min. Excess water from the test tank was returned through (f) tubing to the reservoir where it was re-cooled.

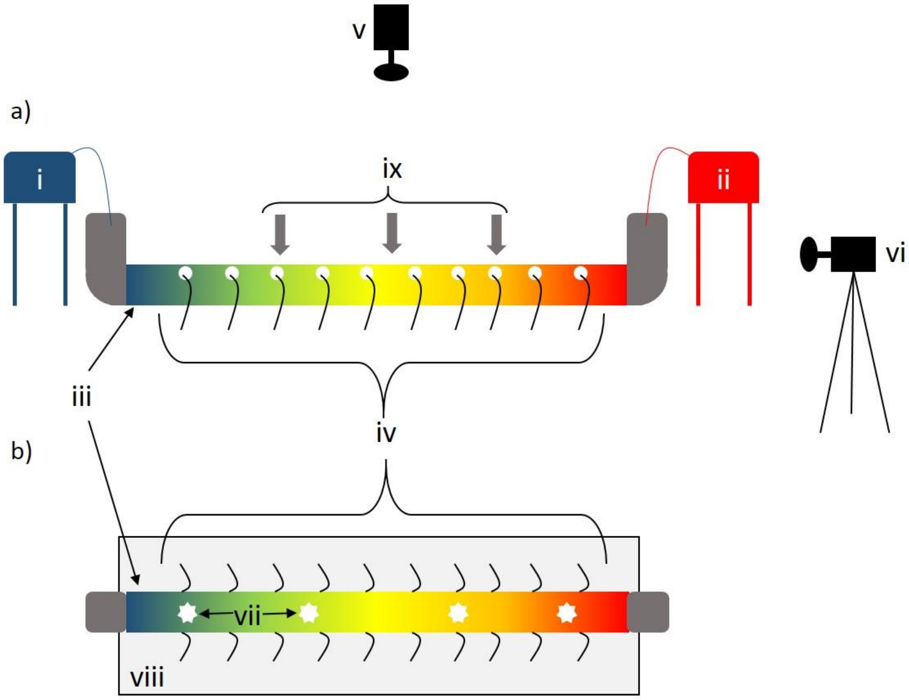


Figure 2.3: Temperature preference experimental design. a) Side and b) overhead view of the apparatus. Chilled and heated water in i) cold and ii) warm water reservoirs respectively was dripped into opposite ends of a iii) longitudinally cut piece of PVC pipe with iv) drain holes cut along its length and pieces of rope placed in the holes that acted as segment markers in v) webcam and vi) infrared camera images. Four vii) airstones were placed in the pipe to mix water preventing stratification and also aerating it. A viii) drip pan was placed below the apparatus to catch water as it spilled through the iv) holes. Fish were entered into the apparatus at one of three ix) entry points: cold, intermediate and warm.

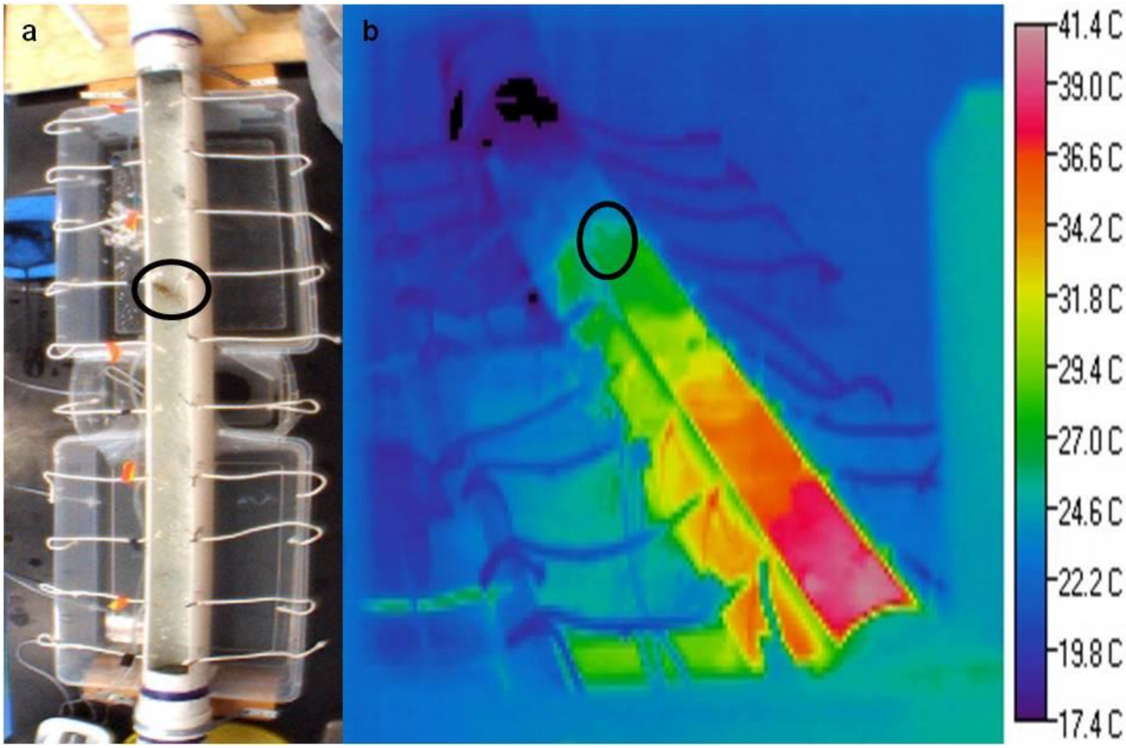


Figure 2.4: Representative webcam and corresponding IR camera images from a temperature preference trial. (a) The webcam picture shows the pieces of rope used to mark segments in the horizontal temperature gradient, and a circle shows the location of a fish in the apparatus. (b) The corresponding IR camera image shows the temperature gradient that was achieved. The pieces of rope from the webcam image can be seen here and were used to match corresponding segments and identify the location of the fish (circled area). In Mikrospec 4.0, a rectangle was drawn around the approximate location of the fish in the IR image and the mean pixel value (temperature) in the rectangle was calculated and used as the temperature for a particular fish at a specific time point. There were 8 such images per fish (at 0, 3, 6, 9, 12, 15, 18 and 21 minutes). The preferred temperature of a fish was the mean of these 8 images. The overall temperature preference was the grand mean of all the individual fish's preferred temperatures.

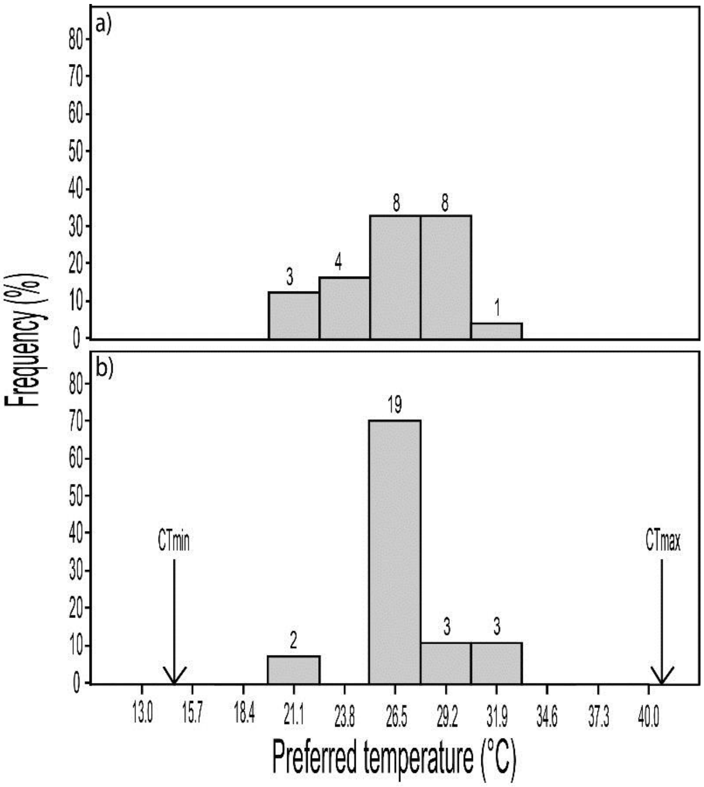


Figure 2.5: Distribution of preferred temperatures for (a) hungry and (b) satiated *Hemichromis guttatus*. The bins represent standard deviations above and below the mean preferred temperature for all fish combined, which was 26.5 ± 2.7 °C. Numbers above the bars are counts. The two distributions are significantly different as was shown by a log-likelihood chi-square test ($X^2 = 13.59$, $df = 4$, $p = 0.009$). Critical thermal minimum (CT_{min}, 14.7 ± 0.8 °C) and maximum (CT_{max}, 40.7 ± 0.5 °C) are noted for comparison of the preferred range to the critical limits.

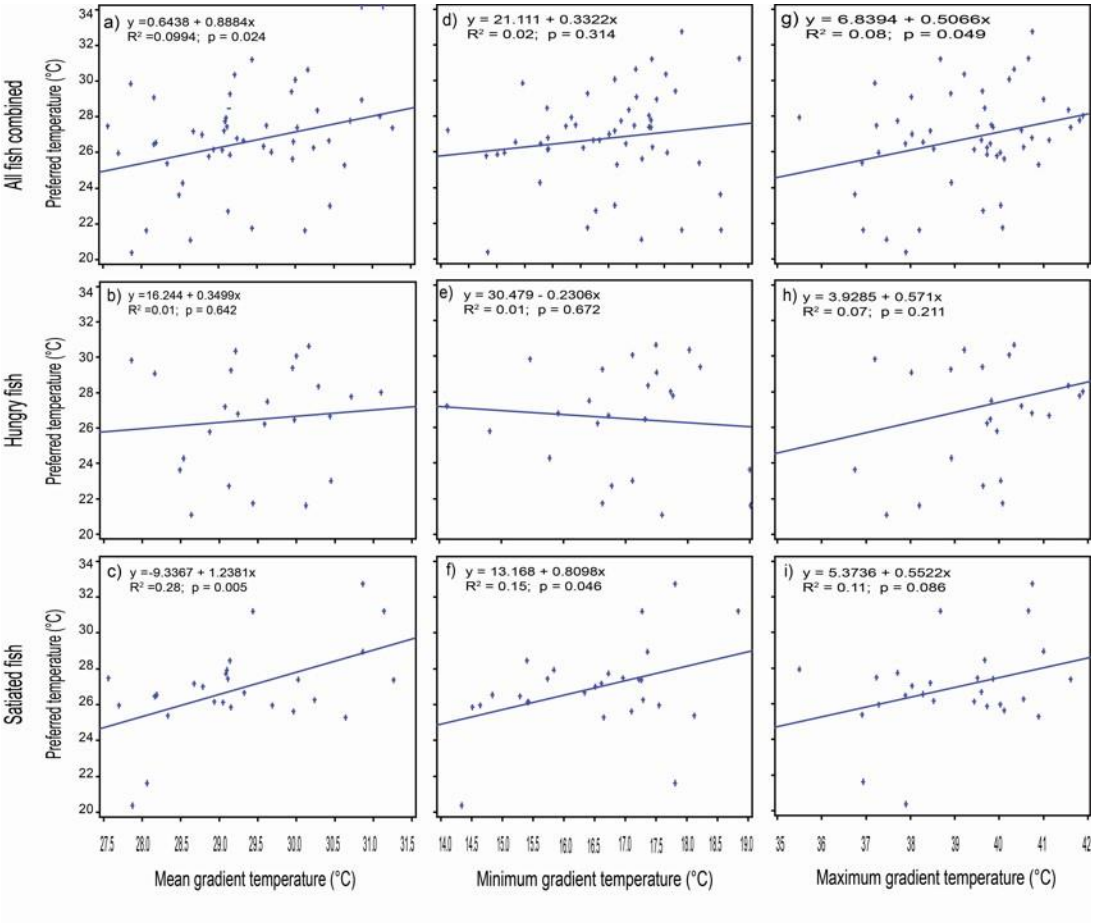


Figure 2.6: Linear regression of temperature preference on three measures of gradient temperature (mean: a-c, minimum: d-f and maximum: g-i) for all fish combined (first row), hungry fish (second row) and satiated fish (third row). Dots are individual observations, and the best fit line is overlain. There was a significant positive association between preferred temperature and mean and maximum gradient temperature for all fish combined and for mean and minimum gradient temperature for the satiated fish, but not the hungry fish.

Chapter 3: Predicting invasion risk of an exotic aquarium trade fish in a desert spring system

INTRODUCTION

One of the major issues currently under study in invasion biology is how to predict invasions. Much work has been done to identify characteristics of species that make them good invaders. Characteristics such as broad physiological tolerances (Kolar & Lodge, 2002, Moyle & Marchetti, 2006), high propagule pressure (Sax & Brown, 2000), history of prior invasion (Rejmánek & Richardson, 1996), commensal symbioses with or desirability by humans (Sax & Brown, 2000, Moyle & Marchetti, 2006), and taxonomic relatedness to a known invader (Lockwood, 1999) have repeatedly been shown to increase the probability that a species will become established and potentially invasive.

The West African jewel cichlids (genus *Hemichromis*) are a group of fishes that have many of these characteristics. All ten species are popular aquarium trade fishes (desirable by humans) and those that have had their physiological limits studied have been shown to have wide tolerances to salinity (*H. letourneuxi*, Langston et al. 2010) , temperature (*H. letourneuxi*, Schofield et al. 2010; *H. guttatus*, Chapter 1) and hypoxia (*H. letourneuxi*, Schofield et al. 2007). Two species have become invasive, one in Canada (*H. bimaculatus*, Hornung and Pacas 2006) and another in the Everglades of south Florida, USA (*H. letourneuxi*, Schofield et al. 2013). A third, *H. guttatus*, has been introduced in México and though detailed censuses of native populations have not yet been done, this species has been shown to have potential negative impacts on native fishes through dietary overlap, competition (Marks *et al.*, 2011) and potential inhibition of reproduction (see

Chapter 4). Elsewhere, several other congeners have been introduced including in the United States (*H. elongatus* in Hawaii, Eldredge 2000), Italy (*Hemichromis* sp. in Tuscany, Piazzini *et al.*, 2010), Austria (*H. bimaculatus* and *H. fasciatus*, Rabitsch and Essl 2006) and Australia (*H. guttatus*, Koehn and MacKenzie 2004; *H. bimaculatus*, Olden *et al.* 2008; *H. lifalili*, Webb 2008), though, studies of their potential impacts has not yet been conducted. *Hemichromis guttatus*, introduced into a biodiversity hotspot, Cuatro Ciénegas (Abell *et al.*, 2000) in northern Mexico and potentially in the early stages of invasion there, provides an opportunity to study an invasion in progress and to make predictions and subsequently test these predictions of where it will establish, spread and integrate based on characteristics of the environment.

Cuatro Ciénegas is an oasis in the Chihuahua Desert that has been compared to the Galapagos Islands due to its high level of endemism (Dinerstein *et al.*, 2000). This World Wildlife Fund conservation priority (Abell *et al.*, 2000), UNESCO biosphere preserve (UNESCO, 2010), RAMSAR wetland of international importance (RAMSAR, 2013), and federally-protected reserve for flora and fauna (Secretaría de Desarrollo Social, 1994) consists of an intermontane valley, nestled amongst the Sierra Madre Oriental. The valley contains hundreds of springs, or "pozas" as they are locally called, and other aquatic habitats including rivers, evaporative lakes, streams, man-made canals and marshes (or ciénegas, for which the valley was named) (Calegari, 1997). There are 72 endemic species reported from here, the highest density of endemics in the world (Abell *et al.*, 2000), 10 of which are fishes (of the total 21 species of fishes present in the valley). Eleven of the fish species present here are listed as threatened or endangered by the Mexican Federal

Government by the NOM-059-ECOL-1994 (Secretaría de Desarrollo Social, 1994), and the presence of exotics is listed as one of the factors contributing to this status in all but one of these species (Contreras-Balderas *et al.*, 2003). Anecdotal evidence from local landowners suggests declines in populations of native fishes following the arrival of exotic *H. guttatus* to springs on their properties. More formal evidence of negative impacts by this fish have been shown by Marks *et al.* (2011) who through stable isotope analysis found potential dietary overlap between *H. guttatus* and two threatened, endemic fishes, the Cuatro Ciénegas cichlid (*Herichthys minckleyi*) and the two-line pupfish (*Cyprinodon bifasciatus*). Additionally, Marks *et al.* (2011) conducted *in situ* field enclosure experiments that showed a greater impact (in terms of lesser growth) on juveniles of *H. minckleyi* when in interspecific competition with *H. guttatus* than in intraspecific competition suggesting competitive exclusion of the native fish as a potential outcome of the interaction of these two species. Further, in Chapter 4, I present data suggesting potential negative impacts of *H. guttatus* on *H. minckleyi*, including possible inhibition of reproduction and behavioral changes induced by aggressive interactions with the jewel cichlid along with alterations to the macroinvertebrate community when the exotic is present.

Several pairs of *H. guttatus* were first discovered in the Cuatro Ciénegas valley in 1996 (Contreras-Balderas & Ludlow, 2003), localized in a single spring (Poza Churince). Since that time, other populations have been detected, including one in a river (Río Mesquites, Figure 3.1) that covers a large area and is connected to many tributaries and springs, yet there are still many areas where this exotic is not present. In this study, I conducted a valley-wide survey of fishes and collected environmental data to attempt to identify biotic and/or abiotic characteristics of the environment that predict *H. guttatus* presence. Observations of established populations of *H. guttatus* in an intermediate temperature spring and lack thereof in a connected but warmer spring generated the hypothesis that temperature is an important factor determining *H. guttatus* presence. Likewise, observations of *H. guttatus* presence in vegetated areas and its rarity in open areas generated a second hypothesis that this fish's presence is associated with vegetation. I thus sought to determine whether temperature and vegetation are indeed good predictors of jewel cichlid presence and to identify other potential important predictors of invasion risk that could be used to aid management of this exotic in Cuatro Ciénegas and elsewhere.

METHODS

Study site

The Cuatro Ciénegas valley is an oasis in the Chihuahua Desert situated within the Sierra Madre Oriental Mountains in the Mexican state of Coahuila (Figure 3.1). It occupies just under 1500 km^2 and sits at 740 m elevation. Though it receives less than 200 mm rainfall per year on average (Minckley, 1969), there are many aquatic habitats found here fed primarily by discharge from the Cupido-Aurora aquifer, as well as some run-off from the surrounding mountains (Johanneson *et al.*, 2004). There are more than 500 springs and other water bodies in the valley that have varying levels of connectivity ranging from some small, isolated springs to one relatively large river system with many tributaries and associated springs (Carson & Dowling, 2006). For the purpose of this study, a poza is defined as a habitat that directly receives its water from an underground spring, a pool is a body of still water typically intermediate in size between springs and lakes, a lake is a large body of still water that is the terminal point for a hydrological system, a stream is a narrow channel of moving water typically flowing out of or into a poza, pool or lake, and a river is a larger channel of moving water fed by springs and streams. Minckley (1969) delineated 7 separate, isolated hydrologic systems based on visual inspection of the land surface (i.e., Churince, El Garabatal, Río Mesquites, Puente Chiquito, Tío Candido-Escobedo, Santa Tecla and Río Salado), 6 that reside in the valley and the 7th (Río Salado) that lies just outside the valley in the northeast. In a study using water chemistry, however, Evans (2005) determined there to be 5 separate, isolated hydrologic systems (Figure 3.1): Churince (the same as Minckley), Río Mesquites (which combined the Río Garabatal, Escobedo and Río Mesquites systems from Minckley's delineation), Tío Candido-Los Hundidos (different from Minckley), Santa Tecla (the same as Minckley) and Anteojo (not delineated by Minckley).

Data collection

Samples were obtained from all five discrete hydrologic systems as delineated by Evans (2005). Within each system, one or more sites were sampled for a total of twenty-two sites and were chosen based on the following criteria: known locations of *H. guttatus*, locations with known or potential surface connections to known *H. guttatus* sites, and sites with high human use. Across all sites, wire-mesh funneled minnow traps were set at a total of 251 locations. Trap locations were not standardized across sites because the various types of water bodies (pozas, pools, evaporative lakes, streams and rivers) called for different sampling approaches. Further, within those sites that were of the same water body type, there was a lot of heterogeneity in terms of terrain and accessibility for moving along/through them, and for these reasons the number of trap locations at each site varied as well (Table 3.1). Some generalities can be made for specific habitat types, however, including the following: i) for rivers and streams, the number of trap locations and distance between them depended on a combination of how quickly the bank could be traversed (or in certain cases, how easily the river could be kayaked) and the maximum number of trap locations that could be set with enough time to return to the first location within 1-2 hours to check for *H. guttatus* (varying between 5-12 traps and 30-75 m respectively, Table 3.1), ii) for springs, 5-6 locations per hour were sampled from the perimeter at 15 – 45 m intervals depending on the size of the spring (with trap locations more closely spaced in smaller springs and vice-versa) as well as haphazardly throughout the body of the spring, and iii) for lakes and pools, 5-6 locations per hour were sampled at regular intervals in a line away from the edge of the lake to 1/3-1/2 the diameter of the habitat. Most sites were sampled over the course of one day (approximately 8:00 AM – 6:00 PM), though, two sites, Churince stream and the Río Mesquites, were sampled over the course of 3 - 4 days respectively. The decision of where to sample on a specific day was not standardized and often depended on local field assistants' availability.

Each trap was baited with dry dog food and left for 1-2 hours. At each trap location, measurements of pH, temperature and salinity were taken with a Hydrolab data logger every 2 minutes for 10 minutes, depth was measured, vegetation presence and type and all other fish species seen in the area as well as the number and species of every fish trapped were noted. For vegetation, traps directly on or within one trap length of vegetation (an arbitrary designation) were given a '1' and traps further than one trap length from vegetation were given a '0', and the type of vegetation (*Nymphaea ampla*, *Chara* sp., species with grass-like architecture (e.g., *Eleocharis* sp. and *Typha dominguensis*), other or none) was noted. While abundant rains had occurred just prior to the start of this study (possibly influencing water chemistry data), no rains fell during the time of actual data collection.

After the allotted time, traps were checked and *H. guttatus* and any other fish captured were enumerated before the traps were reset further down/around the habitat being sampled. All *H. guttatus* caught were preserved and deposited in the ichthyology collection at the Universidad Autónoma de Nuevo León in Monterrey, Nuevo León, and all native fishes caught were released at their capture location. All data were collected between July 20 – August 18, 2010.

Analysis

All environmental measurements per trap set except depth (measured only once) were averaged to give one average value of pH, temperature and salinity per trap location.

Grand means for temperature and salinity (henceforth called mean temperature and salinity respectively), mean depth and median pH was then calculated for all twenty-two sites. Additionally, a chi-square tests was performed to determine whether *H. guttatus* presence was related to the type of vegetation present. Post-hoc analysis was conducted to determine which cells were driving this pattern by calculating the adjusted standardized residuals (ASR) for each cell, which returns the number of standard deviations away from the mean an observed count is. Those cells with an ASR > |2| were considered significant. ASR was calculated as follows:

$$\text{ASR} = \frac{O_{ij}-E_{ij}}{\sqrt{E_{ij}*p_i*p_j}},$$

where O_{ij} is the observed value in row *i*, column *j*, E_{ij} is the expected value in row *i*, column *j*, p_i is the row *i* marginal probability and p_j is the column marginal probability.

Model Building

Five different datasets were created from the data collected at each trap location, and are as follows: i) 'all sites', which includes data from all trap locations except from San José de Anteojo due to the high possibility of obtaining false negatives there as an established population of *H. guttatus* once persisted there, but has since been eradicated (de Lourdes Lozano-Vilano *et al.*, 2006); ii) 'all sites w/o LS', which is the 'all sites' dataset minus data from Las Salinas, a hypersaline and fishless pool with salinities more than an order of magnitude greater than anywhere else in the valley; iii) 'systems', which includes the subset of data from sites within those hydrologic systems where at least one *H. guttatus* was caught; iv) ‘systems w/o LS’, which is the ‘systems’ dataset minus data from Las Salinas; and v) ‘Hg sites’, which is the subset of data from those sites where at least one *H. guttatus* was caught. Logistic regression analysis was performed separately on each of these five datasets (described below).

Logistic regression was performed to determine which factors, if any, predict the presence of *H. guttatus* in a trap and was preferred over discriminant analysis or other such analyses because it is a predictive test yet does not make any assumptions about linearity, normality or homogeneity of variances of the independent variables. It is also robust to differences in sample size (Sokal & Rohlf, 1995). Potential variables to be used in the models were the 4 measured quantities (temperature, pH, depth and salinity), their squared variants and the presence of vegetation for a total of 9 possible variables. Squared terms were used to detect potential non-linear relationships between the quantitative environmental characteristics and *H. guttatus* presence and were created by first centering the raw variable (by subtracting the mean from each observation), and then squaring that term. A Pearson correlation test was conducted on the 9 variables, and salinity and salinity centered and squared (salinity^2) as well as depth and depth centered and squared (depth^2) were highly correlated, thus the squared terms were dropped and only the raw values used. Raw temperature and pH were not highly correlated with their centered and squared variants (temperature^2 and pH^2 respectively) and so these squared terms were kept as potential variables to be entered into the model. Squared terms were never entered into a model without their un-squared counterpart. Other fish species caught was not used as a variable in the model because a) the type of minnow trap used, while very efficient at trapping *H. guttatus*, excludes larger species and more trap-shy species from entering and would thus bias the results and b) the sample size of most of the other fish species caught was too small to provide usable information on the relationship between their presence and *H. guttatus* presence. The number of observations (n = 242 trap locations) allowed for enough statistical power for up to 5 variables to be entered into a model (Hosmer & Lemeshow, 2000), and all possible combinations of the 7 variables (with 1, 2, 3, 4 or 5 variables) were run.

Model Selection

Akaike's Information Criteria (AIC) was used to determine which combination of 5 or fewer variables produced the best model fit. AIC as a model selection statistic was used because it both maximizes model fit while discounting for the number of parameters in the model (Wagenmakers & Farrell, 2004). Once AIC scores were obtained, Akaike weights (relative likelihood of the model) were calculated to determine relative performance of each model compared to the best model, and then used to calculate evidence ratios (a ratio of Akaike weights). Difference in AIC score between a given model and the best model (Δ_i) was calculated for each model and used to identify the subset of best models. Substantially supported models have $\Delta_i \leq 2$ and moderately supported models have $2 \geq \Delta_i \leq 4$ (Burnham & Anderson, 2004). Akaike weights of the parameters for the set of best models, determined as those models which were within 10% of the Akaike weight of the best model (Wagenmakers & Farrell, 2004) for all analyses were also calculated. All analyses were performed in SAS 9.2 (Version 9.2 of the SAS system for Windows).

Prediction of Invasion Risk in Cuatro Ciénegas

Probabilities of presence of *H. guttatus* from the logistic regression analyses were used to determine invasion risk of the exotic in sampled sites in Cuatro Ciénegas. Values of the quantitative variables were arbitrarily categorized as low risk (< 0.5 probability of presence, or less than expected by random), moderate risk (0.5 to 0.75 probability of presence) and high risk (> 0.75 probability of presence). To determine the risk category of the unsquared terms, the raw values of that variable were used. To determine the risk category of squared terms, the process of producing the centered and squared term was reversed, e.g., if the temperature^2 plot values relating to a probability of presence > 0.75 was 4 and mean temperature overall for all the data used in that analysis was 28.0 °C, the temperatures relating to a potential high risk of invasion were 28.0 °C $\pm \sqrt{4}$ or 26.0 °C – 30.0 °C. Based on the results of the models, vegetation was found to be a highly significant predictor of *H. guttatus* presence, thus invasion risk based on vegetation was qualitatively assigned by inspecting photos and from personal observation of the sites and was classified as follows: i) a high risk ranking was assigned to those sites with vegetation throughout the site, ii) a moderate risk was assigned to those sites with spotty vegetation or only vegetation around the edges, and iii) a low risk was assigned to those sites with little to no vegetation.

Overall invasion risk was assigned by synthesizing the individual variable risk rankings for each site.

RESULTS

Sampling data

Two hundred and fifty-one trap locations were sampled in twenty-two sites covering all five hydrologic systems. The locations sampled consisted of 10 springs (pozas), 2 pools, 2 lakes, 5 streams and 3 rivers (Table 3.1). Overall, 11 fish species were caught including 818 *H. guttatus* individuals, which were taken from two hydrologic systems (Churince and Río Mesquites) at seven sites (Poza Churince, Churince stream, Poza Juan Santos, Poza Azul (previously called Mojarral Oeste), Mojarral Este, Río Mesquites, and Los Remojos, Table 3.2). No *H. guttatus* were recovered from San José de Anteojo, the spring where an established population had existed but was eradicated, thus, the data from the 9 trap locations from this site were left out of all analyses to avoid including probable false negatives since past conditions are known to have been suitable for *H. guttatus* presence. Thus, the 'all sites' dataset included 242 trap locations total in twenty-two sites with 56 traps catching and 186 not catching *H. guttatus*, and in the 'all sites w/o LS' dataset, there were 237 trap locations total in twenty-one sites with 56 traps catching and 181 not catching *H. guttatus*. In the 'systems' dataset, there were 205 traps total in fourteen sites with 56 traps catching and 149 not catching *H. guttatus*, and the 'systems w/o LS' dataset, there were 200 trap locations total in thirteen sites with 56 traps catching and 144 not catching *H. guttatus*. Lastly, in the 'Hg sites' dataset, there were 144 trap locations total in seven sites with 56 traps catching and 88 not catching *H. guttatus* (Table 3.3).

Environmental data

Temperature, pH, depth and salinity varied widely among sites (Table 3.1), though they varied only slightly between trap locations where *H. guttatus* was and was not caught (Table 3.3), however certain combinations of variables were better than others at predicting presence of *H. guttatus* as shown by the logistic regression model results (see below). The aquatic vegetation encountered included *Chara* sp., *Eleocharis* sp., *Nymphaea ampla, Typha dominguensis* and *Bacopa monnieri,* and *H. guttatus* presence varied significantly between different vegetation types ($X^2 = 26.16$, $df = 4$, $p < 0.001$) (Figure 3.2). The adjusted standardized residuals (ASR) showed that *H. guttatus* was present significantly more often than expected in grass-like vegetation (*Eleocharis* sp. and *Typha dominguensis*) and *Chara* sp. (ASR = -6.28 and -15.29 respectively), less often than expected by random in 'other' vegetation types and when there was no vegetation present (ASR = 3.06 and 3.98 respectively), and as often as expected in *Nymphaea ampla* (ASR = -0.80).

The values used to determine invasion risk classification varied among the five analyses and can be seen in Table 3.4

Model results

All sites with and without Las Salinas

The final logistic regression model for the 'all sites' analysis that minimized the AIC score included pH, pH^2, depth, salinity and vegetation (Table 3.5), predicted *H. guttatus* presence correctly 80.1% of the time and was relatively 36.8% likely to be the best model (Table 3.6). In this model, probability of *H. guttatus* presence was negatively associated with pH^2 indicating a concave quadratic curve such that as the difference in pH from the mean pH determined in this study (8.17) increased, probability of *H. guttatus* presence decreased. Probability of presence also decreased with increasing depth and salinity, and increased with vegetation presence.

Two other models had substantial support based on difference in AIC score (Δi) from the best model ($\Delta i \leq 2$, Burnham & Anderson, 2004). The second best model included pH, pH^2, depth and vegetation and had a 24.8% chance of being the best model. The third best model was the same as the best but replaced salinity with temperature and had a 20.6% chance of being the best model (Table 3.6). A fourth model had moderate support ($2 \leq \Delta i \leq 4$, (Burnham & Anderson, 2004); 11.9% likely to be the best model) and included temperature, temperature^2, pH, pH^2 and vegetation. All models identified pH^2 and vegetation as significantly affecting probability of *H. guttatus* presence, and the fourth model also identified temperature^2 as significant predictor.

Depth and salinity were not significantly related to the probability of presence of *H. guttatus*, though, depth was included in the top three models, and removing depth from the best model resulted in one with only a 0.5% chance of being the best model (though removing salinity did not greatly decrease model performance, see above). The model with just the variables common to the best three models, pH, pH^2 and vegetation, was only 0.3% as probable of being the best model (Table 3.7, model 32). The Akaike weights of parameters, which denote relative importance of the parameters, suggest that pH, pH^2, depth and vegetation are relatively the most important variables affecting probability of *H. guttatus* presence (Table 3.8).

Salinities at one site, Las Salinas, were an order of magnitude greater than at any other site (Table 3.1) and no fish species were seen or caught in this site, so the analysis was repeated with this site removed ('all sites w/o LS'), and one model was clearly the best (75.1% probability of being the top model) that included temperature, $temperature^2$, pH, pH^2 and vegetation (Table 3.6). In this model, probability of presence was significantly negatively associated with $temperature^2$ and pH^2 and significantly positively associated with vegetation presence (Table 3.5). No other models in this analysis had high support. All models tested and their relative Akaike weights can be seen in Table 3.7.

The invasion risk map for the 'all sites' analysis is the most divergent from all other maps (Figure 3.4). Comparing the 'all sites' and 'all sites w/o LS' analyses, the 'all sites' analysis had fewer moderate risk sites than the 'all sites w/o LS' analysis, and the two sites where *H. guttatus* is present in the highest densities (Poza Churince and Churince stream) were classified by the 'all sites' model as moderate and high respectively, whereas they were both high in the 'all sites w/o LS' analysis (Table 3.9).

Systems with and without Las Salinas

The final logistic regression model for the systems analysis that minimized AIC score included temperature, temperature^2, pH, pH^2 and presence of vegetation (Table 3.10), predicting *H. guttatus* presence correctly 82.5% of the time and having a 48% chance of being the best model (Table 3.6). A negative relationship between temperature^2 and pH^2 with probability of presence indicates a concave quadratic association between these two variables similar to what was found between pH^2 and probability of presence in the 'all sites' analysis. Probability of presence increased with the presence of vegetation. Temperature, pH^2 and vegetation were all significant predictors of *H. guttatus* presence. The Akaike weights of parameters calculated for this model suggest that temperature, temperature^2, pH, pH^2, and vegetation are relatively the most important variables affecting probability of presence (Table 3.8). A second model had substantial support ($\Delta_i = 1.293$) and contained temperature, temperature^2, salinity and vegetation, and a third model had moderate support ($\Delta_i = 2.588$) with temperature, temperature^2, depth, salinity and vegetation (Table 3.6). In both models, temperature^2, salinity and vegetation were significant predictors. The same results were obtained when data from Las Salinas were removed ('systems w/o LS') except for the prediction of Las Salinas itself (Table 3.9), and the best model from the two 'systems' analyses and the 'all sites w/o LS' analysis was the same. All models tested and their relative Akaike weights can be seen in Table 3.7.

The resulting invasion risk maps were similar to that for the 'all sites w/o LS' analysis except for five sites (Figure 3.4). In the two 'systems' analyses, Tierra Blanca, Puente Dos Cuates and Escobedo stream were all classified with a lower level of invasion risk based on the logistic regression output while the Río Mesquites and Río Mesquites downstream were both classified as having a higher risk (Table 3.9).

Hg sites

The logistic regression model for the Hg sites that minimized the AIC score included temperature, temperature^2, pH, depth and presence of vegetation (Table 3.11), predicted *H. guttatus* presence correctly 81.1% of the time and had a 61% chance of being the best model (Table 3.6). Temperature^2 was negatively associated with probability of presence, again indicating a concave quadratic relationship (Figure 3.3). Probability of presence also decreased with increasing pH and depth and increased with vegetation presence (Figure 3.3). One other model had moderate support ($\Delta_i = 2.695$) with a 16% chance of being the best model and included temperature, temperature^2, pH, pH^2 and vegetation (Table 3.6). Akaike weights of the variables for the best set of models suggest that temperature, temperature^2, pH, depth and vegetation are the most important predictor variables (Table 3.8). All models tested and their relative likelihoods (Akaike weights) can be seen in Table 3.7.

Of all analyses performed, this analysis conferred the fewest number of 'low' (n=4) and 'moderate' (n=3) invasion risk classifications to sites. All sites near the Sierra San Marcos with the exception of Poza Azul (a warm spring where < 10 individual *H. guttatus* have ever been caught despite surface flow to a nearby, cooler spring with an established population, Arturo Contreras Arquieta, *pers. comm.*) were classified as having either a ‘high’ or ‘very high’ invasion risk (Figure 3.4). Poza Azul was classified as having a ‘moderate’ risk (Table 3.12).

Prediction of invasion risk in Cuatro Ciénegas

Only the ‘Hg sites’ analysis resulted in probability of presence values greater than 0.75 for any of the four quantitative variables (Figure 3.3), thus the Hg sites analysis resulted in the highest invasion risk scores for individual sites (Table 3.4), although the pattern of relative invasion risk among sites for all analyses was generally the same. Three sites (Charcos Prietos, Puente Chiquito and Laguna Grande) had the same invasion risk determination in all 5 analyses. The ‘all sites w/o LS’ was similar to the ‘systems’ and ‘systems w/o LS’ analyses, and the two ‘systems’ analyses were the same except for one site (LS), while the ‘all sites’ analysis was the most different from the other four (Table 3.4). In all analyses, invasion risk was generally higher closer to the Sierra San Marcos and decreased with distance from the mountain (Figure 3.4).

Generally, across all analyses, Poza Churince (CHp), Churince stream (CHs), Los Remojos (RE), and the Río Mesquites (RMa-c) had a high invasion risk; Tierra Blanca (TB), Mojarral Este (ME), Escobedo stream (ESs), Tío Candido stream (TCs) and San José de Anteojo (SJAO) had a moderate to high invasion risk; Poza Juan Santos (JS), Anteojo (AO) and Poza Tío Candido (TCp) had a moderate invasion risk, Río Mesquites tributary (RMtrib) and Puente Dos Cuates (PC) had a moderate to low invasion risk; and Laguna Grande (LG), Poza Azul (PA), Puente Chiquito (PCh), Las Salinas (LS), Charcos Prietos

(CP) and Las Playitas (LP) had a low invasion risk. Two sites, Poza Escobedo (ESp) and Las Teclas (LT) were variable, with a low risk indicated by the ‘all sites’ analysis, a moderate risk by the ‘all sites w/o LS’, ‘systems’ and ‘systems w/o LS’ analyses and a high risk by the ‘Hg sites’ analysis (Figure 3.5).

DISCUSSION

Different datasets were analyzed to determine potential invasion risk of the sampled sites in Cuatro Ciénegas, and while different results were obtained from the different analyses, some generalities can be discerned. Generally, sites closer to the Sierra San Marcos in the center of the valley had a higher invasion risk than those farther away. This could be due in part to temperature as the warm water that comes up from underground near the mountain cools as it flows away from the mountain. Also, although different levels of invasion risk were assigned to the different sites based on the modeling results of the various datasets, the overall pattern of relative invasion risk was similar across all models with certain sites always having a higher or lower or more moderate risk of invasion (Figure 3.4).

To determine which analysis resulted in the most probable invasion risk classifications, we can compare the known presences to the invasion risk maps (Figure 3.4). For example, Poza Churince is where *H. guttatus* was first seen and where the largest and most dense population exists (density determination is based on the numbers of *H. guttatus* caught at each site and personal observation, but has not been rigorously calculated, Table 3.2). The ‘all sites’ analysis, however, classified this spring as having only a ‘moderate’ level of invasion risk (and this discrepancy is seen again in Poza Juan Santos and Mojarral Este, both of which also have long-sustained *H. guttatus* populations). This inconsistency between classification and observation is likely due to false absences (or places with favorable environmental conditions where *H. guttatus* could persist but where it has not yet had the opportunity to reach) in the ‘all sites’ dataset. There is also the possibility of false absences in the ‘all sites w/o LS’, ‘systems’ and ‘systems w/o LS’ analyses, e.g., in some of the downstream sites in the Río Mesquites and Poza Escobedo, which are part of the same hydrologic system as determined by Evans (2005), but that may only be accessible via underground waterways that limit dispersal. Therefore, the ‘Hg sites’ analysis is likely the most appropriate for predicting invasion risk valley-wide as it both i) accurately represents the known locations of *H. guttatus* populations and ii) contains the least number of potential false absences of all the analyses run. Thus, after a brief discussion of the others, the results of the ‘Hg sites’ analysis are discussed in more detail below.

All sites and systems models

The best model in the ‘all sites’ analysis included pH, pH^2, depth and salinity, though while the inclusion of depth made a substantial difference, salinity played a minor role in predicting *H. guttatus* presence. Further support for the insignificance of salinity in *H. guttatus* comes from a study by Langston *et al.* (2010) who found through physiological tolerance tests that salinity would likely not limit the spread of an invasive congener, *Hemichromis letourneuxi* in southern Florida. Furthermore, when considering the best model with Las Salinas (LS) (a hypersaline pool) removed, I show that measuring temperature, pH and vegetation result in the best predictive power and in this case, the top model performed considerably better than any other model. Since no fishes live in these brine pools, the model produced by removing Las Salinas appears to be the best model for this set of sites. The top model for both systems-based analyses was identical barring one site (LS, Table 3.9) and to the 'all sites w/o LS' analysis, thus further supporting temperature, pH and vegetation as important predictor variables for *H. guttatus* presence.

'Hg Sites' model

As with all other models, pH and vegetation were significant predictors of *H. guttatus* presence, and in concordance with the 'all sites w/o LS' and both 'systems' analyses, temperature was also a significant predictor (though the relationship between these variables and presence differed from the other models in some cases, i.e., squared vs unsquared variants, Tables 3.5, 3,10 and 3.11). One difference was the presence of depth in the top-performing model. Though it did not significantly affect probability of presence *per se*, the probability of the model without depth being the best was only 7.4% (Table 3.7), and it was only moderately supported. This could indicate that there is a threshold depth above which presence of *H. guttatus* is highly unlikely, and below which presence is possible, though with no specific pattern of association to depth. This interpretation is supported by the data shown in Figure 3.3 where all instances of probability of presence over 0.75 are found at depths less than 0.7 m. Thus, a high risk site would have a depth less than 0.7 m, although whether it was 0.2 m or 0.6 m, for example, would not affect the probability of presence in any predictable way. In the ‘Hg sites’ analysis, the top model (with temperature, temperature^2, pH, depth and vegetation) performed substantially better than any other, and is the recommended model for predicting potential risk of invasion of *H. guttatus* in Cuatro Ciénegas.

Environmental characteristics associated with probability of presence

Temperature is known to be an important factor controlling species ranges and ability to spread (Kimball *et al.*, 2004). On the day of sampling in this study, a spring with an established population of *H. guttatus*, Mojarral Este (ME), was measured to have a mean temperature of 32.8 °C, or 3.1 °C above the overall mean temperature of ‘Hg sites’. This spring also has a surface flow connection to a nearby, warmer spring, Poza Azul (PA) where fewer than ten individuals have ever been caught (Arturo Contreras Arquieta *pers. comm.*) and no established population persists and with a mean temperature of 33.5°C, or 3.8°C above mean temperature for the Hg sites. This discrepancy in difference from the mean does not seem large, but when the quadratic relationship of temperature and probability of presence are taken into account and these values are squared, there is a sizeable difference in estimated potential probability of presence. Interestingly, mean annual temperature of ME calculated from the hourly piezometer data is 29.7 °C (INECC, 2013), though the piezometer is situated in the warmer end of the spring, and so is likely an overestimate of the spring-wide mean temperature. Annual mean temperature calculated from hourly piezometer data from PA was 32.2 °C, and this is likely an underestimate of the overall mean temperature due to the piezometers placement at the opposite end from where the actual warm water spring is in the pool (INECC, 2013). Thus, the difference in mean temperature of these two springs is likely greater than the mean temperatures measured from one day of sampling in this study, further supporting temperature as an important factor influencing *H. guttatus* establishment and subsequent spread in the valley.

In Chapter 2, I determined the high risk temperature range related to invasion to be 23.8 °C – 29.2 °C. The fish in this experiment had been housed at 26 °C, or approximately the mean annual temperature of three sites where *H. guttatus* is currently found (Poza Churince, Poza Juan Santos and Río Mesquites) (INECC, 2013). The data for this field study were collected in the summer, so the mean temperature was likely higher than the annual mean. As acclimation is known to affect thermal preference (Fry, 1947), this could explain the higher temperatures relating to high risk of invasion in this study. Thus, *H. guttatus* distribution may change slightly throughout the year and surveys similar to the one in this study but conducted in other seasons are recommended.

Other environmental factors important in the top-performing models are known from other studies to be important influences on fish habitat use. Environmental pH influences many vital biochemical reactions, and so has been shown to be an important factor in determining distributions in other fishes (Moyle & Cech, 2004). This could be because optimal pH for many enzymatic reactions are species-specific (Hidalgo *et al.*, 1999), thus making pH a potentially range-limiting factor. Further, Schofield and Driscoll (1987) found that pH contributed to species numbers and community structure, and Rahel (1984) found that species assemblages varied along a pH gradient in 43 northern Wisconsin lakes, reinforcing that pH may be an important factor affecting invasion into new areas. Vegetation has been associated with the presence of various *Hemichromis* spp. in their native ranges including *H. bimaculatus* (Ewer, 1966, Holden & Reed, 1972, Welcomme, 1979), *H. letourneuxi* (Bailey, 1994) and *H. guttatus* (Loiselle, 1979). Hyslop (1987) found the opposite in a survey of a northern Nigerian stream where there are no large higher plants present, yet abundant *H. bimaculatus*, although there was an abundance of filamentous algae on the benthos, which is where this benthopelagic fish resides. Vegetation likely provides protection from potential predators, protects eggs/fry and/or is a resource island where there is more invertebrate prey. Depth was included in the top three best models for 'all sites', the third best 'systems' model (with and without LS), and the 'Hg sites' best model, though it did not significantly affect probability of presence and was not in the best model for all sites when LS was removed. This suggests that while depth *per se* is not a good predictor of *H. guttatus* presence, when taken into account with other factors, it can greatly improve model predictive ability. *H. guttatus* has been reported to be found in shallow habitats in its native range (Loiselle, 1979), and all *Hemichromis* species are benthopelagic (Baensch & Riehl, 1985, 1991). Their affinity for vegetation could be driving their residence in shallower habitats where vegetation is more prominent, which could be driving depth's presence in some of the top performing models.

Prediction of the course of invasion in Cuatro Ciénegas

It is unknown how *H. guttatus* arrived in the other two systems (Anteojo and Río Mesquites, Figure 3.1) after first being detected in the Churince system in 1996 (Contreras-Balderas & Ludlow, 2003). It is possible there were multiple introductions by humans, movement of eggs by birds (though there is no evidence currently to support this), subterranean hydrologic connections through which they traveled, or flooding events that temporarily connected normally isolated systems. Since the time of the first discovery of the invasive in Cuatro Ciénegas in 1996, there has been one major flooding event caused by rains from Hurricane Alex in the summer of 2010 (immediately preceding this field survey) that could have allowed *H. guttatus* to move between systems, and a follow-up survey to detect it in new areas (e.g., Poza la Becerra and downstream Río Mesquites) such as those in close geographic proximity or with hydrologic connections to sites where it is known to persist is recommended. Apart from this event, there have been other smaller-scale, more localized flooding events within the valley that may have created opportunities for movement between systems (Juan Carlos Ibarra, Sub-Director of the reserve, *pers.comm.*), though the extent or frequency of flooding has not been consistently documented. A population genetics study of the *H. guttatus* populations in each site could help answer the question of how many distinct introductions have occurred and the mechanism(s) by which it arrived there.

With the results from this study, however, we can predict how the *H. guttatus* invasion may proceed in Cuatro Ciénegas in terms of unassisted spread and establishment from its current distribution and thus identify areas where efforts and limited resources should be focused to try to stop or at least slow the invasion. The areas of greatest immediate concern are those sites that have direct surface connections to sites with known occurrences of *H. guttatus*, so I address those first. The discussion below is based on the 'Hg sites' invasion risk classification.

Churince System

The Churince system (Figure 3.1) is the site where *H. guttatus* was first seen and where it is in the greatest abundance. It is found throughout the spring and outflowing stream, but not in the evaporative lake, Laguna Grande (Figure 3.1). The lake has nearly completely dried up in recent years except when the heavy rains from Hurricane Alex in the summer of 2010 filled it temporarily. Even with this large rain event, though, the lake was dry again by the end of this study period (mid-August 2010). Even if it were to refill, however, despite direct connection to the most densely populated sites, having scored low for three of the four variables considered in this analysis, the lake has a very low risk of invasion (Table 3.12)

Rio Mesquites System

Four separate systems identified by Minckley (1969) based on visual inspection of surface drainage paths (the Garabatal/Becerra system, the Río Mesquites system, Puente Chiquito system, and Poza Escobedo) were combined by Evans (2005) into one (the Río Mesquites system, Figure 3.1) due to similar chemical composition of the waters suggesting likely subterranean connections between them, and Evans' delineation will be considered here. The Rio Mesquites system is large with both surface and subterranean flow that is not well understood in many areas. The historical flow of the water from the La Becerra/Tierra Blanca headsprings in the west to its terminal lake (Las Playitas) in the east has been disrupted by canals, most prominently and currently the Canal de la Becerra in the west removing much of the La Becerra/Río Garabatal flow before it would have a chance to follow its presumed subterranean path to the Río Mesquites, and the Saca Salada canal, which diverts Río Mesquites water at Puente Dos Cuates out of the valley to the Río Salado. Beyond the point of diversion of flow into the Saca Salada canal lie Charcos Prietos and Las Playitas, the latter of which is the terminal lake for this system, though it receives little to no, or only seasonal, inflow from the upstream drainage as the results of the Saca Salada diversion. Just upstream of this diversion in Puente Dos Cuates enters the Escobedo spring outflow via a canal built in 1898 (Minckley, 1969) that Evans considers to be part of this system as well, though, before reaching the river, the water in this canal goes underground (Figure 3.1).

West of the Sierra San Marcos in this system, *H. guttatus* is known only, thus far, from Poza Juan Santos that was part of Minckley's Garabatal/Becerra system (Figure 3.1), though, no sampling was done in Poza La Becerra, a previously heavily used tourist attraction and swimming hole, before being closed for conservation purposes, and a thorough survey there is recommended. Conditions in the Tierra Blanca River (Figure 3.1) (part of Minckley's Río Mesquites system), also in the west and the headwaters for the Río Mesquites, ranked high overall in terms of invasion risk (Table 3.12) and should be closely monitored as well. Dispersal to Tierra Blanca from the Río Mesquites, however, is limited by probable underground flow from the river to Mojarral Este (a spring that has a surface connection to Poza Azul and drains into the Río Mesquites, Figure 3.1), so unassisted dispersal may take longer than expected based on its invasion risk. Similarly, dispersal from Poza Juan Santos in the west to Tierra Blanca is inhibited by the lack of surface connections. In the past, Poza Juan Santos drained into the Río Garabatal, which passed west and then south of Tierra Blanca before going underground and likely resurfacing somewhere near Poza Azul or Mojarral Este (Figure 3.1). Thus, unknown underground hydrologic connections or future flooding events are required to create a pathway for *H. guttatus* to move into Tierra Blanca from Poza Juan Santos.

In the central part of the system, *H. guttatus* was found at all sites sampled (Poza Azul, Mojarral Este and Los Remojos as well as the main stem of the river itself, Figure 3.1), though, in Poza Azul, only one individual was caught, and an established population is not thought to exist there. Interestingly, all sites also had a high risk of invasion except for Poza Azul, which only had an intermediate level of risk due to high temperature and little vegetation, both of which are possibly excluding the establishment of a population here (Table 3.12), though this needs to be empirically tested. Downstream from these sites and upstream of Puente Dos Cuates is an area referred to as ‘El Laberinto’ due to the maze-like path of the river, which contains many forks and confluences as it passes through an extensive and dense *Typha dominguensis*-dominated marshland. I did not sample this area, but it seems likely that *H. guttatus* could invade here, though, it is possible that winter temperatures in the furthest downstream reach (far away from the thermal inputs of the headwater springs) could preclude populations from remaining here year-round as temperatures have been observed near currently occupied areas far below the mean determined in this study and well within the potential ‘low’ risk of invasion range of temperatures (13.5 °C, INECC 2013). This also suggests a potential seasonal shift in occupied area with a possible range contraction occurring in colder months, and a valley-wide winter survey is recommended to address this issue.

Puente Chiquito (Figure 3.1) lies to the north of the main part of the Río Mesquites, and though Minckley (1969) determined this to be a separate system due to drainage patterns on the surface, water chemistry links it to the central river system (Evans, 2005). The subterranean connection to the Río Mesquites reinforces the low-risk evaluation, as determined by its environmental characteristics (Table 3.12). Further, this site also contains fast-flowing water, and while water velocity was not measured in this study, observations of other such sites (e.g., canals, parts of Puente Dos Cuates) suggest that water velocity and *H. guttatus* presence are negatively related, and further investigation to test this is of interest.

Hemichromis guttatus is not found in the eastern part of the valley in Puente Dos Cuates, Charcos Prietos or Las Playitas and while this could be due to not enough time for

H. guttatus to arrive there yet, these sites also have a low risk of invasion based on their habitat characteristics (Table 3.12), and so it is unlikely that *H. guttatus* will establish there in the future. Just upstream of Puente Dos Cuates, the canal from Escobedo enters (Figure 3.1), and this spring's outflow does have a potentially favorable environment for *H. guttatus* invasion (Table 3.12), though, similarly to Poza Azul, this spring stays warm year-round (mean temperature measured in this study = 32.5 °C and annual mean temperature calculated from hourly piezometro readings = 31.9 °C; INECC, 2013). Additionally, the water in the Escobedo canal goes underground before reaching this point, so *H. guttatus* spread to here is likely to be slow if it occurs at all.

Anteojo System

An isolated system not delineated by Minckley (1969), the Anteojo system (Figure 3.1) is found in the northwest part of the valley, with at least its surface drainage currently well-isolated from the other systems. San José de Anteojo was found to have a high level of invasion risk even though it had a high mean temperature (Table 3.12). This is not surprising as an established population was eradicated from here in 2002 (de Lourdes Lozano-Vilano *et al.*, 2006). A nearby spring, El Anteojo, had a high risk of invasion (Table 3.12), yet it was never invaded despite its close proximity to San José de Anteojo. Both springs have had their outflows diverted into canals for irrigation of crops, though these irrigation canals dry before converging with any other water body, thus greatly reducing the risk of invasion unless by unnatural dispersal.

Tío Candido –Los Hundidos System

Though the Tío Candido-Los Hundidos system (Figure 3.1) is connected to the Río Mesquites system via an underground canal that enters the river at Puente Dos Cuates where the Saca Salada canal diverts water, different source springs and terminal lake (Los Gatos) put these springs in a different hydrologic system (Evans, 2005). Tío Candido and its outflow stream are highly susceptible to an invasion (Table 3.12), though the canal linking them to the Río Mesquites system where the closest known occurrences of *H. guttatus* are dries before arriving there, so, like Escobedo and the springs of the Anteojo system, invasion is possible but dispersal limited and potentially only possible only with assistance from humans.

Santa Tecla System

The Santa Tecla system (Figure 3.1) ranked as having a high risk of invasion (Table 3.12), however, due to the great distance from the closest known occurrence of *H. guttatus* and its hydrographic isolation from the rest of the valley, it is unlikely that, without assistance from humans, *H. guttatus* will arrive here on its own. There are connections via canals connecting the Río Mesquites to Las Teclas, but the Santa Tecla canal is very long and the flow of the water fast along its entirety making it highly unlikely that a small fish like *H. guttatus* could arrive there unaided. That said, however, if it were to be released here, there is a high likelihood it would be able to establish and spread throughout the system.

Conclusion

This study was conducted in a desert spring system, and it is possible that the conditions for successful invasion of *H. guttatus* could differ in other regions, but the factors identified here as promoting probability of presence align with the habitat characteristics in its native habitat: warm (close to 29.7 °C), slightly alkaline, shallow waters with aquatic vegetation (Loiselle, 1979) and are somewhat similar to the habitat of the invaded range of *H. letourneuxi,* the warm, shallow, seasonally flooded wetlands of southern Florida. Further, all analyses performed regardless of how the data were categorized resulted in generally the same relative invasion risk among sites. Thus, I conclude that these factors confer a high risk of establishment and spread of *H. guttatus* and potentially other *Hemichromis* species elsewhere. I further suggest that all species of this genus are potential invaders due to prior invasion success of some of its members, broad physiological tolerances by at least two of the species (temperature in *H. guttatus* (Chapter 2); and salinity (Langston *et al*. 2010), hypoxia (Schofield *et al*. 2010), and low temperature (Shafland and Pestrak 1982, Schofield *et al*. 2010) in *H. letourneuxi*), desirability by humans and the related high propagule pressure from the aquarium pet trade. I further suggest that for the other four *Hemichromis* species that overlap with *H. guttatus* in its native habitat (*H. bimaculatus*, *H. elongatus*, *H. fasciatus* and *H. letourneuxi*) if introduced into a habitat with favorable conditions, such as those presented here, they would have a high probability of establishment and spread and ensuing invasion.

Future directions

This system gives us an opportunity to monitor an invasion in progress and to make predictions about how it may unfold and then to test those predictions. Here, I have outlined the characteristics that I determined are related to a low, moderate and high risk of invasion. It will thus be interesting to continue to monitor these populations over time to see if *H. guttatus* does indeed spread throughout the valley as predicted and how native fish populations respond to this spread. Of course, this will require a lot of time, and efforts will hopefully be made in the meantime to prevent such movement. Thus, another way to test these predictions would be to create experimental mesocosms in which the environmental characteristics predicted here to correspond to low, moderate and high invasion risk could be manipulated while monitoring populations of *H. guttatus* to see if/how survival, growth, reproduction and/or dispersal are affected.

Table 3.1: Summary statistics of environmental factors, location and type of each site sampled. Abbreviations below site names refer to the abbreviations used on the maps (Figure 3.1). Coordinates are given in UTM, Zone 13.

		Poza Churince (CHp)	Churince (stream) (CHs)	Laguna Grande (LG)	Poza Juan Santos (JS)	Río Tierra Blanco (TB)	Poza Azul (PA)
Coordinates		E 784825 N 2971945	E 784305 N 2972636	E 783133 N 2973271	E 783259 N 2978325	E 784184 N 2980990	E 785762 N 2981119
Hydrologic system (Evans 2005)		Churince	Churince	Churince	Río Mesquites	Río Mesquites	Río Mesquites
Water body type		Poza	Stream	Lake	Poza	River	Poza
Number of Traps		10	34	7	12	13	11
Distance covered - (streams and rivers)		NA	1,747 m (3 sample days)	NA	NA	359 m	NA
Temperature (°C)	Mean	28.8 ± 0.5	28.4 ± 1.8	25.7 ± 0.9	28.3 ± 2.3	30.2 ± 0.2	33.5 ± 0.2
	Range	28.3 – 29.5	25.6 – 32.0	24.6 – 27.0	23.1 – 32.0	29.8 – 30.4	33.1 – 33.9
pH	Median	7.76	8.10	8.92	8.00	7.69	7.70
	Range	7.62 – 7.88	7.64 – 8.86	8.8 – 9.0	7.58 – 8.21	7.62 – 7.76	7.61 – 7.93
Depth (m)	Mean	1.1 ± 0.5	0.4 ± 0.2	0.3 ± 0.1	0.4 ± 0.2	0.8 ± 0.4	0.6 ± 0.5
	Range	0.5 – 2.0	0.2 – 0.9	0.2 – 0.4	0.2 – 0.9	0.4 – 1.9	0.2 – 2.0
Salinity (ppt)	Mean	1.50 ± 0.01	1.78 ± 0.26	3.73 ± 0.77	1.61 ± 0.19	2.09 ± 0.28	1.56 ± 0.0
	Range	1.48 – 1.52	1.48 – 2.58	2.31 – 4.19	1.49 – 1.78	1.17 – 2.26	1.50 – 1.57

Table 3.1, continued

		Mojarral Este (ME)	Río Mesquites (RM a-c)	Los Remojos (RE)	Río Mesquites tributary (RM trib)	Las Salinas (LS)	Escobedo (stream) (ESs)
Coordinates		E 786135 N 2981175	E 787401 N 2980569	E 787268 N 2980244	E789346 N 2979486	E 789972 N 2981893	E 789407 N 2977776
Hydrologic system (Evans 2005)		Río Mesquites	Río Mesquites	Río Mesquites	Río Mesquites	Río Mesquites	Río Mesquites
Water body type		Poza	River	Poza	River	Pool	Stream
Number of traps		18	32	9	11	5	4
Distance covered - (streams and rivers)		NA	1,342 m (3 sample days)	NA	391 m	NA	240 m
Temperature (°C)	Mean	32.8 ± 0.9	29.4 ± 0.4	31.6 ± 0.8	28.5 ± 0.28	35.2 ± 2.7	32.4 ± 0.3
	Range	31.0 – 34.1	27.1 – 31.0	30.2 – 32.7	28.2 – 29.1	32.7 – 39.3	32.2 – 32.7
pH	Median	7.85	8.51	8.24	8.65	8.77	7.88
	Range	7.71 – 8.18	8.13 – 8.72	8.02 – 8.41	8.59 – 8.72	8.19 – 8.93	7.82 – 7.98
Depth (m)	Mean	0.7 ± .5	0.7 ± 0.5	0.7 ± 0.5	0.40 ± 0.11	0.5 ± 0.2	0.7 ± 0.3
	Range	0.3 – 7.8	0.2 – 2.3	0.3 – 2.0	0.18 – 0.58	0.3 – 0.8	0.5 – 1.0
Salinity (ppt)	Mean	1.86 ± 0.21	2.60 ± 0.50	1.54 ± 0.20	2.24 ± 0.11	42.18 ± 10.60	2.22 ± 0.05
	Range	1.65 – 2.4	2.05 – 3.21	1.38 – 2.05	2.11 – 2.33	34.04 – 58.73	2.16 – 2.27

Table 3.1, continued

		Escobedo (poza) (ESp)	Puente Chiquito (PCh)	Puente Dos Cuates (PC)	Charcos Prietos (CP)	Las Playitas (LP)
Coordinates		E 789376 N 2977740	E 792387 N 2980628	E 791543 N 2979696	E 794493 N 2979971	E 796501 N 2980223
Hydrologic system (Evans 2005)		Río Mesquites	Río Mesquites	Río Mesquites	Río Mesquites	Río Mesquites
Water body type		Poza	Stream	River	Pool	Lake
Number of traps		6	5	13	5	10
Distance covered - (streams and rivers)		NA	213 m	436 m	NA	NA
Temperature (°C)	Mean	32.5 ± 0.2	24.5 ± 0.0	27.3 ± 0.3	33.0 ± 1.4	30.3 ± 1.4
	Range	32.2 – 32.7	24.5 – 24.5	26.9 – 27.9	31.5 – 35.0	28.0 – 32.2
pH	Median	7.86	7.96	8.30	8.76	8.95
	Range	7.81 – 7.97	7.94 – 7.97	8.20 – 8.38	8.51 – 8.82	8.89 – 8.99
Depth (m)	Mean	0.9 ± 0.7	0.6 ± 0.1	0.6 ± 0.2	0.3 ± 0.1	1.0 ± 0.8
	Range	0.5 – 2.1	0.5 – 0.7	0.1 – 0.9	0.2 – 0.4	0.2 – 2.1
Salinity (ppt)	Mean	2.27 ± 0.02	3.85 ± 0.00	2.96 ± 0.03	3.66 ± 0.10	4.05 ± 0.00
	Range	2.22 – 2.29	3.85 – 3.86	2.92 – 3.00	3.57 – 3.78	4.04 – 4.06

Table 3.1, continued

		Anteojo (AO)	San José de Anteojo (SJAO)	Tío Candido (stream) (TCs)	Tío Candido (poza) (TCp)	Las Teclas (LT)
Coordinates		E 785145 N 2986208	E 785823 N 2986282	E 790395 N 2975427	E 790254 N 2975363	E 798051 N 2966472
Hydrologic system (Evans 2005)		Anteojo	Anteojo	Tío Candido – Los Hundidos	Tío Candido – Los Hundidos	Santa Tecla
Water body type		Poza	Poza	Stream	Poza	Poza
Number of traps		8	9	10	6	13
Distance covered - (streams and rivers)		NA	NA	397 m	NA	NA
Temperature (°C)	Mean	31.4 ± 1.6	33.8 ± 0.5	31.9 ± 0.5	30.7 ± 0.2	30.7 ± 0.5
	Range	29.8 - 33.9	33.2 – 34.6	31.2 – 32.6	30.5 – 31.1	29.9 – 31.5
pH	Median	7.72	8.38	7.74	7.55	7.77
	Range	7.55 – 8.79	8.29 – 8.79	7.70 – 7.77	7.52 – 7.61	7.68 – 7.87
Depth (m)	Mean	1.0 ± 0.8	0.5 ± 0.3	0.3 ± 0.2	0.8 ± 0.9	1.5 ± 0.7
	Range	0.1 – 2.1	0.2 – 1.0	0.2 – 0.6	0.3 – 3.3	0.5 – 2.8
Salinity (ppt)	Mean	4.05 ± 0.00	1.19 ± 0.33	1.03 ± 0.26	1.69 ± 0.07	0.81 ± 0.10
	Range	4.04 – 4.06	0.99 – 1.73	0.93 – 1.73	1.54 – 1.8	0.67 – 0.96

Table 3.2: Fish species and abundances caught at each site sampled.

	Poza Churince (CH1)	Churince (stream) (CH2)	Laguna Grande (LG)	Río Tierra Blanco (TB)	Poza Juan Santos (JS)	Anteojo (AO)	San José de Anteojo (SJAO)	Poza Azul (PA)	Mojarral Este (ME)	Río Mesquites (RM a-c)	Río Mesquites trib (RM trib)	Los Remojos (RE)	Las Salinas (LS)	Puente Dos Cuates (PC)	Puente Chiquito (PCh)	Charcos Prietos (CP)	Las Playitas (LP)	Tío Candido (stream) (TCs)	Tío Candido (poza) (TCp)	Escobedo (stream) (ESs)	Escobedo (poza) (ESp)	Las Teclas (LT)	Totals
Centrarchidae																							
Lepomis megalotis†	0	2	0	0	10	0	0	0	1	12	0	3	0	0	0	0	0	0	0	0	0	1	29
Micropterus salmoides†	0	2	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	3
Characidae																							
Astyanax mexicanus	0	0	0	3	2	5	264	0	0	3	0	7	0	3	0	0	0	0	3	0	1	186	477
Cichlidae																							
*Hemichromis guttatus**	356	176	0	0	81	0	0	1	34	130	0	40	0	0	0	0	0	0	0	0	0	0	818
Herichthys cyanoguttatus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	8

Table 3.2, continued

	Poza Churince (CH1)	Churince (stream) (CH2)	Laguna Grande (LG)	Río Tierra Blanco (TB)	Poza Juan Santos (JS)	Anteojo (AO)	San José de Anteojo (SJAO)	Poza Azul (PA)	Mojarral Este (ME)	Río Mesquites (RM a-c)	Río Mesquites trib (RM trib)	Los Remojos (RE)	Las Salinas (LS)	Puente Dos Cuates (PC)	Puente Chiquito (PCh)	Charcos Prietos (CP)	Las Playitas (LP)	Tío Candido (stream) (TCs)	Tío Candido (poza) (TCp)	Escobedo (stream) (ESs)	Escobedo (poza) (ESp)	Las Teclas (LT)	Totals
Herichthys minckleyi†	1	7	0	29	2	101	605	15	29	9	0	2	0	0	0	0	0	0	0	20	66	51	937
Cyprinidae																							
Cyprinella xanthicara†	0	5	0	13	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	1	23
Cyprinodontidae																							
Cyprinodon bifasciatus†	0	0	0	27	0	0	0	12	9	0	0	0	0	0	0	0	0	0	0	0	0	0	48
Cyprinodon atrorus†	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	4	0	5
Poeciliidae																							
Gambusia spp.†	0	2	0	3	0	50	245	11	0	0	0	0	0	0	0	0	0	0	0	1	10	0	322

Notes: † = endemic; * = introduced

Table 3.3: Summary statistics for environmental characteristics at locations where traps were set for *Hemichromis guttatus*. For all analyses, Hg=0 refers to locations where traps did not catch *H. guttatus* and Hg=1 refers to those that did. *Temperature, salinity and depth values are given in mean ± sd, and pH values shown are medians.

Analysis	Variable	*H. guttatus* presence	N	Mean/Median*	Range
All locations	Temperature (ºC)	Total	242	30.0 ± 2.4	23.1 – 39.3
		Hg=0	186	30.2 ± 2.6	23.1 – 39.3
		Hg=1	56	29.7 ± 1.7	26.1 ± 34.0
	Salinity (ppt)	Total	242	2.98 ± 5.91	0.67 – 58.73
		Hg=0	186	3.28 ± 6.73	0.67 – 58.73
		Hg=1	56	2.00 ± 0.63	1.38 – 3.21
	pH	Total	242	8.06	7.52 – 9.04
		Hg=0	186	8.06	7.52 – 9.04
		Hg=1	56	8.10	7.62 – 8.83
	Depth (m)	Total	236	0.71 ± 0.53	0.12 – 3.32
		Hg=0	183	0.74 ± 0.57	0.12 – 3.32
		Hg=1	53	0.61 ± 0.34	0.21 – 1.77
All locations w/o LS	Temperature (ºC)	Total	237	29.9 ± 2.31	23.1 – 35.0
		Hg=0	181	30.0 ± 2.46	23.1 – 35.0
		Hg=1	56	29.7 ± 1.72	26.1 – 34.0
	Salinity (ppt)	Total	237	2.16 ± 0.85	0.67 – 4.19
		Hg=0	181	2.21 ± 0.91	0.67 – 4.19
		Hg=1	56	2.00 ± 0.63	1.38 – 3.21
	pH	Total	237	8.13	7.52 – 9.04
		Hg=0	181	8.14	7.52 – 9.04
		Hg=1	56	8.08	7.62 – 8.83

Table 3.3, continued

Analysis	Variable	*H. guttatus* presence	N	Mean/Median*	Range
All locations w/o LS	Depth (m)	Total	231	0.71 ± 0.53	0.12 – 3.32
		Hg=0	178	0.74 ± 0.57	0.12 – 3.32
		Hg=1	53	0.61 ± 0.34	0.21 – 1.77
Systems	Temperature (ºC)	Overall	205	29.8 ± 2.6	23.1 – 39.3
		Hg=0	149	29.9 ± 2.8	23.1 – 39.3
		Hg=1	56	29.7 ± 1.7	26.1 – 34.0
	Salinity (ppt)	Overall	205	3.29 ± 6.39	1.17 – 58.73
		Hg=0	149	3.77 ± 7.43	1.17 – 58.73
		Hg=1	56	2.00 ± 0.63	1.38 – 3.21
	pH	Overall	205	8.19	7.58 – 9.04
		Hg=0	149	8.23	7.58 – 9.04
		Hg=1	56	8.10	7.62 – 8.83
	Depth (m)	Overall	200	0.64 ± 0.44	0.12 – 2.35
		Hg=0	147	0.65 ± 0.44	0.12 – 2.35
		Hg=1	53	0.61 ± 0.34	0.21 – 1.77
Systems w/o LS	Temperature (ºC)	Overall	205	29.7 ± 2.4	23.1 – 35.0
		Hg=0	149	29.7 ± 2.6	23.1 – 35.0
		Hg=1	56	29.7 ± 1.7	26.7 – 34.0
	Salinity (ppt)	Overall	205	2.32 ± 0.81	1.17 – 4.19
		Hg=0	149	2.44 ± 0.84	1.17 – 4.19
		Hg=1	56	2.00 ± 0.63	1.38 – 3.21
	pH	Overall	205	8.19	7.58 – 9.04
		Hg=0	149	2.44	7.58 – 9.04
		Hg=1	56	2.00	7.62 – 8.83

Table 3.3, continued

Analysis	Variable	*H. guttatus* presence	N	Mean/Median*	Range
Systems w/o LS	Depth (m)	Overall	200	0.64 ± 0.44	2.12 – 2.34
		Hg=0	147	0.66 ± 0.48	0.12 – 2.34
		Hg=1	53	0.61 ± 0.34	0.21 – 1.77
Hg sites	Temperature (ºC)	Total	144	29.7 ± 2.3	23.1 – 34.1
		Hg=0	88	29.8 ± 2.7	23.1 – 34.1
		Hg=1	56	29.7 ± 1.7	26.1 – 34.0
	Salinity (ppt)	Total	144	2.07 ± 0.67	1.38 – 4.19
		Hg=0	88	2.11 ± 0.69	1.44 – 4.19
		Hg=1	56	2.00 ± 0.63	1.38 – 3.21
	pH	Total	144	8.18	7.58 – 9.04
		Hg=0	88	8.22	7.58 – 9.04
		Hg=1	56	8.10	7.62 – 8.83
	Depth (m)	Total	140	0.61 ± 0.42	0.15 – 2.35
		Hg=0	87	0.61 ± 0.46	0.15 -2.35
		Hg=1	53	0.61 ± 0.34	0.21 – 1.77

Notes: 'All sites' = all trap location data used in the analysis except those placed in San José de Anteojo (see methods); 'All w/o LS' = all trap location data excluding those placed in San José de Anteojo and the hypsersaline Las Salinas (LS); 'Systems' = data from trap locations in hydrologic systems where at least one *Hemichromis guttatus* was caught; 'Sys w/o LS' = same as for 'Systems' but excluding those in Las Salinas; 'Hg sites' = data from trap locations in the subset of sites where at least one *H. guttatus* was caught.

Table 3.4: Values used to determine high, moderate (mod) and low invasion risk for a particular site for 5 different analyses:

Analysis	Temp (°C)			pH			Sal (ppt)			Depth (m)		
	high	mod	low	high	mod	low	high	mod	low	high	mod	low
All sites	na	na	na	none	7.22 - 8.94	other	none	< 3.5	other	none	< 0.9	other
All w/o LS	none	26.9 - 32.9	other	none	7.75 - 8.35	other	na	na	na	na	na	na
Systems	none	27.8 - 31.8	other	none	7.72 - 8.66	other	na	na	na	na	na	na
Sys w/o LS	none	27.2 - 31.9	other	none	7.71 - 8.65	other	na	na	na	na	na	na
Hg sites	28.0 - 31.4	26.5 - 28.0; 31.4 - 32.9	other	7.62 - 7.94	7.94 - 8.46	other	na	na	na	< 0.7	0.7 - 1.7	other

Notes: 'All sites' = all trap location data used in the analysis except those placed in San José de Anteojo (see methods); 'All w/o LS' = all trap location data excluding those placed in San José de Anteojo and the hypsersaline Las Salinas (LS); 'Systems' = data from trap locations in hydrologic systems where at least one *Hemichromis guttatus* was caught; 'Sys w/o LS' = same as for 'Systems' but excluding those in Las Salinas; 'Hg sites' = data from trap locations in the subset of sites where at least one *H. guttatus* was caught..

Table 3.5: Best logistic models predicting presence of *H. guttatus* in its invaded range across all sites sampled both with and without a hypersaline site, Las Salinas (LS). The 'all sites' model correctly predicted exotic presence 80.1% of the time and had a final whole model AIC = 209.780. The 'all sites w/o LS' model correctly predicted exotic presence 80.9% of the time and had a final whole model AIC of 212.007.

Analysis	Variable	d.f.	Parameter estimates	Wald chi-square	P >chi-square	Odds Ratio	95% Wald CL
All sites	pH	1	0.035	0.0023	0.958	1.035	0.282 – 3.795
	pH^2	1	-7.118	13.557	<0.001	0.001	0.001 – 0.036
	Depth	1	-0.497	1.440	0.230	0.608	0.27 – 1.370
	Salinity	1	-0.308	1.071	0.301	0.735	0.411 – 1.316
	Vegetation	1	1.853	15.082	<0.001	6.378	2.504 – 6.250
All sites w/o LS	Temperature	1	-0.147	2.267	0.132	0.863	0.712 – 1.045
	$Temperature^2$	1	-0.093	6.240	0.013	0.911	0.847 – 0.980
	pH	1	-0.249	0.174	0677	0.780	0.242 – 2.511
	pH^2	1	-6.810	12.523	<0.001	0.001	<0.001 – 0.048
	Vegetation	1	1.838	14.613	<0.001	6.282	2.448 – 6.116

Notes: 'All sites' = all data used in the analysis except those placed in San José de Anteojo (see methods); 'All w/o LS' = all data excluding that from San José de Anteojo and the hypsersaline Las Salinas (LS). Neither analysis included data from San José de Anteojo (see text).

Table 3.6: Summary of model selection statistics for the best models predicting *Hemichromis guttatus* presence. The difference in AIC score of a model from the best model is represented with Δ_i. The Akaike weight can be interpreted as the probability a given model is the best model (of the models analyzed), and the evidence ratio can be interpreted as how many more times likely the best model is over a given model. Only substantially ($\Delta_i \leq 2$) and moderately ($2 \leq \Delta_i \geq .4$) supported models are shown. Models with lower AIC scores (within a data group) performed better than those with higher AIC scores.

Data	Model No.	No. Variables	AIC	Δ_i	Akaike Weight	Evidence Ratio
All sites	64	5	209.780	0.000	0.368	1.00
	52	4	210.572	0.792	0.248	1.49
	62	5	210.937	1.157	0.206	1.78
	57	5	212.032	2.252	0.119	3.08
All sites w/o LS	57	4	212.007	0.000	0.751	1.00
Systems	57	5	192.913	0.000	0.481	1.00
	43	4	194.164	1.251	0.257	1.87
	60	5	195.459	2.546	0.135	3.57
Systems w/o LS	57	5	192.871	0.000	0.478	1.00
	43	4	194.164	1.293	0.250	1.91
	60	5	195.459	2.588	0.131	3.65
Hg sites	59	5	152.722	0.000	0.651	1.00
	57	5	155.417	2.695	0.169	3.85

Notes: 'All sites' = all trap location data used in the analysis except those placed in San José de Anteojo (see methods); 'All w/o LS' = all trap location data excluding those placed in San José de Anteojo and the hypsersaline Las Salinas (LS); 'Systems' = data from trap locations in hydrologic systems where at least one *Hemichromis guttatus* was caught; 'Sys w/o LS' = same as for 'Systems' but excluding those in Las Salinas; 'Hg sites' = data from trap locations in the subset of sites where at least one *H. guttatus* was caught. For model descriptions, see Table 3.6

Table 3.7: All models run for the different subsets of data. All possible combinations of variables were tested with the caveats that a squared term had to appear with its un-squared counterpart and no more than 5 variables could be included in any given model. A ‘1’ or ‘0’ indicates if a particular variable was used in a particular model. Values under the 5 different datasets are Akaike weights (relative likelihoods of the models). *Denotes a model with substantial support ($\Delta_i \leq 2$). †Denotes a model with moderate support ($2 \leq \Delta_i \leq 4$).

Model no.	No. Variables	Temp	$Temp^2$	pH	pH^2	Depth	Salinity	Veg	All sites	All sites w/o LS	Systems	Systems w/o LS	Hg sites
1	5	1	0	1	0	1	1	1	0	0	0	0	0
2	1	1	0	0	0	0	0	0	0	0	0	0	0
3	1	0	0	1	0	0	0	0	0	0	0	0	0
4	1	0	0	0	0	1	0	0	0	0	0	0	0
5	1	0	0	0	0	0	1	0	0	0	0	0	0
6	1	0	0	0	0	0	0	1	0	0	0	0	0
7	2	1	1	0	0	0	0	0	0	0	0	0	0
8	2	1	0	1	0	0	0	0	0	0	0	0	0
9	2	1	0	0	0	1	0	0	0	0	0	0	0
10	2	1	0	0	0	0	1	0	0	0	0	0	0
11	2	1	0	0	0	0	0	1	0	0	0	0	0
12	2	0	0	1	1	0	0	0	0	0	0	0	0
13	2	0	0	1	0	1	0	0	0	0	0	0	0
14	2	0	0	1	0	0	1	0	0	0	0	0	0
15	2	0	0	1	0	0	0	1	0	0	0	0	0
16	2	0	0	0	0	1	1	0	0	0	0	0	0
17	2	0	0	0	0	1	0	1	0	0	0	0	0
18	2	0	0	0	0	0	1	1	0	0	0.001	0.001	0
19	3	1	1	1	0	0	0	0	0	0	0	0	0

Table 3.7, continued

Model no.	No. Variables	Temp	$Temp^2$	pH	pH^2	Depth	Salinity	Veg	All sites	All sites w/o LS	Systems	Systems w/o LS	Hg sites
20	3	1	1	0	0	1	0	0	0	0	0	0	0
21	3	1	1	0	0	0	1	0	0	0	0	0	0
22	3	1	1	0	0	0	0	1	0	0.001	0.019	0.013	0.003
23	3	1	0	1	1	0	0	0	0	0	0	0	0
24	3	1	0	1	0	1	0	0	0	0	0	0	0
25	3	1	0	1	0	0	1	0	0	0	0	0	0
26	3	1	0	1	0	0	0	1	0	0	0	0	0
27	3	1	0	0	0	1	1	0	0	0	0	0	0
28	3	1	0	0	0	1	0	1	0	0	0	0	0
29	3	1	0	0	0	0	1	1	0	0	0	0	0
30	3	0	0	1	1	1	0	0	0	0	0	0	0
31	3	0	0	1	1	0	1	0	0	0	0	0	0
32	3	0	0	1	1	0	0	1	0.003	0.041	0.005	0.002	0.002
33	3	0	0	1	0	1	1	0	0	0	0	0	0
34	3	0	0	1	0	1	0	1	0	0	0	0	0.001
35	3	0	0	1	0	0	1	1	0	0	0	0	0
36	3	0	0	0	0	1	1	1	0	0	0	0	0
37	4	1	1	1	1	0	0	0	0	0	0	0	0
38	4	1	1	1	0	1	0	0	0	0	0	0	0.001
39	4	1	1	1	0	0	1	0	0	0	0	0	0
40	4	1	1	1	0	0	0	1	0	0	0.034	0.029	0.079
41	4	1	1	0	0	1	1	0	0	0	0	0	0
42	4	1	1	0	0	1	0	1	0.017	0.010	0.013	0.009	0.011
43	4	1	1	0	0	0	1	1	0	0	0.250*	0.257*	0.001

Table 3.7, continued

Model no.	No. Variables	Temp	Temp^2	pH	pH^2	Depth	Salinity	Veg	All sites	All sites w/o LS	Systems	Systems w/o LS	Hg sites
44	4	1	0	1	1	1	0	0	0	0	0	0	0
45	4	1	0	1	1	0	1	0	0	0	0	0	0
46	4	1	0	1	1	0	0	1	0.003	0.025	0.002	0.001	0.002
47	4	1	0	1	0	1	1	0	0	0	0	0	0
48	4	1	0	1	0	1	0	1	0	0	0	0	0.001
49	4	1	0	1	0	0	1	1	0	0	0	0	0
50	4	1	0	0	0	1	1	1	0	0	0	0	0
51	4	0	0	1	1	1	1	0	0	0	0	0	0
52	4	0	0	1	1	1	0	1	0.248*	0.045	0.002	0.009	0.021
53	4	0	0	1	1	0	1	1	0.005	0.031	0.015	0.016	0.001
54	4	0	0	1	0	1	1	1	0	0	0	0	0
55	5	1	1	1	1	1	0	0	0.007	0.001	0	0	0.007
56	5	1	1	1	1	0	1	0	0	0	0.001	0.001	0
57	5	1	1	1	1	0	0	1	0.119†	0.751*	0.478*	0.481*	0.169†
58	5	1	1	1	0	1	1	0	0	0	0	0	0.001
59	5	1	1	1	0	1	0	1	0.008	0.004	0.031	0.027	0.651*
60	5	1	1	0	0	1	1	1	0.012	0.005	0.131†	0.135†	0.008
61	5	1	0	1	1	1	1	0	0	0	0	0	0
62	5	1	0	1	1	1	0	1	0.206*	0.029	0.001	0.001	0.030
63	5	1	0	1	1	0	1	1	0.004	0.025	0.011	0.012	0.001
64	5	0	0	1	1	1	1	1	0.368*	0.031	0.006	0.006	0.010

Notes: 'All sites' = all trap location data used in the analysis except those placed in San José de Anteojo (see methods); 'All w/o LS' = all trap location data excluding those placed in San José de Anteojo and the hypsersaline Las Salinas (LS); 'Systems' = data from trap locations in hydrologic systems where at least one *Hemichromis guttatus* was caught; 'Sys w/o LS' = same as for 'Systems' but excluding those in Las Salinas; 'Hg sites' = data from trap locations in the subset of sites where at least one *H. guttatus* was caught.

Table 3.8: Summary of relative importance of parameters for the best set of models predicting *Hemichromis guttatus* presence. Akaike weights (relative importance) of each variable used in the models for all sites combined (all sites) with hypersaline Las Salinas (LS).

Variable	All sites w/LS	All sites w/o LS	Systems w/LS	Systems w/o LS	Hg sites
Temperature	0.33	0.75	0.90	0.86	0.82
Temperature^2	0.12	0.75	0.90	0.86	0.82
pH	0.94	0.75	0.51	0.48	0.82
pH^2	0.94	0.75	0.48	0.48	0.17
Depth	0.82	0.00	0.13	0.13	0.65
Salinity	0.37	0.00	0.39	0.38	0.00
Vegetation presence	0.94	0.75	0.90	0.86	0.82

Notes: 'All sites' = all trap location data used in the analysis except those placed in San José de Anteojo (see methods); 'All w/o LS' = all trap location data excluding those placed in San José de Anteojo and the hypsersaline Las Salinas (LS); 'Systems' = data from trap locations in hydrologic systems where at least one *Hemichromis guttatus* was caught; 'Sys w/o LS' = same as for 'Systems' but excluding those in Las Salinas; 'Hg sites' = data from trap locations in the subset of sites where at least one *H. guttatus* was caught.

Table 3.9: Summary of invasion risk for all sites based on the five different models run. VL =; very low = low; M = moderate; H = high; VH = very high.

Site	All sites	All sites w/o LS	Systems	Systems w/o LS	Hg sites
Anteojo	M	M	M	M	VH
San José de Anteojo	H	M	M	M	H
Churince (poza)	M	H	H	H	VH
Churince (stream)	H	H	H	H	VH
Laguna Grande	L	L	L	L	L
Juan Santos	M	M	M	M	H
Tierra Blanca	H	H	M	M	VH
Poza Azul	L	M	L	L	M
Mojarral Este	M	M	M	M	H
Los Remojos	H	H	H	H	H
Río Mesquites (a-c)	H	M	H	H	H
Río Mesquites (trib)	L	L	M	M	M
Las Salinas	L	M	L	M	L
Puente Chiquito	L	L	L	L	L
Puente Dos Cuates	L	M	L	L	M
Charcos Prietos	L	L	L	L	L
Las Playitas	VL	L	L	L	L
Escobedo (stream)	H	H	M	M	H
Escobedo (poza)	M	M	M	M	H
Tío Candido (stream)	H	M	M	M	H
Tío Candido (poza)	M	M	M	M	H
Las Teclas	L	M	M	M	H

Notes: 'All sites' = all trap location data used in the analysis except those placed in San José de Anteojo (see methods); 'All w/o LS' = all trap location data excluding those placed in San José de Anteojo and the hypsersaline Las Salinas (LS); 'Systems' = data from trap locations in hydrologic systems where at least one *Hemichromis guttatus* was caught; 'Sys w/o LS' = same as for 'Systems' but excluding those in Las Salinas; 'Hg sites' = data from trap locations in the subset of sites where at least one *H. guttatus* was caught.

Table 3.10: Best logistic regression model predicting presence of *Hemichromis guttatus* in its introduced range based on the 'Systems' and 'Systems w/o LS' datasets. Parameter estimates and Wald chi-square tests for all variables entered into the model are given. The 'Systems' model correctly predicted *H. guttatus* presence 82.5 % of the time with an AIC score = 192.913. The 'Systems w/o LS' model correctly predicted *H. guttatus* presence 81.9 % of the time with an AIC score = 192.871.

Analysis	Variable	d.f.	Parameter estimates	Wald chi-square	P >chi-square	Odds Ratio	95% Wald CL
Systems	Temperature	1	-0.109	1.268	0.260	0.896	0.741 – 1.084
	Temperature^2	1	-0.118	8.428	0.003	0.889	0.821 – 0.963
	pH	1	-0.958	2.507	0.113	0.384	0.117 – 1.256
	pH^2	1	-4.840	6.065	0.014	0.008	<0.001 – 0.372
	Vegetation	1	-0.873	12.904	0.003	0.175	0.067 – 0.456
Systems w/o Las Salinas	Temperature	1	-0.109	1.249	0.264	0.897	0.741 – 1.085
	Temperature^2	1	-0.117	8.382	0.004	0.889	0.821 – 0.963
	pH	1	-0.948	2.440	0.114	0.387	0.118 – 1.273
	pH^2	1	-4.799	5.881	0.015	0.008	<0.001 – 0.398
	Vegetation	1	-0.873	12.915	0.003	0.175	0.067 – 0.452

Notes: 'Systems' = data from those hydrologic systems where at least one *Hemichromis guttatus* was caught; 'Sys w/o LS' = same as for 'Systems' but excluding data from Las Salinas.

Table 3.11: Best logistic regression model predicting presence of *Hemichromis guttatus* in its invaded range based on the 'Hg sites' dataset. Parameter estimates and Wald chi-square tests for all variables entered into the model are given. This model correctly predicted invasive presence 81.1% of the time. Final whole model AIC = 152.722.

Variable	d.f.	Parameter estimates	Wald chi-square	P >chi-square	Odds Ratio	95% Wald CL
Temperature	1	-0.0512	0.1859	0.6663	0.950	0.7530 - 1.199
Temperature^2	1	-0.1529	10.0083	0.0016	0.858	0.781 – 0.943
pH	1	-2.0134	9.1175	0.0025	0.134	0.036 – 0.493
Depth	1	-0.6436	1.4624	0.2265	0.525	0.185 – 1.491
Vegetation	1	1.6492	10.2937	0.0013	5.203	1.900 – 14.248

Table 3.12: Invasion risk of the sites sampled in this study based on results of the 'Hg sites' analysis. Sites with a mean temperature, mean depth or median pH within the range of temperatures that could be related to a probability of presence > 0.75 were assigned a high (H) risk of invasion, those within 0.5 to ≤ 0.75 were assigned a moderate (M) risk, and all others a low (L) risk (see Table 3.4 for specific values). Sites with abundant vegetation throughout were assigned a 3, those with little vegetation or vegetation only around the edges were assigned a moderate risk, and all others a low risk.

Site	Temperature	pH	Vegetation	Depth	Invasion Risk
Anteojo	H	H	M	H	VH
San José de Anteojo	L	M	H	H	H
Churince (poza)	H	H	H	M	VH
Churince (stream)	H	M	H	H	VH
Laguna Grande	L	L	L	H	VL
Juan Santos	H	M	M	H	H
Tierra Blanca	H	H	H	M	VH
Poza Azul	L	H	L	H	M
Mojarral Este	M	H	M	M	H
Los Remojos	M	M	H	M	H
Río Mesquites (a-c)	H	M	H	M	H
Río Mesquites (trib)	H	L	L	H	M
Puente Chiquito	L	M	L	H	L
Las Salinas	L	L	M	H	L
Puente Dos Cuates	M	M	L	H	M
Charcos Prietos	L	L	L	H	VL
Las Playitas	H	L	L	M	L
Escobedo (stream)	M	H	H	M	H
Escobedo (poza)	M	H	M	M	H
Tío Candido (stream)	M	H	H	M	H
Tío Candido (poza)	H	L	H	M	H
Las Teclas	H	H	M	M	H

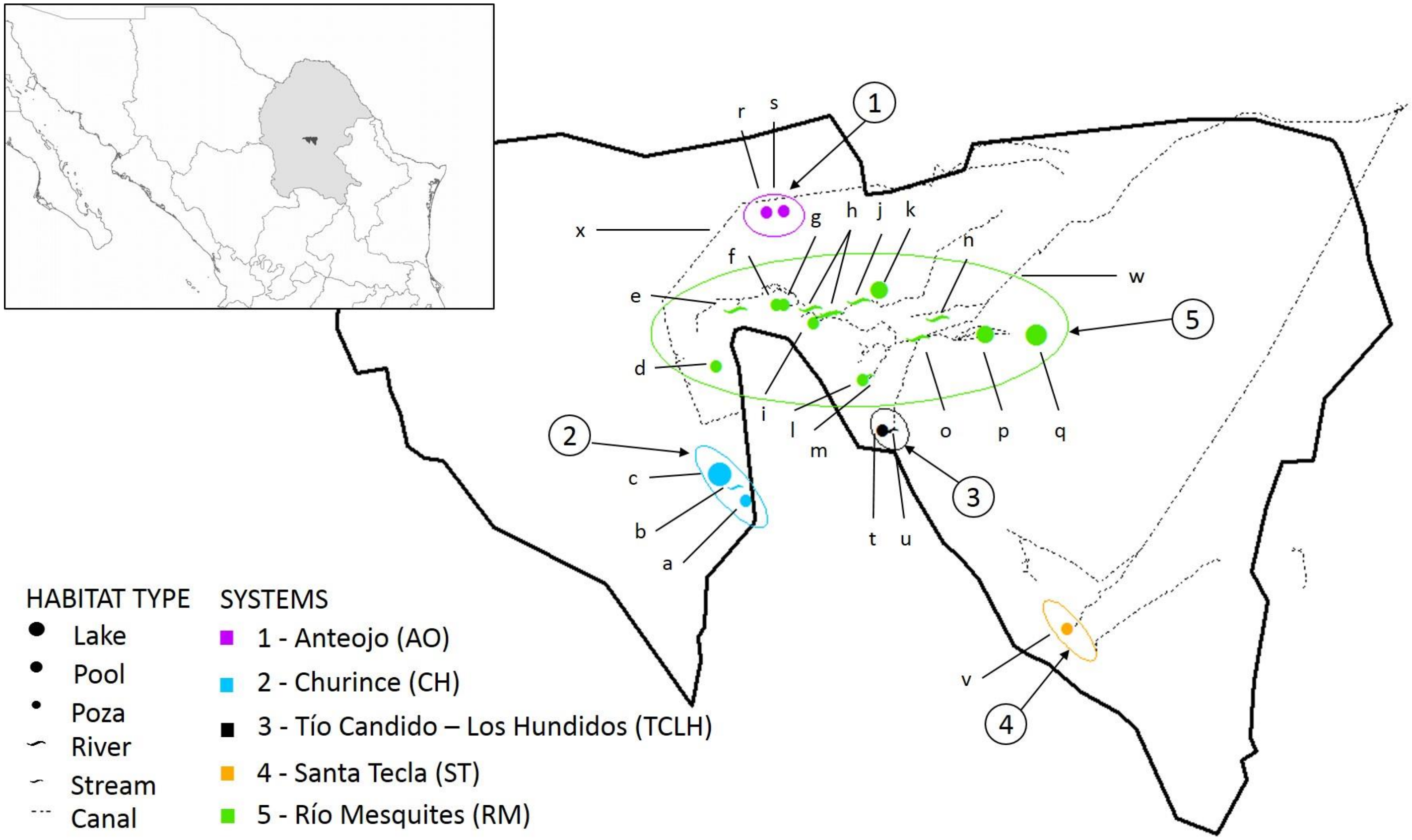


Figure 3.1: Map of the study area in Cuatro Ciénegas, México. The site is located in the state of Coahuila (shaded area in inset). The black outline delineates the protected area. Colors and associated numbers denote isolated hydrologic systems. Symbols denote habitat type. Letters identify sampled sites and canals: a) CHp, b) CHs, c) LG, d) JS, e) TB, f) PA, g) ME, h) $RM_{a\text{-}c}$, i)RE, j) RM_{trib} k) LS, l) ESp, m) ESs, n) PCh, o) PC, p) CP, q) LP, r) AO, s) SJAO, t) TCp, u) TCs, v) LT, w) the Saca Salada canal and x) the Río Garabatal canal. See Table 3.1 for site abbreviation definitions.

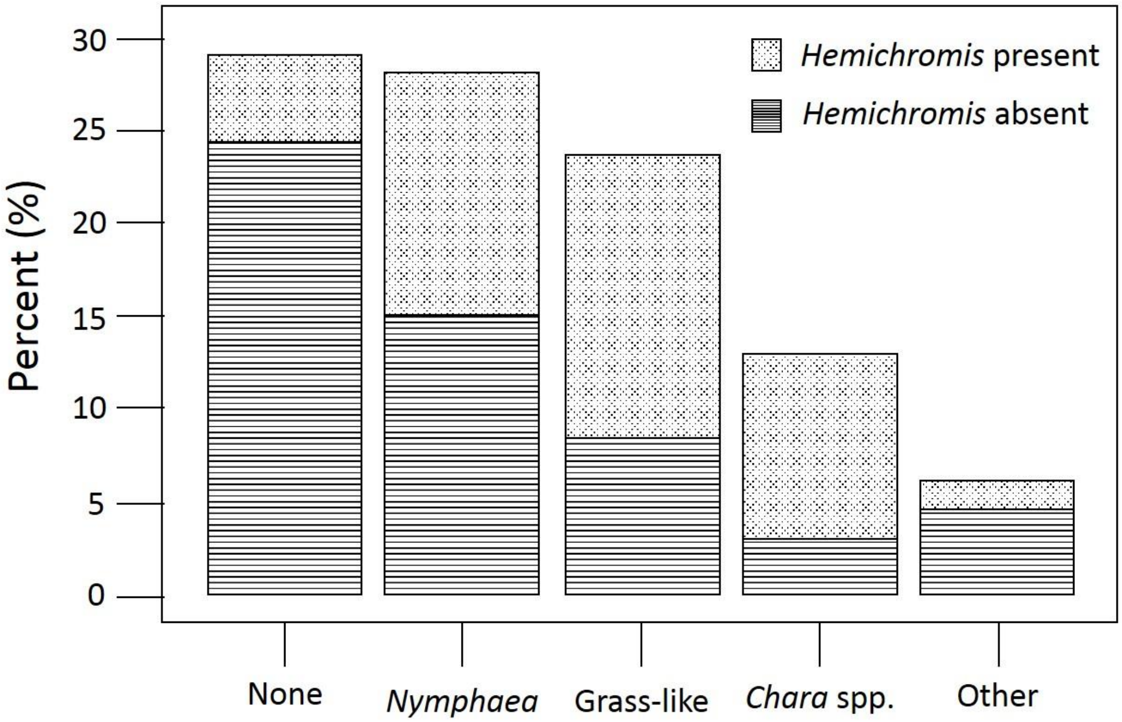


Figure 3.2: Presence of *Hemichromis guttatus* by vegetation type. *H. guttatus* presence was significantly associated with vegetation type (X^2=26.16, *df*=4, p<0.001). Adjusted standardized residuals showed that *H. guttatus* was present more often than expected by chance in *Chara* sp. and vegetation with grass-like architecture, *H. guttatus* was absent in other or no vegetation more often than expected by chance and present as expected in *Nymphaea*.

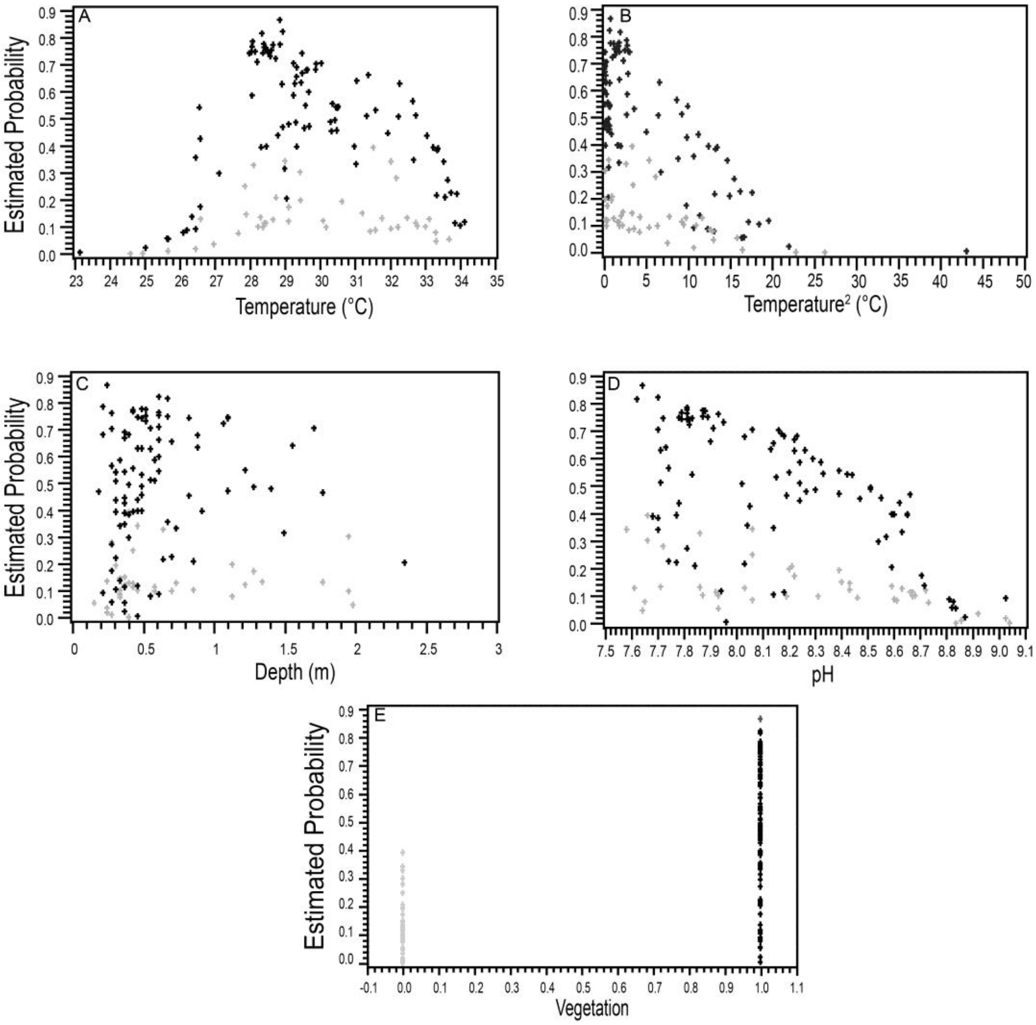
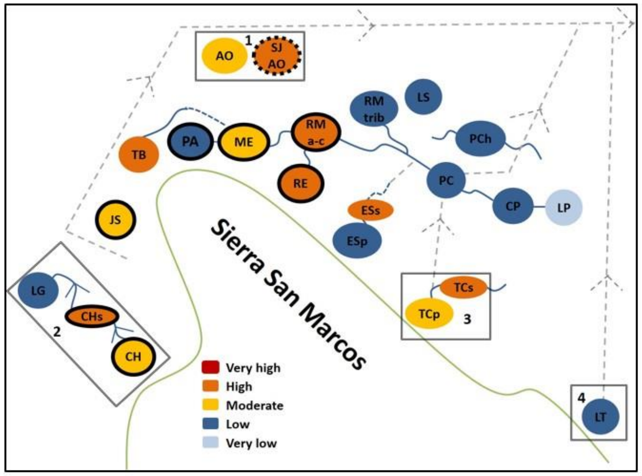
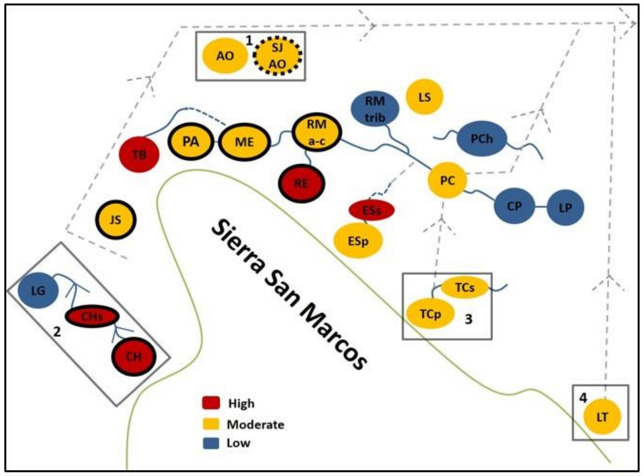


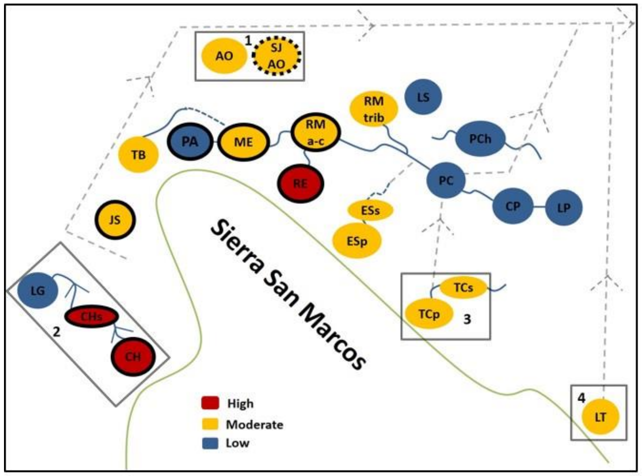
Figure 3.3: Probability of presence of *Hemichromis guttatus* by 5 variables. Logistic regression was performed to determine the best model predicting *H. guttatus* presence at the subset of sites where at least one individual was caught, which included (a) temperature, (b) temperature^2, (c) depth, (d) pH, and (e) vegetation. Squared temperature, pH and vegetation were significant predictors of invasive presence, though the best model predicting *H. guttatus* presence also included depth. Grey marks represent trap locations not associated with vegetation and black marks represent those traps on or near (within one trap length) vegetation.



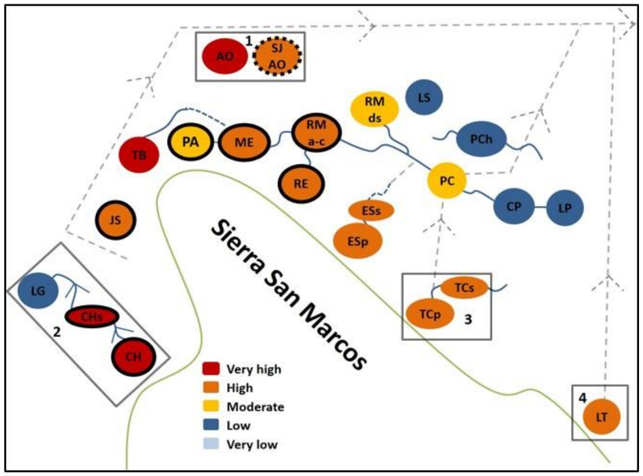
All



All w/o LS



Systems; Systems w/o LS



Hg sites

Figure 3.4 (caption on next page)

Figure 3.4: Stylized maps of the invasion risk of *Hemichromis guttatus* of surveyed sites in Cuatro Ciénegas based on 5 different analyses (see text for descriptions). The 'Systems' and 'Systems no LS' resulted in the same invasion risk maps with the exception of the invasion risk prediction for Las Salinas (LS, low for 'Systems' and 'moderate for 'Systems w/o LS') and so are presented together here (with the 'Systems' determination for LS). Dashed gray lines show the placement of canals. Dotted blue lines show where an uncanalized waterway goes underground. Solid blue lines denote streams and rivers. Boxes show separate hydrologic systems (see Figure 3.1 for key). All remaining sites not enclosed in a box are part of the Río Mesquites system. Circles/ovals are sampled sites, and those sites outlined in black are sites where at least one *H .guttatus* was caught. The dotted line around San José de Anteojo (SJAO) indicates that a population of *H. guttatus* existed here in the past, but has since been eradicated and none were found there during this study. The 'All' and 'Hg sites' analyses use 5 levels of risk due to containing more environmental characteristics in the top models for each analysis (3 each) versus fewer (2 each) for the other analyses. Site abbreviations are defined in Table 3.1. Maps are not to scale.

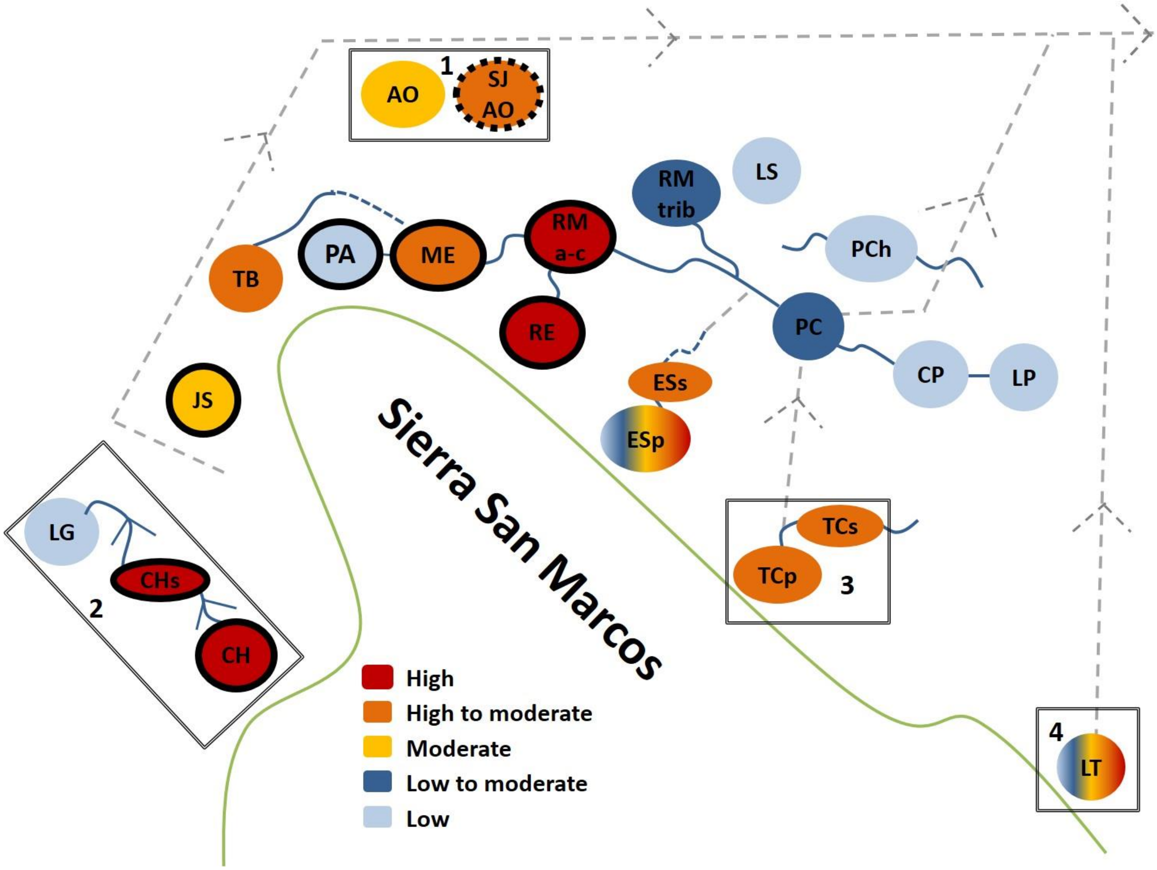


Figure 3.5: Summary stylized map of invasion risk of all sites sampled compiled from five separate analyses categories (see text for details). Three sites (Laguna Grande (LG), Puente Chiquito (PCh) and Charcos Prietos (CP)) had the same invasion risk level across all five analyses. Two sites (Poza Escobedo (ESp) and Las Teclas (LT)) were variable. All others were classified similarly by all analyses, and the pattern of relative risk overall was withheld across all analyses. Site abbreviations are defined in Table 3.1. Map not to scale.

Chapter 4: Community-level effects of an introduced aquarium trade fish to a biodiversity hotspot

INTRODUCTION

Invasive species, or those species that are present outside of their native range and which are negatively impacting native biota, are a global problem (Vitousek *et al.*, 1997) and affect biodiversity in a variety of ways at multiple levels of biological organization. Hybridization between invasive and native species can cause homogenization of gene pools with a subsequent loss of local adaptations and reduction in fitness, waste of energetic resources on 'dead-end' reproductive events, and the loss of genetically pure native species through introgression (Simberloff, 1996). Invasives may bring with them novel parasites and pathogens (Solheim, 1995) or act as reservoirs for emerging pathogens (Gozlan *et al.*, 2010). They may also compete for resources with native species (Human & Gordon, 1996) or predate on them (Wiles *et al.*, 2003). In some cases, they can alter ecosystem functioning through changes in fire regimes (Salo, 2004), hydrologic regimes (Anderson *et al.*, 2009) and nutrient cycling patterns (James *et al.*, 1997).

There is no doubt that through these various mechanisms and their interactions invasive species are effecting global change (Vitousek *et al.*, 1997), though, the degree of impact varies among mechanisms. While there are several examples of invasive predators causing extinctions of native species, e.g., the brown tree snake, *Boiga irregularis* in Guam and the rosy land snail, *Euglandina rosea* in Polynesia, very few extinctions have been directly attributed to competition with invasives. Lodge (1993) attributes roughly 4% of the impacts on native communities to competition alone. This is potentially due to competition requiring a longer time-scale to play out, which allows for other mechanisms to ‘beat competition to the punch’, native species to adapt, or for the environment to fluctuate and shift the competitive balance between invasive and native species (Davis, 2003). Regardless, competition can still reduce population sizes making them more susceptible to other impacts imposed by invasive species as well as environmental and demographic stochasticity (Davis, 2003, Gurevitch & Padilla, 2004).

In this study, we investigate both the competitive and predatory effects of a potentially invasive cichlid on an endemic, threatened cichlid (Contreras-Balderas & Ludlow, 2003) and a macroinvertebrate community. The West African jewel cichlid, *Hemichromis guttatus*, is native to coastal rivers from Sierra Leone to Cameroon where it shares a similar distribution with four other *Hemichromis* spp. All these species (and six other congeners distributed throughout southwestern Africa) are popular aquarium trade species and are transported globally for the pet trade, which is one of the top five vectors for introductions of aquatic species (Chang *et al.*, 2009) and the source of a third of the world’s invaders, and which has very little regulation and enforcement (Padilla & Williams, 2004). *Hemichromis* spp. introductions have been documented globally in Canada (*H.bimaculatus*, Welcomme 1986), the United States *(H. letourneauxi* in Florida, Shafland 1996; and *H. elongatus* in Hawaii, Eldredge 2000), Mexico (Contreras-Balderas and Ludlow 2003), Italy (*Hemichromis* spp., Piazzini et al. 2010), Austria (*Hemichromis* spp., Petutschnig et al. 2008) and Australia (*H. guttatus*, Koehn and MacKenzie 2004; *H. bimaculatus*, Olden *et al.* 2008; *H. lifalili*, Webb 2008). *Hemichromis letourneuxi* is a known invader in the Everglades in Florida, and *H. guttatus*, under investigation here, has been shown to have some of the general characteristics of invasive species (e.g., wide physiological tolerance, Chapter 2; close relationship to a known invader; desirability by humans (Moyle & Marchetti, 2006) and parental care (Marchetti *et al.*, 2004a)), thus warranting investigation of this highly dispersed group of fishes as potential invaders.

Hemichromis guttatus, introduced in Cuatro Ciénegas, an intermontane valley in the Chihuahua Desert and an oasis consisting of over 300 water bodies, was prehistorically, isolated from other surface waters, resulting in high endemism in the flora and fauna. Because of this, it has been named a World Wildlife Foundation conservation priority (Abell *et al.*, 2000), a UNESCO biosphere reserve (UNESCO, 2010) a RAMSAR convention wetland of international importance (Instituto Nacional de Ecología (Mexico) & U.S. Fish and Wildlife Service, 2007), and is a federally protected area for flora and fauna (Secretaría de Desarrollo Social, 1994). The valley hosts 17 native fishes, 11 that are endemic. Of these native fishes, 11 are considered threatened by the Mexican Federal Government (Secretaría de Desarrollo Social, 1994), due, in part, to the presence of exotics for nearly all of them (Contreras-Balderas, 1999). The first *H. guttatus* individuals seen in Cuatro Ciénegas were in one spring (Poza Churince) in 1995 (Contreras-Balderas & Ludlow, 2003), and since that time, the population has exploded and is beginning to spread throughout the valley (Hendrickson *et al.*, 2008).

Using isotope analysis, Marks *et al.* (2011) found dietary overlap between *H. guttatus* and two threatened endemic fishes, the Cuatro Ciénegas cichlid (*Herichthys minckleyi*) and the two-line pupfish (*Cyprinodon bifasciatus*). Further, one of these endemics, *H. minckleyi*, has two pharyngeal jaw morphologies, one with small, thin pharyngeal teeth (papilliform) that eats primarily detritus, algae and macroinvertebrates and the other with large, flat pharyngeal teeth (molariform) used to add snails to its diet (Swanson *et al.*, 2003). *Hemichromis guttatus* has a papilliform pharyngeal jaw dentition, and since its appearance in Poza Churince, the proportion of papilliform *H. minckleyi* has decreased (Hendrickson *et al.*, 2008) further supporting the hypothesis that there is dietary overlap between at least the papilliform morph of *H. minckleyi* with *H. guttatus*. This shift (increase) in the proportion of molariform Cuatro Ciénegas cichlids could potentially start a trophic cascade as populations of its endemic snail prey could be affected, which in turn could affect the stromatolites upon which the snails graze. Both species are known to associate with aquatic vegetation (*H. guttatus,* Chapter 3, *H. minckleyi*, Cohen et al. 2005), although, only *H. minckleyi* has been shown to also be associated with open areas of habitat, such as travertine or broken travertine and snail shells (Cohen *et al.*, 2005). Further, *H. guttatus* could itself be imposing an impact on the macroinvertebrate community upon which it preys directly.

In this study, I addressed two questions regarding the impacts (and corresponding invasive status) of *H. guttatus* (henceforth called *Hemichromis*) on its invaded system. Firstly, is there a differential effect of competition type (i.e., inter- versus intraspecific) and presence of cover on *H. minckleyi* (henceforth called *Herichthys*) or *Hemichromis*? I hypothesize on the basis of the results from Marks *et al.*(2011) and field data (Chapter 3) that *Herichthys* will be more impacted when in interspecific competition with *Hemichromis* and when cover is present. Secondly, do *Hemichromis* and *Herichthys* alter the structure of macroinvertebrate communities in different ways? Regarding this question, I hypothesize that the macroinvertebrate community will be affected differentially by the two species.

METHODS

Study animals

The *Herichthys minckleyi* stock used in this study was descended from individuals collected in Cuatro Ciénegas in 1993 (Konings, 1994) and subsequently housed at México's National Autonomous University (UNAM) in Mexico City. In 1995, nine individuals were given to the fish collection at the University of Texas at Austin (UT) where they were housed and bred in aquaria until being moved in 2000 to concrete-lined artificial ponds (24 m x 24 m x 1.75 m) that were populated by *Typha* plants and used as irrigation reservoirs at the J. J. Pickle Research campus (Dean Hendrickson, *pers. comm.*). In May 2011, juvenile *Herichthys minckleyi* were collected from these ponds with a 1/8 in (32 mm) seine net and transferred to 38 L and 208 L aquaria for one year before use in this study. The tanks were kept at ambient temperature, and the light:dark schedule was not standardized and followed that of the external environment via windows in the fish lab. Fish were fed with New Life Spectrum® cichlid pellets once daily.

The *Hemichromis* individuals used in this experiment were collected in February 2008 from Poza Churince in Cuatro Ciénegas and transported to the Bracknridge Field Laboratory at UT where they were housed in a PVC-lined pond (4.4 m x 1.4 m x 0.4 m depth) with constant running well water from the Edwards Aquifer. They remained here until August 2008 when they were transported to 38 L aquaria in a climate-controlled chamber in a lab on UT's main campus until used in this study. The tanks were kept at 26 °C, under a 12h:12h light:dark cycle, and each contained a cracked flower pot to provide structure. *Hemichromis* were fed with New Life Spectrum® cichlid pellets once daily.

In May 2012, 96 individuals of each species were transported to the Brackenridge Field Laboratory (BFL) of the University of Texas at Austin, where they were individually weighed (g), measured (standard length (SL, mm)), right or left pelvic fin clipped and photographed. Fin clips and photographs were taken to aid in matching before and after data for individuals (see 'Analysis' below). *Herichthys* exists in four different trophic morphs in the Cuatro Ciénegas valley including a papilliform morph having papilliform or comb-like pharyngeal teeth, a molariform morph having molariform or larger, flat pharyngeal teeth, a less common intermediate morph with both molariform and papilliform pharyngeal teeth, and a piscivorous morph with either papilliform or molariform teeth, but with an elongated head and body (Hulsey *et al.*, 2006). No laboratory-raised *Herichthys* have been documented as developing molariform pharyngeal dentition to the extent seen in Cuatro Ciénegas (Trapani, 2003). Likewise, in this stock, all individuals that have been checked have been papilliforms or at most slightly intermediate. To verify this, however, each individual used in this study large enough to safely insert an otoscope into its mouth was inspected. Only 8 individuals were too small to inspect (<4 cm SL).

Experimental design

Twenty-six mesocosms were established in May 2011 at BFL in 1,135 L capacity cattle tanks. Each tank was filled with 984 L of well water that was maintained via a continuous inflow ranging from approximately 0.1 L/min – 0.25 L/min. Outflow was through a 51 cm length of 1” (2.5 cm) PVC drainpipe attached to the cattle tank 6 cm from the bottom of the tank. A nylon stocking was placed over the outflow to prevent escape of fishes. Each mesocosm was covered with a 0.85 cm mesh nylon fabric supported in the middle by a 1.8 m length of 2” x 1” (19 x 38 mm) pine lumber and secured to the edges of the tank with clothespins. This allowed sunlight to penetrate the water, but prevented fish from jumping out of and birds from landing in the tanks. Each mesocosm was filled with approximately 38 L of sediment collected from the Colorado River adjacent to BFL (Figure 4.1).

Mesocosms were initially inoculated with macroinvertebrates collected from an established, artificial pond on the BFL property on May 19, 2011. Three samples were collected from the shallow edge of the pond with a d-net, mixed and added to a tank. This process was repeated for each tank. On June 6, 2011, two d-net samples were taken from each tank in the first row, mixed together in a 19 L bucket, and an equal portion of the mixed sample was distributed into each tank in the second row to increase homogeneity of macroinvertebrate communities between tanks (Figure 4.2). The following spring (May 18, 24 and 30, 2012), this procedure was repeated to prepare the tanks for the experiment.

Two treatments were randomly assigned to the mesocosms: competition type and presence/absence of cover. Competition type (intra- or interspecific) was applied in a substitutive manner such that in the intraspecific treatments (all *Herichthys* or all *Hemichromis*), eight individuals of the same species were used, and in the interspecific treatments (a mix of both species), four individuals of each species were used for a total of eight fish in every tank. Tanks randomly assigned to contain cover received six large and six small fake plants made from synthetic window screen material. Each large 'plant' was made from 0.56 m^2 piece of the material and was gathered and tied around a weight secured in its center (to prevent it from floating). The material was cut from the edges toward the center in the shape of long, broad leaves, half of which were tied to a fishing bobber with string to hold them upright while the remaining leaves were allowed to fold over (Figure 4.1). The small plants were made in the same way from a 0.19 m^2 piece of the screen material. Twenty-four tanks were randomly assigned one of the six possible treatment combinations (i.e., all *Herichthys* with cover, all *Herichthys* without cover, all *Hemichromis* with cover, all *Hemichromis* without cover, mixed species with cover, mixed species without cover) resulting in four replicates of each treatment combination, and two more tanks were randomly assigned to be no fish/no cover controls (Figure 4.2).

Macroinvertebrate samples were collected from all mesocosms on June 8, 2012 with a d-net, sweeping throughout the tank twice and depositing the sample in water from the tank in a 0.95 L Ziploc bag that was stored in a refrigerator for 1-2 days before processing in the lab. In the lab, sub-samples (hereafter referred to as the "samples") were collected as follows: i) contents of the Ziploc bag were emptied into a dissecting tray (29.2 cm x 19.1 cm), ii) using soft forceps and a pipette, organisms were detected without the aid of a dissecting microscope, removed from the tray and put into a 50 mL centrifuge tube containing 70% EtOH. Organisms were collected from the samples during two 5-minute sessions. During the first session, easily seen macroinvertebrates were collected including shelled, large and moving organisms. During the second session, more care was taken to look for smaller, slower or non-moving and hiding organisms. Macroinvertebrates were identified to various levels of taxonomic organization following McCafferty (1981) and Furtado and Gilroy (1995) and the abundance of each taxon in each sample was recorded.

On June 8, 2012, fish were added to their randomly assigned tanks. Following addition of the fish, mesocosms were checked every other day to assure the large-mesh coverings and drain pipes were in place and that drainpipes were unclogged. At that time, any algal mats built up were removed and inflow monitored and adjusted as needed. Observations were noted regarding the general appearance of the tanks (e.g., clarity of water, algal build up), mortalities and the presence of fry. On July 22, a second set of macroinvertebrate samples were collected and identified in the same manner as the first set, and finally on July 27, the mesocosms were drained by turning down the drain pipes and all remaining fishes (including as many fry as possible in those tanks where reproduction occurred) from both tanks and stocking drainpipe caps were removed.

Recovered adult fishes were euthanized with clove oil (Javahery *et al.*, 2012), weighed, measured, and photographed, and the pelvic fins were inspected and the clipped fin (if it could be determined) was noted. Fry were also euthanized with clove oil, and all fry collected from a particular tank were counted and weighed together, and the mean SL was determined for each cohort.

Analysis

Survival

The number of individuals of each species surviving in each tank was counted and a log-likelihood ratio test was used to determine whether survival amongst all possible combinations of species (*Hemichromis* or *Herichthys*), competition type (intra- or interspecific) and cover (present or absent) varied more than would be expected by chance. Post-hoc analysis was conducted by calculating the adjusted standardized residuals (ASR) for each cell, which returns the number of standard deviations away from the mean for an observed count. Those cells with an ASR > |2| were considered significant. ASR was calculated as follows:

$$\mathrm{ASR} = \frac{O_{ij}-E_{ij}}{\sqrt{E_{ij}*p_i*p_j}},$$

where O_{ij} is the observed value in row *i,* column *j,* E_{ij} is the expected value in row *i,* column *j,* p_i is the row *i* marginal probability and p_j is the column marginal probability.

Condition

The length-weight relationship was determined for each species independently using initial standard lengths of the individuals used in the experiment as the reference group for each species' predicted mass. For each individual, observed SL was used to determine predicted mass from the species-specific length-weight relationship, and LeCren's relative condition factor (LeCren, 1951) was then calculated as observed divided by predicted mass. A condition factor less than 1 indicated the fish was in poorer condition than would be expected for their length and vice-versa, and a condition factor equal to 1 indicated the fish is in the exact condition predicted by their SL.

To get data on individual fish, before and after photographs (that were linked to size and weight data) were matched. Pictures were matched using the iridescent blue dots on the flanks of both species, which are unique to individuals. The pelvic fin clips (either right or left) served to reduce the number of potential pictures to match. Once individual before and after data were matched, an ANOVA was performed with species as fixed variable, condition as a repeated variable and tank as a random variable to account for any potential tank effects to determine if condition changed differentially between the two species (all treatments combined). Lastly, to test whether competition type, cover or their interaction differentially affected condition in either species, a two-way repeated measures ANOVA was performed on each species separately with competition type and cover as fixed variables, tank as a random variable and individual fish as subjects.

Reproduction

The number of tanks in which reproduction did and did not occur was counted, and the species to which the fry belonged noted. Due to a low sample size (8 tanks with reproduction occurring), a 3-way test of species x competition type x cover was not possible (Table 4.1). A visual inspection of the contingency table, however, prompted two *a posteriori* hypotheses about potential effects of the treatments on reproduction. The first hypothesis tested was that *Hemichromis* reproduction is affected by the presence or absence of cover. The second hypothesis tested was that *Herichthys* reproduction was affected by the presence of *Hemichromis*. Separate 2x2 contingency tables were constructed, one for each hypothesis, and a separate Fisher's exact test was performed on each table.

Macroinvertebrates

Community data are notoriously difficult to analyze using standard parametric multivariate techniques. This is due to the presence of many zeroes in these datasets as well as the tendency for communities to contain few species that are very abundant and many species that are rare. Thus, abundance distributions are typically skewed to the left and truncated at zero. Statistical methods that use Euclidean distances are preferred because of their interpretability. However, raw abundance data cannot be used in Euclidean distance-based methods due to the issues listed above creating a "species abundance paradox" where communities that share fewer species will appear more similar than those that share more species (Legendre & Gallagher, 2001). To solve this, the Hellinger distance can be calculated from the raw data by calculating relative abundances and then weighting them proportionately (Legendre & Gallagher, 2001). Thus, a Hellinger transformation was applied and a redundancy analysis conducted with the transformed data. A triplot was created to view the relationship between experimental treatments and community composition.

Calculation of predicted masses for LeCren's condition and the redundancy analysis of macroinvertebrate communities were performed in R V 2.15.1 (R Development Core Team, 2011), and all other analyses were performed using SAS software (Version 9.2 of the SAS system for Windows).

RESULTS

Effects of *Hemichromis* were detected on survival of itself, reproduction of *Herichthys* and the structure of the macroinvertebrate communities but not condition of either species. Cover was directly related to *Hemichromis* and *Herichthys* survival and to reproduction of *Hemichromis* but not *Herichthys*. These results are presented in detail below.

Survival

Of the 192 individuals initially placed in the mesocosms (96 of each species), one mixed, no cover tank was removed from analysis because the outflow pipe tipped over and inflow tubing pulled off (perhaps by a passing deer) draining the tank completely and no fish were recovered. Thus, total survivorship was calculated on the basis of the remaining 184 starting individuals. Of the 106 (57.6%) that survived, 50 were *Hemichromis* (47.2% survival) and 56 were *Herichthys* (52.8% survival). Species, competition type and the presence of cover was not significantly related to survival, though it was marginal ($X^2 = 13.90$, $df = 7$, $p = 0.05$, Table 4.2). This trend toward significance was driven by *Hemichromis* in intraspecific competition and with cover having lower survival than expected and both *Hemichromis* and *Herichthys* in interspecific competition with cover present having higher survival than expected (Table 4.2).

Condition

The equation best describing the length-weight relationship for *Hemichromis* at the start of the experiment was $\log(\text{mass}) = 10^{(-1.623 + 3.157 \log (SL))}$ and for *Herichthys* was $\log(\text{mass}) = 10^{(-1.467 + 2.925 \log(SL))}$. These equations were used to obtain predicted masses for LeCren's condition for each individual, which decreased on average over the course of the study period for *Hemichromis* (from 1.001 before to 0.950 after), and increased for *Herichthys* (from 1.001 before to 1.079 after, Figure 4.3), and the effect of species on change in condition was significant (F - = 17.19, *df* = 1, $p < 0.0001$).

Before and after images of each fish were matched for 87 of the surviving 106 individuals (46 *Hemichromis* and 41 *Herichthys*). For *Herichthys*, condition (using the data from matched individuals) was not significantly affected by competition, cover or their interaction. Similarly, *Hemichromis* was not affected by either main effect or their interaction, though the interaction was trending toward significance (Table 4.3). Thus, while condition did change for both species, this change was apparently not related to the treatments applied.

Reproduction

Reproduction occurred in eight tanks overall, three with *Hemichromis* in intraspecific competition with cover, one with *Herichthys* in intraspecific competition with cover, two with *Herichthys* in intraspecific competition without cover, and three with both species (interspecific competition) with cover. In the three mixed tanks with reproduction occurring, only *Hemichromis* fry were discovered. Thus, *Hemichromis r*eproduction occurred in 6 tanks in both intra- and interspecific competition, and *Herichthys* reproduction occurred in 3 tanks all with intraspecific competition. Seven of the nine tanks in which reproduction occurred contained cover, and *Hemichromis* only reproduced when cover was present, whereas *Herichthys* reproduced both with and without cover present.

The *a posteriori* test of the effect of cover on *Hemichromis* reproduction was significant (p = 0.026, Table 4.4), though the *a posteriori* test of the effect of *Hemichromis* presence on *Herichthys* reproduction was not (p = 0.200, Table 4.5).

Macroinvertebrates

Twenty-seven taxa were collected over the course of the study (Table 4.6), 5 mollusks, 2 crustaceans, 16 insects, 2 annelids, 1 planarian and 1 nematode. The most abundant taxa included the invasive Asian clam (*Corbicula fluminea*), orb snails (family Planorbidae), pouch snails (family Physidae), the invasive snail *Melanoides tuberculata*, amphipods, dragonflies (order Anisoptera) and midges (family Chironomidae). Initially, the most abundant taxa were *M. tuberculata* (39.4%), amphipods (25.5%), orb snails

(19.2%), dragonflies (5.4%) and pouch snails (4.8%). At the end of the study, the most abundant taxa were *M. tuberculata* (59.6%), chironomid flies (17.3%), orb snails (7.3%) and amphipods (5.4%).

Within the specific treatment combinations (competition type x cover presence), a few generalities were also observed. While orb snails and amphipods decreased across all treatments, the greatest drop in orb snails occurred in *Hemichromis* with cover tanks while for amphipods, it was in the *Herichthys* tanks (with and without cover) and the mixed no cover tanks. Chironomids had their largest increase in *Hemichromis* with cover, *Herichthys* without cover and the no fish control tanks, whereas *M. tuberculata* had the greatest increase in the two *Herichthys* treatments (with and without cover) (Table 4.7).

The redundancy analysis showed that the initial communities were not significantly different from each other (F = 1.097, *df* = 4, p = 0.370). However, the final communities were significantly different (F = 1.718, *df* = 4, p = 0.039), with 17% of the variation in final macroinvertebrate communities explained by the treatments applied. The *Hemichromis* only and *Herichthys* tanks were orthogonal to each other, while the mixed species tanks fell in between and were the only significant term in the model (F = 3,190, *df* = 1, p = 0,03). Further, the control treatments resulted in different macroinvertebrate communities than those experiencing fish predation in general (Figure 4.4).

DISCUSSION

I tested two questions in this study. The first asked if survival, condition or reproduction was affected differentially by competition type or cover. Several pieces of evidence were either significant or showed a trend that was nearly significant and together suggest that *Hemichromis* is highly associated with cover causing an increase in mortality and decrease in condition. These impacts could be due to increased aggression as a result of adults having a higher chance of becoming reproductive when cover is present or from higher fish densities occurring in these covered areas. Further, potential inhibition of reproduction by *Hemichromis* on *Herichthys* is suggested, though further work with larger sample sizes is needed to ascertain this. The second question asked if competition type or presence of cover differentially affected the macroinvertebrate community. Analyses indicated that tanks containing both species altered the macroinvertebrate community in a way not predicted by the individual effects each species alone had. All of these results are explained in more detail below.

Fish

Mortality was high for all fishes with only 57.6% surviving, which could be due to various reasons. Cichlids are known to be aggressive fishes and are often kept separate from other species in aquaria for this reason (Lamboj & Schmettkamp, 2004). It is possible that aggressive interactions between individuals resulted in their death in these mesocosms. Also, although measures were taken to prevent bird predation by placing netting over the tops of the tanks, on a few occasions, we arrived at the site to find that the netting had been dislodged possibly either by wind or by deer moving through the area. Green herons (*Butorides virescens*) were observed in the area during the course of the study, and it is possible that they or other predatory birds took advantage of these occasions when the netting was off.

Though not significant (possibly due to the high overall mortality reducing sample sizes), the effect of the competition and cover treatments on survival trended toward significance (Table 4.2). Somewhat surprisingly, in tanks with interspecific competition, survival was significantly higher than in tanks with intraspecific competition. This might be explained, however, in terms of heterogeneity of habitat and fish densities. Tanks with cover present provided a heterogeneous habitat for the two species to use. *Hemichromis* in its native range is associated with vegetation (Loiselle, 1979) and this association has been observed in its introduced range in Cuatro Ciénegas as well (Chapter 3) Further, *Herichthys* occupies both open and covered areas in Cuatro Ciénegas (Cohen *et al.*, 2005). Thus, in the mixed species tanks with cover present, it is possible that habitat segregation (with *Hemichromis* occupying the covered areas and *Herichthys* occupying the open areas) is reducing the amount of potentially aggressive interactions the two species have and accordingly the realized densities of fish in the two microhabitats within a tank. Cohen *et al.* (2005) found that although detritus (an open habitat) was by far the most abundant in the particular spring used in their study in Cuatro Ciénegas (Poza Azul), it was one of the least elected habitats by *Herichthys,* and overhanging terrestrial vegetation (a covered habitat), broken travertine and snail shells, and travertine (both open habitats) were the most commonly selected. It would be interesting then to know if *Herichthys* has a preference for open areas and that is what was driving its presence in open areas of the mesocosm tanks, or if *Hemichromis* is interacting aggressively with *Herichthys* and driving it out of the covered areas in opposition to its preference.

On the other hand, tanks with *Hemichromis* in intraspecific competition and cover had lower survivorship than expected. This could also be explained by habitat and fish densities. Because *Hemichromis* is highly associated with cover, in tanks with cover and only conspecifics, the absolute area used is greatly decreased as all eight fish would be congregating in this covered patch, thus increasing density and the number of potential aggressive interactions between individuals. This agrees with the condition data, which was trending toward significance in the competition x cover interaction effect in *Hemichromis* only tanks. Overall condition of *Hemichromis* decreased over the duration of the experiment, and it seems plausible that, as with survival, the decrease was driven by increased aggressive interactions between *Hemichromis* individuals. Similarly, Marks *et al.* (2011) found that *Hemichromis* growth was negatively affected by increased density in intraspecific competition with itself.

Lower survival and decrease in condition of *Hemichromis* in tanks with cover and by itself could also be related not just to higher densities and more opportunity for aggressive interaction, but also to the reproduction that occurred in these treatments. Cichlids, in general, are known to be highly aggressive during times of mating, reproducing and guarding. Fitzgerald and Keenleyside (1978) found that higher densities of convict cichlids, *Cichlasoma nigrofasciatum*, resulted in fewer spawns, more aggressive interactions, lower percentage of eggs surviving to the fry stage and greater mortality than was seen at lower densities. Thus, a potential scenario for what occurred in tanks with cover present and *Hemichromis* only is that individuals congregated in the cover, causing them to engage in aggressive interactions toward one another (more so than in *Herichthys* only or mixed tanks), which resulted in bodily injury, reducing condition or even leading to death in some cases. In some cases a pair formed, and this pair was even more aggressive, inflicting even greater bodily injury while protecting its eggs and subsequent fry. An experiment quantifying the effect of density and reproduction on aggression and the resulting decrease in condition or increase in mortality would aid in verifying this scenario.

Another interesting point is whether the arrangement of the cover in the tank affected survival, condition or reproduction. In the current study, one large patch of cover was present, and the results could have been different if several smaller patches of cover were dispersed throughout the tank. In the latter scenario, fish could spread out more, thus reducing the number of opportunities for direct interaction and potential associated aggressive interactions, though, depending on territory size, multiple patches could be controlled by a single pair and function similarly to one larger sized patch. Alternatively, multiple patches of cover could promote the formation of more reproducing pairs, which would act more aggressively toward each other (FitzGerald & Keenleyside, 1978). A study examining this aspect of the role of cover is needed to resolve this issue.

Unlike *Hemichromis*, *Herichthys* was able to reproduce in tanks both with and without cover. This result would suggest that in the mixed tanks, *Herichthys* could reproduce both in open areas of tanks with the cover treatment as well as in tanks without cover at all, regardless of whether or not *Hemichromis* also reproduced in the covered areas. This was not the case, however, as there were no mixed species tanks in which any *Herichthys* fry were found. This pattern, however, did not have statistical support (Table 4.5). The small sample size (only three tanks total with *Herichthys* reproduction occurring at all) may be masking a real effect, though, and a study focusing specifically on the effect of *Hemichromis* on *Herichthys* reproduction is of great interest to determine if *Hemichromis* really is inhibiting *Herichthys* from reproducing. One way to test this would be to add *Hemichromis* individuals to tanks with breeding pairs of *Herichthys* at different stages of the reproduction process to determine a) if *Hemichromis* is in fact inhibiting reproduction, and b) if so, at what stage and by what mechanism, e.g., inhibition of courtship between *Herichthys* males and females, predation of eggs, or predation of fry. McKaye (1977) observed that aggression by reproductive cichlids in a Nicaragua lake was highest between conspecifics as pairs were forming but aggression between members of different species was highest when fry were present. This suggests the hypothesis that *Hemichromis* could be affecting *Herichthys* reproduction by predating on its fry.

A study on a closely related jewel cichlid, *Hemichromis letourneuxi*, that has invaded the Everglades in Florida, showed that production of fry in two of three native fishes, both live bearers (*Heterandia formosa* and *Gambusia holbrooki*), was not affected by the presence of *H. letourneuxi*, but that a third native fish, *Jordanella floridae*, which happens to have a similar reproductive strategy as *Herichthys* (i.e., laying eggs in guarded nests) experienced reduced reproduction in the presence of *H. letourneuxi* (Schofield *et al.*, 2013). It thus appears that in the Everglades, *H. letourneuxi* is inhibiting reproduction in the native species, though, further work on the specific mechanism is still needed. Additionally, an analysis of *H. letourneuxi* stomach contents showed that small fish make up, by far, the majority of their diet as measured by both volume and frequency across all seasons (Rehage *et al.*, 2013), thus it is possible that *H. guttatus*, like *H. letourneuxi* in Florida, was predating on *Herichthys* fry in mixed tanks.

Macroinvertebrates

A general 'fish effect' was present in the mesocosms in that the presence of fish, regardless of species, altered macroinvertebrate communities as compared to the controls (Figure 4.5). However, the RDA showed that the only treatment to explain a significant amount of variation in the macroinvertebrate communities was the mixed species treatment, which surprisingly altered communities in a way that was not predicted by the contribution of the individual species separately. This could be due to differences in habitat preference that cause the fish to spread out more throughout the mesocosm resulting in fewer refugia for macroinvertebrates. An alternate explanation could be increased aggressive interactions between the two species causing fishes to distribute themselves throughout more of the area of the tanks. For example, if *H. minckleyi*'s behavior is altered in the presence of *H. guttatus*, as I propose based on the condition and mortality data, and it is, in turn, influenced to spend more time in open areas, both benthic and periphytic taxa could be impacted . An example of a group that could be directly affected by this change in behavior in Cuatro Ciénegas is the hydrobiid snails, which reside either on vegetation, in the sediments or on travertine (Hershler, 1983). If *H. minckleyi*'s behavior is altered in the presence of *H. guttatus* to spend more time in open areas, those hydrobiid species in the open areas could decline, while those occupying vegetated areas could flourish, potentially leading to indirect effects on other macroinvertebrate species as well.

Chironomids were rare initially with only 19 total individuals in the samples taken across all tanks, but they became highly abundant (a total of 338 collected) by the end of the study (Table 4.6). This is likely attributed to a change in the abiotic conditions due to the season progressing as chironomid egg hatching and subsequent larval growth are dependent on temperature and other abiotic characteristics of the environment (Oliver, 1971). However, the greatest increase occurred in the no fish controls, which is what would be expected based on gut content analysis studies of the two species. A preliminary analysis of *Hemichromis* gut content in Cuatro Ciénegas found that the most common food items found included filamentous algae, ostracod crustaceans and chironomids (Martínez-Tristán *et al.*, 2005). In a study of *Herichthys* gut content in Cuatro Ciénegas, the most common arthropods found were chironomids, *Hyalella* spp. (amphipods) and ostracods (Hulsey *et al.*, 2006). However, when Hulsey *et al.* (2006) calculated the volumetric contribution of the prey items, arthropods, in general, contributed very little. They proposed that this was not due to a preference for other food sources, but to the low abundances of arthropods in Cuatro Ciénegas. The RDA triplot (Figure 4.4) reinforces the idea that much of the variation in chironomid abundances explained by the treatments is specifically explained by the control treatment. The triplot also indicates a moderate amount of variation is explained by the *Hemichromis* only treatment, which showed the second largest increase of chironomids. A study comparing the predatory effects of each species of fish specifically would aid our understanding of the potential impacts of the increased predation pressure on chironomids in Cuatro Ciénegas with the addition of *Hemichromis*.

Melanoides tuberculata also saw large increases in abundance over the duration of the study. In Cuatro Ciénegas, this exotic snail, which is highly invasive in other areas (Rader *et al.*, 2003), is only known to be abundant in canals and decaying *Typha* in Poza Churince, possibly due to unfavorable environmental conditions elsewhere (Dinger *et al.*, 2005), and thus perhaps not recognized as a food source by the two fishes. More likely, however, is that snails, in general, are less suitable by both species in this study due to the fishes' papilliform pharyngeal teeth dentition. Papilliform *Herichthys* in Cuatro Ciénegas had very few snail opercula (which was used as evidence of snail crushing and thus consumption) in their guts (Hulsey *et al.*, 2006), thus gastropods may just not be a suitable food source for *Hemichromis* or papilliform *Herichthys*.

A decline in planorbid snails occurred overall, and this could be related to the high *M. tuberculata* densities. In the Caribbean, a population of the planorbid snail, *Biomphalaria glabrata*, declined after the introduction of *M. tuberculata* and in Brazil, *B. glabrata* and another native planorbid, *B. straminea*, have been extirpated by the invasive, presumably through competition (Rader *et al.*, 2003). Further, Schofield *et al.* (2013)

found that *H. letourneuxi* reduced populations of *Planorbella duryi*, the Seminole ramshorn snail (family Planorbidae), more than when these species were in treatments with a native predator or no fish at all. However, the largest decline of planorbid snails (40 to 15 individuals on average) in this study was accompanied by a slight decrease in *M. tuberculata* (from 41 to 36 individuals on average), and this occurred in the no fish control tanks. Thus, a more complicated scenario is likely occurring with these species.

Similar to the effect on *P. duryi*, Schofield *et al.* (2013) also found a differential negative effect on the riverine grass shrimp, *Palaemonetes paludosus* in experimental mesocosms, and Rehage *et al.* (2013) found high frequencies (though low volumetric contribution) of this shrimp in the gut contents of *H. letourneuxi* caught in minnow traps in the field (in the spring season). No shrimp were collected in this study, but other crustaceans of the order Amphipoda were, and they experienced a large decline over the course of the study, and while, no obvious differential effect of predator species was seen, this taxon was likely a common food item for both *Herichthys* and *Hemichromis*. There is a native congener (*P. suttkusi*) in Cuatro Ciénegas that overlaps in distribution with *H. guttatus* in at least four sites (Dinger *et al.*, 2005). Monitoring of *P. suttkusi* populations is thus recommended to watch for signs of decline where it coexists with *Hemichromis*.

One potential prey group not represented in this study is other fishes. Anecdotal evidence from Cuatro Ciénegas such as observations by local land owners that the abundance of *Herichthys* decreased in springs on their properties after the arrival of *Hemichromis* as well as observations of apparently partially eaten smaller fish (e.g., *Gambusia*) in minnow traps used to trap *Hemichromis* from the springs by field crew members employed by the Cuatro Ciénegas Flora and Fauna Protected Area Reserve suggests a potential predatory effect of *Hemichromis*. In Florida, *H. letourneuxi* is a known predator on small fishes such as *Gambusia holbrooki*, *Lucania goodei*, *Jordanella floridae* and *Lepomis marginatus*, with up to 97% of their gut content (by volume) and 81% (by count) consisting of fishes. Thus, a potentially important component of *H. guttatus* diet was not accounted for in the present study, and a future study including prey fishes as part of the potential food source is of interest.

The data presented here suggest an impact of *Hemichromis* on the macroinvertebrate community in that when present with *Herichthys*, macroinvertebrate communities are affected in a manner significantly different than expected based on each individual species' effects. While this study is a useful foundation from which to formulate hypotheses of potential impacts on the macroinvertebrate community, the nuances of these impacts are unclear. To untangle the complicated interactions occurring in these communities, more controlled experiments need to be done with known abundances of specific taxa with the same species and cover treatments applied here.

Conclusions

Four conclusions may be drawn from this study about the role of *Hemichromis* in its invaded habitat in Cuatro Ciénegas. First, cover is a requirement for *Hemichromis* reproduction, suggesting that the removal of cover is a potential control method for this species. However, before this can be implemented as a viable solution, much work needs to be done to determine how much removal, at what time of the year, and in what pattern (e.g., complete vs. patchy removal). Second, although *Hemichromis* did not significantly affect *Herichthys* reproduction, the complete lack of reproduction by *Herichthys* in mixed tanks and ability of *Hemichromis* to reproduce in those same conditions suggests that the exotic may potentially be inhibiting reproduction of the native cichlid, which could have great impacts on population sizes. This is an area of great interest for future work. Third, the presence of *Hemichromis* may cause *Herichthys* to move into open areas to escape aggressive interactions when it would otherwise occupy vegetated or covered areas, and this change in behavior could alter macroinvertebrate community structure. Lastly, macroinvertebrate communities are affected differentially when both species are present, though, further work is needed to disentangle the specific ways in which the communities are altered. Overall, these results indicate that *Hemichromis* is likely negatively affecting native aquatic communities in Cuatro Ciénegas and points to its potential to affect the structure of aquatic communities elsewhere.

Table 4.1: The number of tanks in which reproduction occurred. Note that *Hemichromis* reproduction only occurred when cover was present. Also of interest is that *Herichthys* reproduction only occurred when in intraspecific competition; and in the three interspecific competition tanks where reproduction occurred, only *Hemichromis* reproduced.

Treatments	Reproduction	No Reproduction
Hg-intra-cov	2	2
Hg-intra-nocov	0	4
Hm-intra-cov	1	3
Hm-intra-nocov	2	3
Hg-inter-cov	3	1
Hg-inter-nocov	0	3
Hm-inter-cov	0	4
Hm-inter-nocov	0	3

Notes: Hg = *Hemichromis*; Hm = *Herichthy;*,intra= intraspecific competition; inter = interspecific competition; cov = cover present; nocov = cover absent

Table 4.2: The effect of species, competition and cover on survival of *Hemichromis* and *Herichthys* in a substitutive competition experiment. Survival had a trend toward significance ($X^2 = 13.99$, *df* = 7, p = 0.05). Closer inspection showed that *Hemichromis* in intraspecific competition with cover and both *Hemichromis* and *Herichthys* in interspecific competition with cover were driving this pattern. 'obs' = observed; 'exp' = expected; 'Adj SR' = adjusted standardized residual *Indicates significant adjusted standardized residuals (i.e., ASR > |2|). Negative ASR values indicate that the observed frequencies were lower than expected and positive ASR values indicate observed frequencies that are greater than expected.

Treatment combination	Survival (obs)	Survival (exp)	Adj SR surv	Mortality (obs)	Mortality (exp)	Adj SR mort
Hg_intra_cov	11	18.43	-5.47*	21	13.57	2.02*
Hg_intra_nocov	20	18.43	1.16	12	13.57	-1.57
Hm_intra_cov	19	18.43	0.42	13	13.57	-0.57
Hm_intra_nocov	16	9.22	-1.79	16	13.57	2.43*
Hg_inter_cov	12	6.91	4.09*	4	6.78	-5.56*
Hg_inter_nocov	7	9.22	0.06	5	5.09	-0.08
Hm_inter_cov	12	9.22	4.09*	4	6.78	-5.56*
Hm_inter_nocov	9	6.91	1.30	3	5.09	-1.76

Notes: Hg = *Hemichromis*; Hm = *Herichthy;*,intra= intraspecific competition; inter = interspecific competition; cov = cover present; nocov = cover absent

Table 4.3 The effect of competition type, cover and their interaction on the condition of each species separately. An ANOVA showed no effect of competition type ('comp' = intra- or interspecific) or cover (present or absent) on condition in either species, nor was there an interaction effect, though, there was a trend toward significance in the interaction of the two on *Hemichromis* condition.

Species	Effect	*df*	F	P
Hemichromis	comp	1	0.66	0.42
guttatus	cover	1	1.76	0.39
	comp x cover	1	3.04	0.09
Herichthys	comp	1	1.72	0.20
minckleyi	cover	1	0.33	0.57
	comp x cover	1	0.89	0.35

Table 4.4: The effect of cover on *Hemichromis* reproduction. *Hemichromis* reproduction was significantly associated with cover presence (p = 0.026)

	Reproduction	No Reproduction
Cover	5	3
No cover	0	7

Table 4.5: The effect of *Hemichromis* on *Herichthys* reproduction.. *Herichthys* reproduction was not significantly affected by *Hemichromis* presence (p = 0.200), although, interestingly, reproduction only ever occurred in *Hemichromis* when the two species were together. Small sample sizes may be masking an effect and further experiments should test this.

	Reproduction	No Reproduction
Hg0	3	5
Hg1	0	7

Table 4.6: Macroinvertebrate data from the 6 treatment combinations and no fish controls. Mean abundances were calculated for each treatment combination before and after the study.

	Hemichromis w/cover		*Hemichromis* w/o cover		*Herichthys* w/cover		*Herichthys* w/o cover		Mixed w/cover		Mixed w/o cover		No fish control	
	before	after	before	after	before	after	before	after	before	after	before	after	before	after
Mollusca														
Bivalvia														
Corbicula fluminea	--	--	2.5	3.0	1.3	1.0	1.5	--	0.5	1.3	1.0	2.7	0.5	2.0
Gastropoda														
Lymnaeidae	--	--	0.5	--	--	--	--	--	--	0.3	--	0.3	--	--
Physidae	2.0	1.8	7.3	4.5	3.3	3.0	1.8	0.5	0.8	1.5	6.7	5.7	7.0	2.5
Planorbidae	15.8	5.0	16.0	1.8	12.8	2.8	14.5	4.5	12.3	8.8	4.3	1.3	40.0	15.0
Thiaridae (*Melanoides tuberculata*)	11.0	16.8	53.0	48.3	27.0	48.8	11.8	23.5	30.8	34.5	53.3	85.7	41.5	36.0
Crustacea														
Amphipoda	9.5	5.3	11.0	0.5	24.5	3.3	22.3	0.5	33.5	1.3	3.0	--	45.0	24.5
Isopoda	--	--	--	--	--	--	--	--	--	--	--	--	0.5	--
Insecta														
Anisoptera	2.3	--	5.0	--	5.5	0.3	2.0	--	3.5	--	5.3	--	9.0	2.0
Coleoptera														
Dytiscidae	0.3	--	0.8	--	0.3	--	0.8	--	--	--	0.3	--	--	--
Elmidae	--	--	0.3	--	--	--	--	--	--	--	--	--	--	--
Haplidae	--	--	--	--	0.3	--	--	--	--	--	--	--	--	--

Table 4.6, continued

	Hemichromis w/cover		*Hemichromis* w/o cover		*Herichthys* w/cover		*Herichthys* w/o cover		Mixed w/cover		Mixed w/o cover		No fish control	
	before	after	before	after	before	after	before	after	before	after	before	after	before	after
Brachycera pupae	--	--	--	--	--	--	--	0.3	--	--	--	--	--	--
Ceratopogonidae	--	0.3	--	--	--	--	--	--	--	--	--	--	--	--
Chironomidae	0.3	17.0	0.3	11.8	--	7.5	0.8	9.0	1.3	10.0	3.0	8.0	--	24.5
Nematocera pupae	--	3.0	--	0.5	--	1.5	--	1.0	--	0.8	--	0.3	--	2.0
Stratiomyidae	--	--	0.3	--	--	2.0	--	0.3	--	--	--	--	--	--
unknown juv	--	0.3	--	--	--	--	--	--	--	--	--	--	--	--
Epehemeroptera	0.3	--	--	--	--	--	0.5	--	--	0.3	--	--	--	--
Hemiptera														
Hebridae	--	--	--	--	--	--	--	--	--	1.0	--	--	--	--
Mesoveliidae	--	--	--	--	--	--	--	0.3	--	--	--	--	--	--
Notonectidae	--	--	--	--	1.0	--	0.3	--	0.3	--	--	--	--	--
Zygoptera	0.5	--	1.3	--	--	--	--	--	--	--	0.7	--	--	--
Annelida														
Hirudinea	2.0	--	--	--	0.8	--	0.8	--	3.8	--	0.3	--	1.0	--
Oligochaeta	--	--	--	--	0.3	--	--	--	--	0.3	0.3	0.3	0.5	--
Turbellaria														
	--	--	--	--	--	--	--	--	--	--	--	--	--	0.5
Nematoda														
	--	--	--	--	--	--	--	--	--	--	--	0.7	--	--

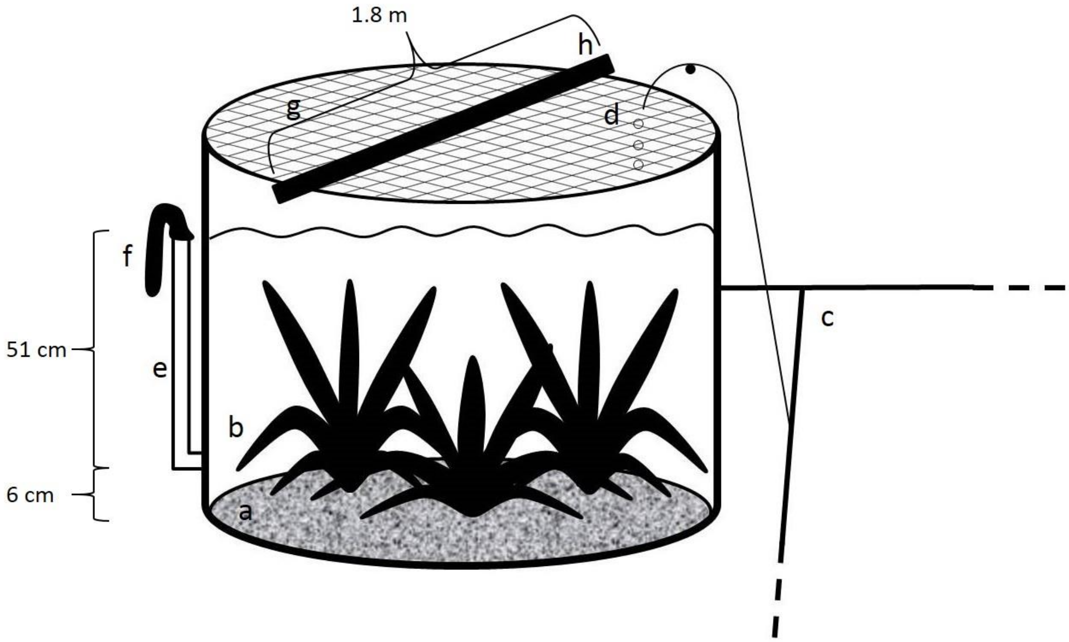


Figure 4.1: Tank set-up for community interactions experiments. The competitive and predatory effects of *Hemichromis guttatus* (exotic) on *Herichthys minckleyi* (native) and macroinvertebrate communities respectively were tested by applying different combinations of species (all invasive, all native, or mixed) in a substitutive design (see text for details). a) River sediments were added as substrate; b) half the tanks received a 'cover' treatment made of screen material and fashioned to emulate submerged aquatic vegetation; c) well water from the Edward's aquifer was d) dripped into the tank and e) flowed out through a PVC drainpipe with f) a nylon stocking over the end to prevent accidental loss of fish through the drainpipe; g) a mesh fabric was applied to the top to prevent both fish from jumping out of the tank and birds from predating on fish in the tank; and h) a 3 m length of 2" x 1" lumber was placed across the center of the tank to help hold up the mesh fabric.

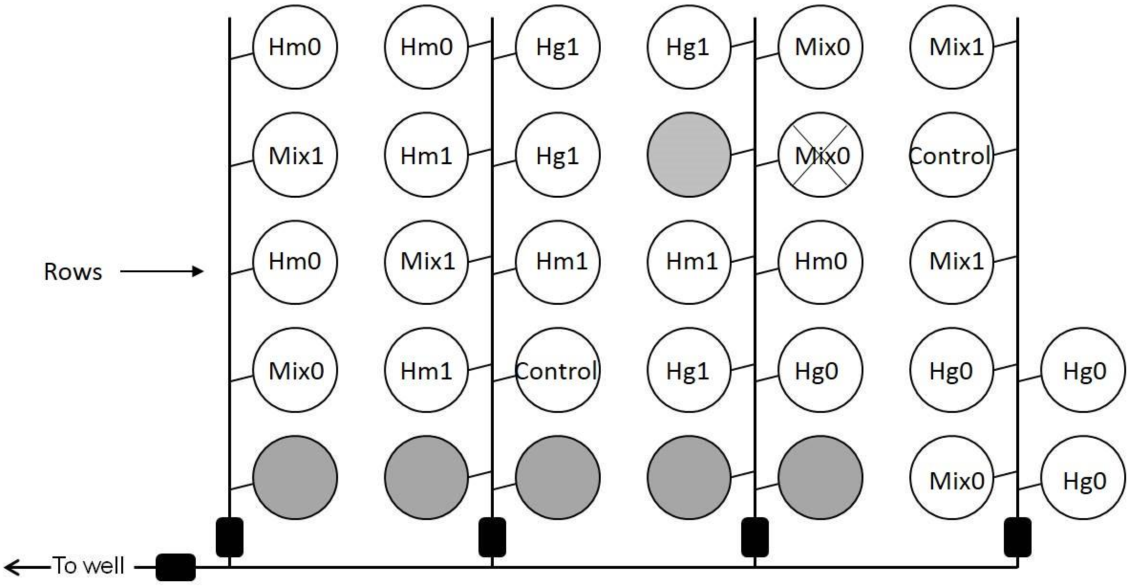


Figure 4.2: Layout of mesocosms. Thirty-two 1,135 L tanks were arranged in rows for a community interactions experiment (see text for details). Six tanks were not used in the experiment (grayed), leaving the remaining twenty-six available for use. A combination of a competition and cover treatment was applied randomly to each tank such that there were four replicates of each of the six possible combinations and two fishless, coverless control tanks. One tank drained unexpectedly (with 'X' over it) and data from this one was not used in analyses. Water flowed to the tanks from a well through garden irrigation tubing (black lines) and could be controlled by valves (black boxes) if needed. Treatment abbreviations are as follows: Hg0 – *Hemichromis* only without cover; Hg1 – *Hemichromis* only with cover; Hm0 – *Herichthys* only without cover; Hm1 – *Herichthys* only with cover; Mix0 – both species without cover; Mix1 – both species with cover; Control – no fish without cover.

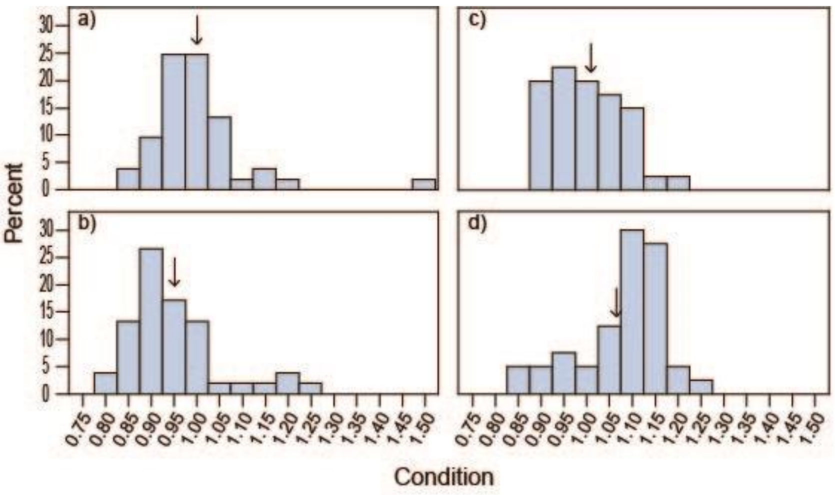


Figure 4.3: LeCren's condition factor of two competing species before and after six weeks in experimental mesocosms. Shown are the distributions of condition of a) *Hemichromis guttatus* condition before the study and b) after the duration of the study. Similarly, c) *Herichthys minckleyi* condition before the study and d) at the end of the study.

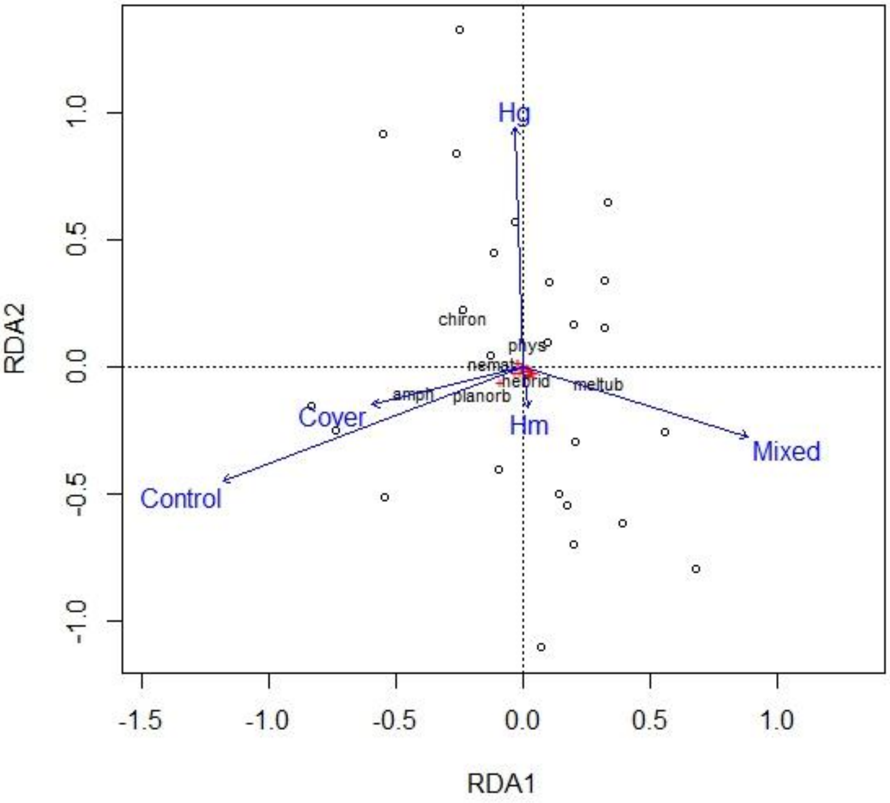


Figure 4.4: Triplot created from redundancy analysis of the effect of treatments on macroinvertebrate community structure in artificial mesocosms. Treatments are as follows: intraspecific with all native fish (Hm), intraspecific with all exotic fish (Hg), interspecific (Mixed), no fish control (Control) and the presence or absence of cover (Cover). Only the mixed treatment had a significant effect on the macroinvertebrate community structure (F = 3.19, df = 1, p = 0.03). Position of arrows is based on eigenvalues and denotes the amount of variance in the community matrix attributed to that axis. It is a measure of importance of the ordination variable. Taxa are plotted according to axes 1 and 2 scores. Only the most abundant taxa are labeled ('chiron' = Chironomidae, 'nemat' = Nematocera pupae, 'phys' = Physidae, 'amph' = Amphipoda, 'planorb' = Planorbidae, 'hebrid' = Hebridae, and 'meltub' = *Melanoides tuberculata*). Circles represent individual tanks. Red plusses represent other taxa sampled

Chapter 5: Conclusion

Whether a particular exotic species is invasive is not as easy a question to answer as it may at first seem. The definition of invasive species as used in this study is a species living outside its native range and that is imposing a negative impact on its invaded community. At what point, though, do we say an impact is occurring, and what kinds of evidence do we need to support such a conclusion? *Hemichromis guttatus* is closely related to a known invader (*H. letourneuxi,* (Schofield *et al.*, 2013)), provides parental care to its young (Marchetti *et al.*, 2004a), is desirable by humans in the aquarium trade and is widely distributed throughout the world because of this creating the potential for high propagule pressure into novel systems. Additionally, in the study presented here, I have shown that *H. guttatus* exhibits a wide physiological tolerance to temperature. All these characteristics mark this species as a potential invasive species if it were to be introduced into a suitable habitat, which it has.

Thus, to determine if *H. guttatus* is an invasive species in Cuatro Ciénegas, we need to determine if this non-indigenous species is having an impact on the native community to which it has been introduced. Anecdotal evidence from local landowners and reserve managers suggests potential declines of native species correlated to the arrival of *H. guttatus* as well as to predatory effects on small fishes. More rigorously collected data from a stable isotope study showed dietary overlap between *H. guttatus* and two endemic, threatened fishes (*Herichthys minckleyi* and *Cyprinodon bifasciatus*), and these same authors found a negative effect of competition on juvenile native cichlids in the presence of *H. guttatus* in *in situ* experiments conducted in Cuatro Ciénegas (Marks *et al.*, 2011).

To add to this list of potential impacts, in this study, I found evidence suggesting that *H. guttatus* may be altering *H. minckleyi* behavior through aggressive interactions causing the native cichlid to spend less time in covered areas when in the presence of the exotic (Chapter 4). This, in turn, could cause changes to the macroinvertebrate communities in covered and open areas, and indeed a difference in these communities was seen between mesocosms with single and mixed fish species assemblages (Chapter 4). I also found evidence of *H. guttatus* potentially inhibiting *H. minckleyi* reproduction, which could have obvious detrimental effects on native cichlid populations (Chapter 4). All of this information suggests that *H. guttatus* could and perhaps already is having a negative impact on *H. minckleyi*, *C. bifasciatus* and/or the native macroinvertebrate community in Cuatro Ciénegas, though we do not have census data of the respective taxa to verify if any of these interactions are affecting population sizes.

So, can we say that *H. guttatus* is an invasive in Cuatro Ciénegas? Invasive species persist in a lag period in their invaded habitats between when they first establish and when they spread and integrate (Sakai *et al.*, 2001). A species in this lag period may interact negatively with native biota, but if populations of the exotic are small, these negative interactions likely won't affect overall population sizes of the natives. However, once the exotic species passes through this lag phase and begins to become widespread and abundant, the negative interactions may lead to negative impacts on and ultimately declines in native populations. In the case of *H. guttatus* in Cuatro Ciénegas, it is localized, but abundant, or at stage IVb as per the classification of Coulatti and MacIsaac (2004) (see Figure 1.1), but based on the characteristics of successful invaders that it possesses, the fact that it is beginning to spread throughout the valley, and the fact that it has achieved such high densities in the original site to where it was introduced, it seems likely that this is a lag stage for this fish. This, mixed with the negative impacts that have been demonstrated by Marks *et al.* (Marks *et al.*, 2011) and this study, lead me to conclude that *H. guttatus* is likely to reach stage V of the invasive process in Cuatro Ciénegas (widespread and abundant, and therefore inflicting a negative impact on the native biota) and is thus a great threat to the aquatic communities there.

The next question, then, is what to do about it. Firstly, those sites that as-of-yet are still without *H. guttatus* present but that are at high risk (as determined in Chapter 3) should be regularly monitored, and if a jewel cichlid is found, intense trapping should be implemented immediately along with, if possible, closure of the newly invaded site from neighboring sites to prevent further spread while the trapping process is occurring. Additionally, determination of invasion risk of sites not sampled in this study should be undertaken, especially those sites in close geographic proximity or with known hydrologic connections to areas with known presences, e.g., Poza la Becerra and downstream reaches of the Río Mesquites amongst others. New sites found possessing the environmental characteristics that classify them as high risk for invasion should be added to the list of sites to closely monitor.

Likewise, at a global scale, habitats that fall within the high risk range of temperature and pH and have vegetation or some sort of cover present are at risk for invasion by *H. guttatus* if it were to be introduced there. Once a species has proven to be invasive in one area, the probability of it being invasive elsewhere increases, and close relation to known invaders also increases the probability that a species will be invasive (Moyle & Marchetti, 2006). Two jewel cichlid species, *H. letourneuxi* in Florida and *H. guttatus* in México, are invasive or likely invasive respectively, suggesting that the various other established populations of *Hemichromis* species globally are at high risk of becoming invasive as well. Studies assessing the potential impacts of these other introduced jewel cichlids is highly recommended.

As with any potentially invasive species however, the best way to prevent an invasion is to stop it at the transport and introduction stages. Therefore, listing of *H. guttatus* on state or national black lists, such as the Lacey Act in the United States (the federal black list for imported species), is recommended for those regions where water temperatures are in the high risk range. For those species that have been introduced and have established, however, while populations are still localized and rare (stage III), eradication may be possible through intense efforts (de Lourdes Lozano-Vilano *et al.*, 2006). After this stage, though, eradication is often not an option and the only option is to try to control and maintain populations at a size at which they do not inflict an impact on native populations (Sakai *et al.*, 2001). For those places where stage IVa, IVb or V *Hemichromis* populations already exist, manipulation of cover may be a feasible control method, however, before this method can be tested in the field, controlled studies are needed to determine what type of manipulation has the greatest effect and how it would affect native taxa where it would be implemented.

In summary, *H. guttatus* is likely a highly detrimental invader that could affect populations of native fishes through competitive, predatory and aggressive interactions.

These interactions may, in turn, cause changes to the macroinvertebrate community with unknown consequences. There are, however, options to stopping/controlling these impacts at various stages of the invasion process. First, legislature can and should be created preventing the transport (stage I) into potentially susceptible regions, and these regions can be determined based on the environmental characteristics conferring high invasion risk as defined here (Chapters 2 and 3). Second, if introduction (stage II) and subsequent establishment (stage III) do occur, intensive trapping efforts can and should be undertaken to prevent spread (stage IVa) and integration (stage IVb) and possibly to eradicate these populations. Lastly, research into the use of cover manipulation to reduce population sizes could lead to efficient control and management strategies for populations that have reached stage V and are inflicting negative impacts on their invaded communities.

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