Temperature-Specific Competition between Invasive Mosquitofish and an Endangered Cyprinodontid Fish

Gerard Carmona-Catot*, Kit Magellan, Emili García-Berthou
Institute of Aquatic Ecology, University of Girona, Girona, Catalonia, Spain

Abstract
Condition-specific competition is widespread in nature. Species inhabiting heterogeneous environments tend to differ in competitive abilities depending on environmental stressors. Interactions between these factors can allow coexistence of competing species, which may be particularly important between invasive and native species. Here, we examine the effects of temperature on competitive interactions between invasive mosquitofish, Gambusia holbrooki, and an endemic Iberian toothcarp, Aphanius iberus. We compare the tendency to approach heterospecifics and food capture rates between these two species, and examine differences between sexes and species in aggressive interactions, at three different temperatures (19, 24 and 29°C) in three laboratory experiments. Mosquitofish exhibit much more aggression than toothcarp. We show that mosquitofish have the capacity to competitively displace toothcarp through interference competition and this outcome is more likely at higher temperatures. We also show a reversal in the competitive hierarchy through reduced food capture rate by mosquitofish at lower temperatures and suggest that these two types of competition may act synergistically to deprive toothcarp of food at higher temperatures. Males of both species carry out more overtly aggressive acts than females, which is probably related to the marked sexual dimorphism and associated mating systems of these two species. Mosquitofish may thus impact heavily on toothcarp, and competition from mosquitofish, especially in warmer summer months, may lead to changes in abundance of the native species and displacement to non-preferred habitats. Globally increasing temperatures mean that highly invasive, warm-water mosquitofish may be able to colonize environments from which they are currently excluded through reduced physiological tolerance to low temperatures. Research into the effects of temperature on interactions between native and invasive species is thus of fundamental importance.

Introduction
Condition-specific competition, a process by which competition between species is mediated by abiotic factors, is widespread in nature (e.g. [1,2]). Species that inhabit spatially or temporally heterogeneous environments tend to have differing competitive abilities and varying tolerance for environmental stressors. Interactions between these factors can allow coexistence of competing species. In one scenario, for example, an inferior competitor may be excluded from part of its range, or for part of the time, by a competitively dominant species but be able to use other parts of its range, or more of its range at different times, through higher tolerance to an abiotic stress (e.g. [3,4,5]). In another scenario, a competitive reversal may occur whereby a competitively dominant species loses its advantage as conditions change along an environmental gradient and the previously subordinate species becomes dominant (e.g. [2,6,7]). Environmental gradients are particularly apparent in aquatic environments [2,8,9], which thus provide ideal situations in which to examine hypotheses concerning condition-specific competition. Several studies have investigated these phenomena in an array of taxa subject to various abiotic influences, including the effects of salinity on salt-marsh plants [6] and fish [1], hydroperiod on mosquitofish [7] and oysters [5], pH on amphibians [3], and temperature on stream fish [2,4,9].

Condition-specific competition may be particularly important when considering invasive species [5,7,10] and the dependence of competitive interactions between native and exotic species on temperature is receiving increasing interest [11,12,13,14]. Temperature is a key factor for poikilothermic organisms and in freshwater and estuarine ecosystems temperature is often considered to be one of the dominant abiotic factors regulating interspecific competition [14,15]. Moreover, growing concern regarding globally increasing temperatures means that research into the effects of temperature is of fundamental importance. In the Mediterranean region, for example, climate change models predict higher annual temperatures and longer droughts [16]. Interannual fluctuations are also expected to be more common, which would result in more exceptionally high temperature events [16]. In addition, continuing habitat alterations may lead to further increases in stream temperatures (e.g. [17]). These factors combined are likely to contribute to an expansion in range and population size of introduced warmwater fishes, and therefore increase predation rates or competitive effects on native species with preferences for cool water [18].

* E-mail: carmona.catot@gmail.com

Citation: Carmona-Catot G, Magellan K, García-Berthou E (2013) Temperature-Specific Competition between Invasive Mosquitofish and an Endangered Cyprinodontid Fish. PLoS ONE 8(1): e54734. doi:10.1371/journal.pone.0054734

Received August 23, 2012; Accepted December 14, 2012; Published January 23, 2013

Copyright: © 2013 Carmona-Catot et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: Gerard Carmona-Catot held a doctoral fellowship (BR2010/10) from the University of Girona during the preparation of the manuscript. Financial support was provided by the Spanish Ministry of Science (projects CGL2009-12877-C02-01 and Consolider-Ingenio 2010 CSD2009-00065). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.
The eastern mosquitofish, Gambusia holbrooki, is a warmwater poeciliid fish native to the United States [19]. Since its introduction to Europe in 1921, G. holbrooki has established stable populations in most Mediterranean countries [20,21]. However, G. holbrooki has not invaded northern Europe, probably because of reduced physiological tolerance, and therefore decreased competitive advantage, at lower temperatures (e.g. [20,22]). Competition from mosquitofish has likely caused the displacement of several Mediterranean fish species, in particular cyprinodontiforms, from much of their native range [23,24,25]. For example, the Iberian toothcarp (Aphanius iberus), a cyprinodontid fish endemic to the Iberian Peninsula, originally occupied most of the Alt Empordá wetlands (NE Spain). Now only isolated populations remain while most of the coastal lagoons, ditches and rivers are inhabited by introduced mosquitofish [26]. From the original 38 Mediterranean populations, 15 are extinct [27] and the toothcarp is listed as Endangered (EN A2ce) under the IUCN Red List, and protected by a number of legislative frameworks such as the Bern Convention on the Conservation of European Wildlife and Natural Habitats [28].

The objective of this study is to examine the role of water temperature in determining the outcome of interspecific competition between invasive mosquitofish and native toothcarp. As mosquitofish are known to be aggressive [19], we predicted that they would exhibit both greater aggression and initiate more encounters, and that they would restrict toothcarp’s access to food. However, as mosquitofish are a warmwater species [19] we further predicted that any competitive advantage would be more evident at warmer temperatures, while at lower temperatures toothcarp would be able to benefit from G. holbrooki’s reduced competitive ability, thus demonstrating condition-specific competition. Finally, as both of these species show marked sexual dimorphism [19,29] and males are generally more aggressive intraspecifically [30] but not always interspecifically (e.g. [31]), we expected sexual differences in aggressiveness.

**Methods**

**Ethics Statement**

All work was performed in compliance with Spanish laws of animal care and experimentation. The experiments were reviewed and approved by the Ethics Committee of the University of Girona.

**General Methods**

Fish used in our experiment were captured using dip nets in September 2011 with scientific permits issued by the relevant authority (Generalitat de Catalunya, Direcció General del Medi Natural i Biodiversitat). Adult mosquitofish came from the Ter, Fluvia and Muga rivers near Girona, Spain, and toothcarp from Fra Ramon lagoon, Baix Empordá salt marshes, Spain [19]. About 200 fish of each species were transported to the laboratory and evenly distributed without mixing species in twelve 60 L species-specific stock aquaria (31 x 31 x 33 cm) containing a gravel substrate, conditioned water, and a filtered air supply. Mosquitofish from all three rivers were housed together. Aquaria were illuminated with 6 W bulbs and maintained at a constant photoperiod (12:12 h light:dark cycle). The temperature was maintained at 24°C and fish were fed to satiation twice daily with commercial food flakes and frozen bloodworms (Chironomus spp.). Fish were adapted to laboratory conditions for at least four weeks prior to the start of temperature acclimation.

The temperature acclimation protocol was conducted in the same 12 aquaria, two for each species at each temperature, and consisted of the progressive adjustment of temperature using aquarium heaters until the three experimental temperatures (19, 24 and 29, ±0.2°C) were reached. These temperatures were selected because they are typical of the range of midsummer water temperatures found in Iberian coastal lagoons (e.g. [32]). Temperature was measured using digital thermometers placed inside the aquaria. After five days, all fish were at the necessary experimental temperature and were maintained at these conditions for at least 14 days before the start of observations. Mortality during acclimation was low (less than 5%) and only one fish died during observations. This trial was restarted after the fish was replaced. Fish acclimated to a specific temperature treatment were maintained at that temperature throughout the experimental period.

Observations were conducted in three 26 L aquaria (45 x 28 x 22 cm) also maintained at 19, 24 and 29°C respectively. Aquaria contained 2 cm of gravel substrate, were filled to a depth of 20 cm with conditioned water and were illuminated by 6 W lights. Dark plastic was attached to the back and sides of the aquaria to minimize disturbance. A removable, transparent methacrylate wall pierced with small holes (216 holes in 12 columns) divided each aquarium into two sides. During the afternoon before observations, fish were placed in the experimental aquaria at the same temperature as their respective acclimation temperatures. Two mosquitofish (visually size matched) of the same sex (50% of trials with males and 50% with females) were placed on one side of the methacrylate divider. Same sex mosquitofish were used to reduce the incentive for male-male competition over females. One toothcarp was randomly selected and its pair was then size matched; both fish were placed on the other side of the divider. The side for each species was swapped in successive trials. The methacrylate divider allowed the two species to visually and chemically respond to each other while preventing physical contact. Fish were fed to satiation with frozen bloodworms and uneaten prey were removed from the experimental aquaria. No food was provided to the experimental fish for at least 20 hours before observations. The series of experimental tests (i.e. Test 1, Test 2 and Test 3) were conducted sequentially the following day. To ensure that individual fish were used only once during the experiments, they were placed into post-experimental aquaria maintained at their specific acclimation temperature after the trials. Each of the three temperature treatments (19, 24 and 29°C) had 30 replicates (i.e. a total of 90 replicates with 360 different fish). All trials were videotaped (two sample videos at contrasting temperatures are provided in Movie S1 and Movie S2).

In test 1, we examined the tendency for mosquitofish and toothcarp to investigate and approach heterospecifics as a function of temperature. Observations began when the methacrylate divider was gently raised to the surface. Every care was taken to avoid disturbing the fish. We recorded the species and the time taken for the first fish to cross to the other side of the aquarium (specifically when the head or tail crossed the center line) and for the first fish to approach within one body length of the other species.

In test 2, following test 1, we studied the effects of temperature and sex on the agonistic interactions between mosquitofish and toothcarp. We waited five minutes after we raised the methacrylate divider to ensure that all fish were behaving normally and then conducted 10-minute observations recording the number of orientations (fish orienting itself and swimming towards another fish), nips (one fish attempts or succeeds at biting another) and chases (rapid chase of one fish by another). We conducted focal
watches of one randomly selected fish per species sequentially, recording the sex of the fish observed for each species.

Test 3 immediately followed test 2. Here we assessed the effects of temperature on food competition between toothcarp and mosquitofish. Four bloodworms were placed at 10 cm intervals on a thin piece of wire and were carefully released at the water surface. Bloodworms were used because they are common prey items in the diet of the two species [33,34]. We recorded the time taken to eat the first prey item and the species that consumed each of the four prey items. Any bloodworms that remained after five minutes were recorded as uneaten.

To assess the tendency for toothcarp and mosquitofish to investigate and approach conspecifics we used generalized linear models (GLMs) in a factorial design with two categorical factors, temperature and species. To analyze the proportion of each species over all trials for each temperature that were first to carry out these behaviors we used separate $\chi^2$ tests for each variable. For the agonistic variables we used separate GLMs for each species and each variable (orientations, nips, and chases) with two categorical factors, temperature and sex. For the last experiment, we also used separate analyses for each species and GLMs for the proportion of prey eaten and the time taken to capture the first prey item with temperature as the single factor. In GLMs, we always used Poisson errors and log-link functions for count variables (i.e. number of nips, chases, and orientations), normal distributions and identity-link functions for time variables and binomial errors and logit-link functions for the proportion of prey eaten. Finally, we conducted two $\chi^2$ tests to assess the difference in the proportion of trials in which each species was the first to capture a prey item. First we included the uneaten prey items and second this category was excluded. All statistical analyses were conducted using the software SPSS 15.

Results

In test 1, the time taken for the first fish to cross the center line of the aquarium (GLM $\chi^2 = 19.4$, d.f. = 2, $P<0.001$) and the time taken for the first fish to approach within one body length of a heterospecific (GLM $\chi^2 = 13.5$, d.f. = 2, $P=0.001$) both decreased significantly with increasing temperature (Figure 1). However, there was no difference between species in the time taken to carry out either of these behaviors (cross: GLM $\chi^2 = 2.43$, d.f. = 1, $P=0.119$; approach: GLM $\chi^2 = 0.086$, d.f. = 1, $P=0.769$), nor were the interactions significant (cross: GLM $\chi^2 = 4.51$, d.f. = 2, $P=0.105$; approach: GLM $\chi^2 = 4.86$, d.f. = 2, $P=0.088$). For the proportion of trials in which each species was the first to carry out these behaviors, toothcarp both crossed the center line first and approached a heterospecific for 19°C, while this response was reversed at higher temperatures (cross: $\chi^2 = 8.30$, d.f. = 2, $P=0.016$; 19°C, 22 toothcarp:mosquitofish, 24°C, 15:13, 29°C, 12:20; approach: $\chi^2 = 7.23$, d.f. = 2, $P=0.027$; 19°C, 20:10, 24°C, 11:19, 29°C, 11:19).

In test 2, mosquitofish exhibited much more aggression than toothcarp, with the majority of aggressive behavior being performed by mosquitofish towards toothcarp (84.02%), whereas only 15.98% was conducted by toothcarp towards mosquitofish. Aggression in both species varied significantly across temperatures (Table 1), with both species showing increased aggression with increasing temperature (Figure 2). There was also a significant difference between the sexes (Table 1). Males of both species exhibited more of all three of the recorded aggressive behaviors than females. Moreover, orientations appear to be the preferred behavior for females while males carried out relatively more nips to the extent that at the highest temperature the frequency of nips equaled or exceeded that of the other behaviors (Figure 2). Temperature × sex interactions were significant for almost all the behavioral variables (Table 1), with the exception of chases performed by toothcarp as female toothcarp did not carry out this behavior. Particularly, toothcarp males changed their preferred behavior type at 29°C from orientations to nips and particularly striking were the differing effects of temperature on male and female mosquitofish. Males exhibited the greatest increase in behaviors performed between 19 and 24°C while for females the major increase in behavior occurred at a higher temperature, between 24 and 29°C (Figure 2).

In test 3 the proportion of prey items captured increased with temperature for both species. However, this relationship was significant only for mosquitofish (mosquitofish: GLM $\chi^2 = 48.2$, d.f. = 2, $P<0.001$; toothcarp: GLM $\chi^2 = 3.05$, d.f. = 2, $P=0.218$; Figure 3). The time required to capture the first prey item decreased substantially between the lowest and highest temperatures (19°C: 29.9±57.1 s, 24°C: 56.2±83.4 s, 29°C: 8.6±15.4 s; mean ± s.d.) although this relationship was not straightforward and was only marginally significant (GLM $\chi^2 = 5.92$, d.f. = 2, $P=0.052$). There was no significant difference between species (GLM $\chi^2 = 3.41$, d.f. = 1, $P=0.52$; toothcarp: 19.0±36.9 s; mosquitofish: 42.8±76.5 s; mean ± s.d.) nor a significant interaction (GLM $\chi^2 = 0.236$, d.f. = 2, $P=0.89$). When all trials at each temperature were considered together, at 19°C toothcarp captured the first prey item significantly more often than mosquitofish but this relationship was reversed for 24 and 29°C ($\chi^2 = 25.2$, d.f. = 4, $P<0.001$). However, this result was mainly due to the inclusion of uneaten prey items (toothcarp:mosquitofish:uneaten; 19°C, 13:61:1; 24°C, 11:18:1; 29°C, 13:17:0). When this variable was removed the relationship between species and temperature was no longer significant ($\chi^2 = 4.61$, d.f. = 2, $P=0.11$).
This study provides evidence for condition-specific competition, both through interference and exploitation, between eastern mosquitofish and Iberian toothcarp, via temperature-mediated changes in competitive abilities. Mosquitofish carried out close to five times as many aggressive acts as toothcarp, and while both species exhibited increased aggression at higher temperatures, this increase was considerably greater for mosquitofish than for toothcarp. Mosquitofish thus have the potential to competitively displace toothcarp through interference competition, and the strength of this interaction is likely to increase at higher water temperatures. Increased aggression at higher temperatures has been proposed as a major factor explaining the relative distribution of several fish species along longitudinal stream gradients [2,4,9]. For example, brook trout (Salvelinus fontinalis) were competitively dominant over cutthroat trout (Oncorhynchus clarki) at higher temperatures (20 versus 10°C), which was related to separation of these species into warmer, downstream (brook trout) and cooler, upstream (cutthroat trout) stretches of river [4]. In another study, brook trout were themselves subject to competitive exclusion by creek chub (Semotilus atromaculatus) at a slightly higher temperature (22°C) resulting in similar upstream-downstream species distributions [2]. In our study, temperature variation occurs more over a temporal rather than spatial scale,

**Figure 2.** Aggressive acts (orientations, nips and chases) performed by Gambusia holbrooki towards Aphanius iberus and vice versa under the different temperature treatments and species combinations. Means and SE (error bar) are shown. Note the different scales on the y-axis for each species. doi:10.1371/journal.pone.0054734.g002

**Table 1.** Generalized linear models for selected response variables (i.e. orientations, nips, chases) of Gambusia holbrooki and Aphanius iberus using temperature and sex as predictors.

| Species   | Orientations | Nips | Chases |
|-----------|--------------|------|--------|
| **Source of variation** | **Gambusia** | **Aphanius** | **Gambusia** | **Aphanius** |
| Temperature | 300.29*** 149.72*** 82.31*** | 35.47*** 31.56*** 37.46*** | 16.46*** 64.81*** 7.36 ** | 16.46*** 26.95*** – |
| Sex | 16.46*** 64.81*** 7.36 ** | 16.46*** 26.95*** – | 13.69** 11.88** – | 13.69** 11.88** – |
| Temperature × Sex | 75.33 *** 24.05*** 11.70** | 75.33 *** 24.05*** 11.70** | 75.33 *** 24.05*** 11.70** | 75.33 *** 24.05*** 11.70** |

Values are \( \chi^2 \).

*** indicates \( P < 0.001; \)

** indicates \( P < 0.01; \)

d.f. are 2 for temperature, 1 for sex, and 2 for their interaction.

doi:10.1371/journal.pone.0054734.t001

**Figure 3.** Effects of temperature on the number of prey items captured by Gambusia holbrooki and Aphanius iberus. Means and SE (error bar) are shown. Note that the totals for both species for 19 and 24°C do not equal 100%. This reflects the prey that remained uneaten. doi:10.1371/journal.pone.0054734.g002

**Discussion**

This study provides evidence for condition-specific competition, both through interference and exploitation, between eastern mosquitofish and Iberian toothcarp, via temperature-mediated changes in competitive abilities. Mosquitofish carried out close to five times as many aggressive acts as toothcarp, and while both species exhibited increased aggression at higher temperatures, this increase was considerably greater for mosquitofish than for toothcarp. Mosquitofish thus have the potential to competitively displace toothcarp through interference competition, and the...
though microhabitat segregation of the two species through competitive interactions is also likely.

The time taken for the fish to both approach a heterospecific and to cross the center line of the experimental aquaria decreased with increasing temperatures although there were no differences between species. However, the frequency with which toothcarp were the first to cross the center line and approach a heterospecific was greater at 19°C, while at higher temperatures this situation was reversed and mosquitofish predominated. This seems to indicate a competitive reversal with toothcarp dominating at lower temperatures and mosquitofish superior at higher temperatures. However, the function of this approach behavior must be taken into consideration. The assumption that approaching a heterospecific is a prelude to attacking that individual is just one of a number of possibilities. For example, closer contact may be necessary to inspect a potential predator and hence assess the degree of threat [35,36]. Alternatively, approaching other individuals may simply be a result of a predisposition for schooling behavior, as mixed species shoals are not uncommon [37,38]. Whatever the purpose of this behavior, it is clear that toothcarp exhibited increased activity relative to mosquitofish at lower temperatures.

Temperature also influenced the potential for exploitative competition. The time taken to capture the first prey item decreased and the proportion of prey items captured by both species increased, with increasing temperature. However, there was no difference between species in capture time and the proportional increase in prey capture was significant only for mosquitofish. Furthermore, while toothcarp captured the first prey item more often at 19°C, this situation was reversed at 24 and 29°C, providing another example of potential competitive reversal. This situation relates to varying total food consumption by both species. Toothcarp captured the first food item with approximately the same frequency at all temperatures while mosquitofish increased their capture frequency at higher temperatures. Therefore, rather than toothcarp being more dominant at lower temperatures, they appear to benefit from reduced exploitative competition from mosquitofish. Release from dominance by a competitively superior species appears to be a common factor in competitive reversal. For example, in the brook trout – cutthroat trout system outlined above both species were nearly equal competitors at 10°C with brook trout becoming dominant only at the higher temperature [4]. A parallel pattern was shown in another study with the white-spotted char (*Salvelinus leucomaenis*) and the Dolly Varden char (*S. malma*) foraging equally well at lower temperatures but the former becoming dominant at a higher temperature [9]. A final consideration is that both these forms of competition, exploitative and interference, may be operating concurrently as in aggression to defend a food resource [39,40]. Thus at higher temperatures mosquitofish have the capacity to restrict toothcarp access to food through exploitative competition and if food was limited, as is often the case, mosquitofish are likely to outcompete toothcarp through interference competition as well.

While aggression in the laboratory does not necessarily imply competition in nature, in this case it is likely. Although interference competition is often more influential and clearer than exploitative competition [41], both types of competition can occur concurrently and interactively and may be difficult to distinguish [42]. *G. affinis* and *G. holbrooki* are well known to produce severe fin damage through nips, which can result in several adverse effects on recipient species [43,44]. For example, swimming performance is likely to be reduced with potential consequent reduction in reproductive success and increased predation risk. Damage is costly in terms of regeneration effort and can increase susceptibility to disease [45,46]. Fin damage can also result in changes in behaviour and prey consumption by the subordinate species [44,47]. In the current study, the increase in aggression together with greater food capture efficiency shown by mosquitofish at higher temperatures indicates that mosquitofish have the capacity to outcompete toothcarp. Moreover, mosquitofish now dominate many of the habitats that were previously occupied by toothcarp [26] and competition is one of the likely mechanisms by which this has occurred.

There was a difference between males and females of both species in both the amount and type of behavior carried out, and for mosquitofish the temperature at which differences became apparent. Males of both species exhibited much more aggression than females. Moreover, females appear to prefer to engage in orientation behavior while males carry out more nips, particularly at higher temperatures, which is arguably a more aggressive behavior than merely observing another fish. These behavioral differences between sexes are likely to be associated with other differences. For example, in many animals, including fish, larger individuals initiate and receive less aggression [48,49]. Both species in this study showed a marked sexual dimorphism with larger females and smaller males [19,29] so this may account for some of the observed difference. In addition, females tend to be more sociable and engage in more shoaling than males [50], an activity incompatible with a high intensity of aggression. Finally, differences in aggression between the sexes may be an indirect consequence of the mating behaviors of these species [50]. Mating in mosquitofish is characterized by male coercion of females via sneaky mating, in which males attempt to insert their intromittent organ into the female’s genital opening by force and males compete aggressively for access to females [51,52]. While reproductive behavior in toothcarp is less well studied, males do court females and will chase away rival males [53]. This may result in male predisposition for aggression [50], which is utilized to the detriment of heterospecifics. Because the temperatures used in this study were typical of the breeding season of both these species [54,55], this effect may be intensified. Although male and female mosquitofish were not tested together in this study, behavior related to reproduction is likely to persist. An interesting result from this study is that male mosquitofish increased their level of aggression at 24°C, while females did not show a similar increase until 29°C. Males show a peak plateau in mating behavior in a comparable temperature range [36] though mating behavior was not quantified in our study. It also may be that males prefer cooler temperatures than females as is the case in two closely related species, *Poecilia sphenops* [57] and *Poecilia reticulata* [58]. Whatever the cause, for females their peak of maximum activity is either shifted to higher temperatures or is narrower compared to males, a factor which may influence the relative impact of males and females on toothcarp.

Temperature may have other effects that can interactively influence aggression. For example, the metabolic rate of ectotherms increases with increasing temperature [e.g. [59]], facilitating increased aggression. However, aggression itself is energetically costly [60] increasing metabolic rate still further, which probably accounts for the rise in food consumption at higher temperatures observed in this study. Another interacting factor is swimming speed which also increases with increasing temperature [e.g. [56]], which again will facilitate intensified aggression and again increase metabolic rate. In addition to these immediate effects, temperature variation may have long term consequences. In this study, fish were allowed to acclimate for four weeks. A longer duration of acclimation, can affect for example growth rate [47] and size at maturity [61]. Finally, temperature
itself may interact with other factors, such as water velocity [62] and salinity [25] to influence aggressive activity. Mosquitofish have been introduced worldwide [61,63] with far reaching effects on native species (reviewed in [64]) and are considered one of the 100 worst invasive species [65]. Therefore, any factor that may aid in ameliorating their effects should be investigated. The influence of temperature on interactions with mosquitofish has been examined in relation to several native species. For example, G. holbrooki aggression towards two Iberian toothcarp species (A. iberus and Valencia hispanica) increased at higher temperatures [23] and increased aggression with temperature has been shown by the closely related G. affinis towards Galaxias maculatus in New Zealand [43] and the least chub, Iothichthys phlegethontis in the USA [31], with effects on the survival of these native fish. In the current study, mosquitofish aggression may have immediate, medium and longer term consequences for toothcarp. In addition to disrupting normal conspecific interactions, mosquitofish can cause considerable fin damage [43,44] and mortality, especially of juveniles [23,43]. Injury, along with decreased food intake [24] and reduced growth rates [47] can lead to increased stress and susceptibility to illness [45,46]. The temperatures used in this study are typical of breeding season temperatures for toothcarp, which is characterized by early offspring that can mature enough to breed later in the summer and late offspring that may overwinter and breed the following year [55]. Restriction of food and disruption of conspecific interactions is likely to reduce the breeding success of early offspring and overwinter survival of poor condition, late offspring could also be reduced. This in turn could result in changes in population demographics (e.g. [31]) through a decline in population density or a shift in breeding season and to the displacement of native species to non-preferred habitats (e.g. [43]). Climate change implies that investigating these types of temperature-mediated interactions between invasive and native species will be increasingly critical to aid in conservation efforts.

Supporting Information

Movie S1 Movie showing an experimental trial at 29°C. (MP4)

Movie S2 Movie showing an experimental trial at 19°C. (MP4)

Acknowledgments

We gratefully thank Carles Alcaraz, Enrie Aparicio, Bruce Hmack and Irene Ylla for their helpful comments, advice and help, and Esther Pérez for assistance with the experiments. We also thank Professor Angelo Bisazza and his lab for introducing GCC to behavioral studies. GCC received all types of support from Joesp Carmona and Joana Catot. Finally, we thank the two anonymous referees whose comments helped improve the quality of this manuscript.

Author Contributions

Conceived and designed the experiments: GCC KM EGB. Performed the experiments: GCC. Analyzed the data: GCC KM EGB. Contributed reagents/materials/analysis tools: GCC KM EGB. Wrote the paper: GCC KM EGB.

References

1. Dunson WA, Travis J (1991) The role of abiotic factors in community organization. Am Nat 138: 1067–1091.
2. Taniguchi Y, Rahel FJ, Nовinger DC, Gerow KG (1996) Temperature mediation of competitive interactions among three fish species that replace each other along longitudinal stream gradients. Can J Fish Aquat Sci 53: 1094–1001.
3. Warner S, Travis J, Dunson WA (1993) Effect of pH variation on interspecific competition between two species of hylid tadpoles. Ecology 74: 183–194.
4. De Stasio J, Rahel B (1996) Influence of water temperature on interactions between juvenile Colorado River cutthroat trout and brook trout in a laboratory stream. J Am Fish Soc 123: 289–297.
5. Kraosi FR, Brown KR, Bishop MJ, Kelaher BP, Summerhayes S (2008) Competition-specific competition allows coexistence of competitively superior exotic oysters with native oysters. J Anim Ecol 77: 5–13.
6. Green LA Perey MK, Grace JB, Hahn E, Mendelsohn IA (2001) The importance of competition in regulating plant species abundance along a salinity gradient. Ecology 82: 62–69.
7. Costanzo KS, Kesavaraj B, Juliano SA (2005) Condition-specific competition in container mosquitos: the role of noncompeting life-history stages. Ecology 86: 3293–3295.
8. Dunson WA, Travis J (1994) Patterns in the evolution of physiological specialization in a marsh marsh animals. Estuaries 17: 102–110.
9. Taniguchi Y, Nakano S (2000) Condition-specific competition: implications for the altitudinal distribution of stream fishes. Ecology 81: 2027–2039.
10. Hobday DA, Suarez AV, Case TJ (2002) Role of abiotic factors in governing susceptibility to invasion: a test with argentine ants. Ecology 83: 1610–1619.
11. Fober E, Fox MG, Ridgway M, Coggi GH (2011) Heated competition: how climate change will affect non-native pumpkimseed Leptus gibbus and native perch Perca fluviatilis interactions in the UK. J Fish Biol 79: 1592–1607.
12. Wensger SJ, Isaak DJ, Luce CH, Neville HM, Fauch KJ, et al. (2011) River flow, temperature, and biotic interactions drive differential declines of trout species under climate change. Proc Natl Acad Sci U S A 108: 14175–14180.
13. Côte BM, Green SJ (2012) Potential effects of climate change on a marine invasion: The importance of current context. Acta Zool Sinica 58: 1–10.
14. Lapointe NVR, Light T (2012) Landscape-scale determinants of non-native fish communities. Divers Distrib 18: 282–293.
15. Ouygi DO, Cucherouset J, Robert Britton J (2012) Temperature-dependent feeding interactions between two invasive fishes competing through interference and exploitation. Rev Fish Biol Fisher 22: 499–513.
16. Giorgi F, Lionello P (2008) Climate change projections for the Mediterranean region. Global Planet Change 63: 98–104.
17. Gisasi D (2006) The thermal regime of rivers: a review. freshwater Biol 51: 1309–1346.
18. Rahel FJ, Olden JD (2008) Assessing the effects of climate change on aquatic invasive species. Conserv Biol 22: 521–533.
19. Mee FJ, F.S (1989) Ecology and evolution of living beetles (Poecilidae). New York: Princen Hall.
20. Benezain L, Alcaraz C, Sasal P, Simon-Levert G, García-Berthou E (2009) Life history and parasites of the invasive mosquitofish (Gambusia holbrooki) along a latitudinal gradient. Biol Invasions 11: 2265–2277.
21. Vidal O, García-Berthou E, Tedesco PA, García-Marin J-L (2009) Origin and genetic diversity of mosquitofish (Gambusia holbrooki) introduced to Europe. Biol Invasions 12: 841–851.
22. Carmona-Catot G, Benito J, García-Berthou E (2011) Comparing latitudinal and upstream-downstream gradients life history traits of invasive mosquitofish. Divers Distrib 17: 214–224.
23. Rincón PA, Correas AM, Morcillo F, Rusario P, Lobón-Cerviá J (2002) Interaction between the introduced eastern mosquitofish and two autochthonous Spanish toothcarps. J Fish Biol 61: 1560–1365.
24. Caioa N, de Sostoa A (2005) Possible reasons for the decline of two native toothcarps in the Iberian Peninsula: evidence of competition with the introduced Gambusia holbrooki. J Appl Ichthyol 21: 358–363.
25. Alcaraz C, Bisazza A, García-Berthou E (2008) Salinity mediates the competitive interactions between invasive mosquitofish and an endangered fish. Oecologia 155: 205–213.
26. Moreno-Amach M, Pou Q, Quintana X, García-Berthou E (1999) Monografía sobre los peces cyprinodontidos ibéricos (ace). Ambiente MdM, editor. Madrid, Spain.
27. Doadrio I (2002) Atlas y libro rojo de los peces continentales de España; Generalitat Valenciana 115–131.
28. Doadrio I (1996) The thermal regime of rivers: a review. freshwater Biol 51: 1309–1346.
29. Oliva-Paterna FJ, Torralva M, Fernández-Delgado C (2006) Threatened fishes of the world: Aphanopus carinatus (Cuvier & Valenciennes, 1816) (Cyprinodontidae). Environ Biol Fish 75: 307–309.
30. Andresson M (1994) Sexual selection. Princeton, New Jersey: Princeton University Press.
31. Priddis E, Rader R, Belk M, Schadde B, Merley S (2009) Can separation along the temperature niche axis promote coexistence between native and invasive species? Divers Distrib 15: 682–691.
32. Oliva Paterna FJ, Torralba Forero M (2008) El fartet en la Región de Murcia: biología y conservación. Murcia, Spain: Dirección General del Medio Natural. Consejería de Desarrollo Sostenible y Ordenación del Territorio.

33. Vargas MJ, de Sostoa A (1997) Life-history pattern of the Iberian toothcarp Aphanius iberus (Pisces, cyprinodontidae) from a Mediterranean estuary, the Ebro delta (Spain). Neth J Zool 47: 143–160.

34. Alcaraz C, García-Berthou E (2007) Food of an endangered cyprinodont (Aphanius iberus): endogenous diet shift and prey elictivity. Environ Biol Fish 76: 195–207.

35. Botham MS, Kerfoot CJ, Losca V, Krause J (2006) The effects of different predator species on antipredator behavior in the Trinidadian guppy, Poecilia reticulata. Naturwissenschaften 93: 431–439.

36. Seda JB, Childres MJ, Ptacek MB (2012) Individual variation in male size and behavioral repertoire in the saflm molly Poecilia latipinnis. Ethology 118: 411–421.

37. Ward A, Axford S, Krause J (2002) Mixed-species schooling in fish: the sensory mechanisms and costs of shoal choice. Behav Ecol Sociobiol 52: 182–187.

38. Karplus I, Zhou B, Rosenfeld L, Grinshpun Y, Slomson T, et al. (2007) Social facilitation of learning in mixed-species schools of common carp Cyprinus carpio L. and Nile tilapia Oreochromis niloticus (L.). J Fish Biol 71: 1023–1034.

39. Huntingford F, Turner A (1987) Animal conflict. London: Chapman and Hall.

40. Ashley PJ (2007) Fish welfare: Current issues in aquaculture. Appl Anim Behav Sci 104: 199–235.

41. Case TJ, Gilpin ME (1974) Interference competition and niche theory. Proc Natl Acad Sci USA 71: 3073–3077.

42. Schoener TW (1983) Field experiments on interspecific competition. Am Nat 122: 240–285.

43. Rowe DK, Smith JP, Baker C (2007) Agonistic interactions between Aphanius iberus (Pisces, cyprinodontidae) from a Mediterranean estuary, the Ebro delta (Spain). Neth J Zool 47: 143–160.

44. Sinclair ELE, Ward AJW, Seebacher F (2011) Aggression-induced fin damage and habitat use in the common carp Cyprinus carpio: a potential threat to the conservation of remnant populations. J Appl Ichthyol 23: 668–674.

45. Portz DE, Woodley CM, Cech JJ (2006) Stress-associated impacts of short-term holding on fishes. Rev Fish Biol Fisher 16: 125–170.

46. Ashely PJ (2007) Fish welfare: Current issues in aquaculture. Appl Anim Behav Sci 104: 199–235.

47. Laha M, Mattingsly HT (2006) Identifying environmental conditions to promote species coexistence: an example with the native Barrens topminnow and invasive western mosquitofish. Biol Invasions 8: 719–725.

48. Arnott G, Echelle RW (2009) Assessment of fighting ability in animal contests. Anim Behav 77: 991–1004.

49. Reddon AR, Voisin MR, Menon N, Marsh-Rollo SE, Wong MYL, et al. (2011) Reproduction of the endangered killifish Aphanius iberus at different salinities. Environ Biol Fish 57: 113–115.

50. Magurran A, Macias GC (2000) Sex differences in behaviour as an indirect consequence of mating system. J Fish Biol 57: 839–857.

51. Pilastro A, Benetton S, Bisazza A (2003) Female aggregation and male competition reduce costs of sexual harassment in the mosquitofish Gambusia holbrooki. Anim Behav 65: 1161–1167.

52. Smith CC, Sargent RC (2006) Female fitness declines with increasing female density but not male harassment in the western mosquitofish, Gambusia affinis. Anim Behav 71: 401–407.

53. Oliva R, Tosdol R (2006) Production of the endangered killifish Aphanius iberus. Environ Biol Fish 57: 113–115.

54. Vargas MJ, de Sostoa A (1996) Life history of Gambusia holbrooki (Pisces, Poeciliidae) in the Ebro delta (NE Iberian peninsula). Hydrobiologia 341: 215–224.

55. Navarro AR, Oliva Paterna FJ (2012) Fartet - Aphanius iberus. In: Salvador A, Elvira B, editors. Museo Nacional de Ciencias Naturales, Madrid: Enciclopedia Virtual de los Vertebrados Españoles.

56. Wilson RS (2005) Temperature influences the coercive mating and swimming performance of male eastern mosquitofish. Anim Behav 70: 1387–1394.

57. Hernández M, Buckle LF, Espina S (2002) Temperature preference and acclimatization in Poecilia reticulata (Pisces, Poeciliidae). Aquac Res 33: 933–940.

58. Johansen PH, Cross JA (1980) Effects of sexual maturation and sex steroid hormone treatment on the temperature preference of the guppy, Poecilia reticulata (Peters). Can J Zool 58: 506–508.

59. Lang B, Rall BC, Brose U (2012) Warming effects on consumption and intraspecific interference competition depend on predator metabolism. J Anim Ecol 81: 516–523.

60. Neat FC, Taylor AC, Huntingford FA (1998) Proximate costs of fighting in male cichlid fish: the role of injuries and energy metabolism. Anim Behav 55: 875–882.

61. Magellan K, Magurran AE (2006) Habitat use mediates the conflict of interest between the sexes. Anim Behav 72: 73–81.

62. Magellan K, Pettersson LB, Magurran AE (2005) Quantifying male attractiveness and mating behaviour through phenotypic size manipulation in the Trinidadian guppy, Poecilia reticulata. Behav Ecol Sociobiol 58: 366–374.

63. García-Berthou E, Alcaraz C, Pou-Rovira Q, Zamora L, Coenders G, et al. (2005) Introduction pathways and establishment rates of invasive aquatic species in Europe. Can J Fish Aquat Sci 62: 453–463.

64. Pyke GH (2008) Plague minnow or mosquito fish? A review of the biology and impacts of introduced gambusia species. Annu Rev Ecol Syst 39: 171–191.

65. Lowe S, Browne M, Boudjelas S, De Poorter M (2006) 100 of the world’s worst invasive alien species. A selection from the global invasive species database New Zealand: The Invasive Species Specialist Group (ISSG), a specialist group of the Species Survival Commission (SSC) of the World Conservation Union (IUCN). 12 p.