



Universitat de Girona

EXPLAINING THE INVASIVE SUCCESS OF THE EASTERN MOSQUITOFISH (GAMBUSIA HOLBROOKI): THE ROLE OF TEMPERATURE

Gerard CARMONA-CATOT

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THE EASTERN MOSQUITOFISH (*GAMBUSIA
HOLBROOKI*): THE ROLE OF TEMPERATURE**



Gerard Carmona-Catot





Universitat de Girona
Institut d'Ecologia Aquàtica

PhD Thesis

**Explaining the invasive success of the eastern mosquitofish
(*Gambusia holbrooki*): the role of temperature**

Gerard Carmona-Catot

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Dr. Emili García-Berthou
Institut d'Ecologia Aquàtica
Universitat de Girona

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Contact information

Gerard Carmona-Catot

carmona.catot@gmail.com

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- Carmona-Catot G., Benito J. & García-Berthou E. 2011. Comparing latitudinal and upstream-downstream gradients: life-history traits of invasive mosquitofish. *Diversity and Distributions* 17: 214–224.

- Àrea temàtica: Biodiversity Conservation

- Factor d' Impacte (any 2012): 6.122

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- Carmona-Catot G., Magellan K. & García-Berthou E. 2013. Temperature-specific competition between invasive mosquitofish and an endangered cyprinodontid fish. *PLoS ONE* 8: e54734.

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SUMMARY; RESUMEN; RESUM

SUMMARY

The variation of biological traits along spatial gradients is among the most important topics of ecology and biogeography. Much research has been done to understand biological patterns along latitude, such as the increase in species richness towards the equator and the increase in body size of species towards higher latitudes or lower temperatures (Bergmann's rule). With the exception of body size, the variation of life-history traits with latitude has been little studied, especially for freshwater fish, despite being crucial to estimate the effects of climate change and their interactions with biological invasions in aquatic ecosystems.

The lack of information regarding spatial variation in life-history traits of fish is also the case for the invasive mosquitofish (*Gambusia holbrooki*), despite recent evidence that invasive success in Europe has been limited partly along spatial gradients.

In the first thesis chapter, we simultaneously sampled along latitudinal and upstream-downstream gradients to evaluate the variation in the life history of invasive mosquitofish. We found that mosquitofish inhabiting environments more favorable to them (i.e. lower latitudes and lower reaches) presented higher reproductive effort and lower body condition, but these patterns explained low percent variation, were nonlinear, and depended on fish age. In addition, the intraspecific version of Bergmann's rule or its converse did not apply to the mosquitofish populations that we studied since mean size-at-age and overall body size did not vary with latitude. In conclusion, we suggest that for life-history traits of freshwater organisms, river longitudinal variation plays a role as important as climate, with often differential effects.

Many ecosystems show large variation across seasons, from biotic characteristics such as species abundance and distribution to temperature and

photoperiod. How seasonality varies along latitudinal gradients is not well understood in freshwater ecosystems, despite being important to predict the effects of climate change. Since fish and most stream organisms are ectotherms, ambient temperatures significantly affect their physiological rates and consequently growth rates. Seasonal variation in fish growth is generally related to changes in water temperature and food resources. **In the second thesis chapter**, we evaluated the extent of seasonality along a latitudinal gradient on a range of variables, from water and air temperatures to physicochemical properties of water, and their relationship with the growth of mosquitofish. We found that most variation in air temperature is explained by latitude and season; however, mosquitofish growth and water features of lowland streams depended more strongly on local factors than latitude and season, even in the case of water temperature. We concluded that latitudinal differences in temperature have the potential to cascade through stream ecosystems influencing water properties and organisms, although local factors and complex interactions often override them.

Mosquitofish is among the most widely introduced fish species worldwide and listed among the world's 100 worst invasive species by the Invasive Species Specialist Group, IUCN (ISSG 2006). There is much research reporting their deleterious impacts on native species and ecosystem processes. There are also many studies on how temperature affects fish ecology and biology, and whether it mediates behavioral interactions. However, the role of temperature on the competition outcome between two species is context-dependent and experimental testing is required. Previously, we explained how mosquitofish life history traits changed along spatial and temporal gradients in which there was variation in variables such as air and water temperatures. **In the third thesis chapter**, we experimentally investigated the role of temperature (19, 24, 29 °C) on the agonistic behaviour and food competition between mosquitofish and

an endangered, endemic fish (fartet, *Aphanius iberus*) that have similar habitat and food uses. We demonstrated that mosquitofish increased its aggressive behaviour towards fartet and captured more prey, increasing its efficiency, with higher temperatures. We also showed a reversal in the competitive hierarchy through reduced food capture rate by mosquitofish at lower temperatures. We suggest that with the ongoing increasing temperatures, invasive mosquitofish might be able to expand their range as streams warm.

RESUMEN

La variación del ciclo vital de los organismos a lo largo de gradientes espaciales es uno de los temas más importantes en ecología y biogeografía. Se ha llevado a cabo mucha investigación para entender los patrones biológicos a lo largo de la latitud, como por ejemplo el aumento de la riqueza de especies hacia el ecuador, o el aumento del tamaño corporal de las especies hacia latitudes más altas o temperaturas más bajas (regla de Bergmann). Aparte del tamaño corporal, las variaciones de la mayoría de rasgos del ciclo vital con la latitud han sido poco estudiadas, sobre todo en los peces de agua dulce, siendo crucial para estimar los impactos del cambio climático y sus interacciones con las invasiones biológicas.

Esta falta de información de la variación espacial de los rasgos del ciclo de vida de los peces también afecta a la gambusia (*Gambusia holbrooki*), aunque hay ciertas evidencias de que su éxito invasor en Europa ha sido desigual a lo largo de gradientes espaciales. **En el primer capítulo**, se hizo un muestreo simultáneo a lo largo de un gradiente latitudinal y de gradientes a lo largo de cursos de ríos para entender mejor la variación del ciclo vital de una de las especies más invasoras mundialmente, la gambusia. Se encontró que la gambusia presentaba un mayor esfuerzo reproductivo y una menor condición corporal en ambientes más favorables (es decir, latitudes bajas y zonas bajas de los ríos); sin embargo, estos patrones de variación explicaban un porcentaje bajo de la variación total, no eran lineales y dependían de la edad de los peces. Además, la versión intraespecífica de la regla de Bergmann o su inverso no es aplicable a las poblaciones de gambusia estudiadas ya que la media del tamaño corporal, para cada edad, y el tamaño corporal en general, no varían con la latitud. En conclusión, se sugiere que para los rasgos de ciclo vital de los organismos de agua dulce, la

variación longitudinal de los ríos tiene un papel tan importante como el clima, a menudo con efectos diferenciales.

Muchos ecosistemas presentan una gran variación estacional, en variables desde la temperatura y el fotoperíodo hasta características biológicas tales como la abundancia y distribución de especies. Cómo varía la estacionalidad a lo largo de la latitud no está todavía bien entendido en los ecosistemas de agua dulce, a pesar de ser importante para predecir los efectos del cambio climático. Dado que los peces y la mayoría de los organismos de agua dulce son ectotermos, las temperaturas ambientales afectan de manera significativa sus procesos fisiológicos y, consecuentemente, sus tasas de crecimiento. La variación estacional en el crecimiento de los peces está en general relacionada con los cambios de temperatura del agua y de los recursos alimentarios. **En el segundo capítulo**, se evaluó el efecto de la estacionalidad a lo largo de un gradiente latitudinal en un conjunto de variables, desde la temperatura del agua y el aire hasta las propiedades fisicoquímicas del agua y el crecimiento de la gambusia. Se encontró que la mayoría de la variación de la temperatura del aire se explica por la latitud y la estacionalidad; sin embargo, el crecimiento de la gambusia y las características del agua en las partes bajas de los ríos dependen más fuertemente de factores locales que de la latitud y la estación, incluso en el caso de la temperatura del agua. Se sugiere que aunque las diferencias latitudinales de la temperatura deben afectar a las características ecosistémicas de los ríos, los factores locales y las interacciones complejas a menudo son más influyentes.

La gambusia es una de las especies de peces más ampliamente introducida en todo el mundo y está incluida en la lista de cien de las peores especies invasoras por el Invasive Species Specialist Group (ISSG 2006). Hay estudios que demuestran sus efectos nocivos en las especies nativas y en los procesos de los ecosistemas. Se ha

estudiado mucho cómo afecta la temperatura a la ecología y la biología de los peces, y si influye en las interacciones entre especies. Sin embargo, el papel de la temperatura en el resultado de la competencia entre dos especies depende del contexto, y por tanto, los estudios experimentales son imprescindibles. En los dos primeros capítulos, hemos explicado como las características del ciclo vital de la gambusia cambiaban a lo largo de gradientes espaciales y temporales donde había una variación de las variables meteorológicas, especialmente en la temperatura. **En el tercer capítulo**, se investigó el papel de la temperatura en el comportamiento agonístico y la competencia de los recursos alimentarios entre la gambusia y el fartet (*Aphanius iberus*), un endemismo ibérico en peligro de extinción, ya que tienen hábitat y dieta similares. Se demostró que la gambusia incrementa el comportamiento agresivo hacia el fartet y captura más presas, aumentando su eficiencia, a medida que la temperatura se eleva. También se puso de manifiesto un cambio en la jerarquía competitiva a través de una reducción del ritmo de captura de alimento de la gambusia a bajas temperaturas. Se predice que, con el actual cambio climático, la gambusia puede ser capaz de ampliar su distribución a medida que la temperatura del agua de los ríos aumente.

RESUM

La variació del cicle vital dels organismes al llarg de gradients espacials és un dels temes més importants en ecologia i biogeografia. S'ha realitzat molta recerca per entendre els patrons biològics al llarg de la latitud, com per exemple l'augment de la riquesa d'espècies cap a l'equador, o l'augment de la mida corporal de les espècies cap a latituds més altes o temperatures més baixes (regla de Bergmann). A part de la mida corporal, les variacions amb la latitud de la majoria de trets del cicle vital han estat poc estudiades, sobretot en els peixos d'aigua dolça, tot i ser crucials per estimar els efectes del canvi climàtic i les seves interaccions amb les invasions biològiques.

Aquesta manca d'informació sobre la variació espacial dels trets del cicle de vida dels peixos és també el cas de la gambúsia (*Gambusia holbrooki*), tot i que hi ha certes evidències que el seu èxit invasor a Europa ha estat desigual al llarg de gradients espacials. **En el primer capítol**, es va fer un mostreig simultani al llarg d'un gradient latitudinal i de gradients al llarg dels cursos dels rius per entendre millor la variació del cicle vital d'una de les espècies més invasores mundialment, la gambúsia. Es va trobar que la gambúsia que habitava ambients més favorables (és a dir, latituds baixes i zones baixes dels rius) presentava un major esforç reproductiu i una menor condició corporal; tot i així, aquests patrons de variació explicaven un percentatge baix de la variació total, no eren lineals i depenien de l'edat dels peixos. A més, la versió intraespecífica de la regla de Bergmann o el seu invers no és aplicable a les poblacions de gambúsia estudiades ja que la mitjana de la mida corporal per a cada edat, i la mida corporal en general, no varien significativament amb la latitud. En conclusió, es suggereix que, pels trets de cicle vital dels organismes d'aigua dolça, la variació longitudinal dels rius té un paper tan important com el clima, sovint amb efectes diferencials.

Molts ecosistemes presenten una gran variació estacional, des de característiques biològiques com ara l'abundància i distribució d'espècies, fins a la temperatura i el fotoperíode. Com varia l'estacionalitat al llarg de la latitud no està encara ben entès en els ecosistemes d'aigua dolça, tot i ser important per predir els efectes del canvi climàtic. Atès que els peixos i la majoria dels organismes d'aigua dolça són ectotèrmes, les temperatures ambientals afecten de manera significativa els seus processos fisiològics i, consegüentment, les taxes de creixement. La variació estacional en el creixement dels peixos està en general relacionada amb els canvis de temperatura de l'aigua i dels recursos alimentaris. **En el segon capítol**, es va avaluar l'efecte de l'estacionalitat al llarg d'un gradient latitudinal en un conjunt de variables, des de la temperatura de l'aigua i l'aire fins a les propietats fisicoquímiques de l'aigua i la seva relació amb el creixement de la gambúsia. Es va trobar que la majoria de la variació de la temperatura de l'aire s'explica per la latitud i l'estacionalitat; no obstant això, el creixement de la gambúsia i les característiques de l'aigua en les parts baixes dels rius depenen més de factors locals que de la latitud i l'estació, fins i tot en el cas de la temperatura de l'aigua. Se suggereix que, tot i que les diferències latitudinals de la temperatura deuen afectar les característiques ecosistèmiques dels rius, els factors locals i les interaccions complexes sovint són més influents.

La gambúsia és una de les espècies de peixos més àmpliament introduïda a tot el món i està classificada com una de les cent pitjors espècies invasores per l'Invasive Species Specialist Group de la UICN (ISSG 2006). Hi ha estudis que demostren els seus efectes nocius en les espècies natives i en els processos dels ecosistemes. S'ha estudiat molt com afecta la temperatura a l'ecologia i la biologia dels peixos, i si influeix en les interaccions entre espècies. No obstant això, el paper de la temperatura en el resultat de la competència entre dues espècies depèn del context, i per tant, els estudis

experimentals hi són imprescindibles. En els dos primers capítols hem explicat com les característiques del cicle vital de la gambúsia canviaven al llarg de gradients espacials i temporals on hi havia una variació de variables com la temperatura. **Al tercer capítol**, es va investigar el paper de la temperatura en el comportament agonístic i la competència dels recursos alimentaris entre la gambúsia i el fartet (*Aphanius iberus*), un endemisme ibèric en perill d'extinció, ja que comparteixen unes hàbitat i dieta similars. Es va demostrar que la gambúsia incrementa el comportament agressiu cap al fartet i captura més preses, augmentant l'eficiència, a mesura que puja la temperatura. També es va posar de manifest un canvi en la jerarquia competitiva a través d'una reducció del ritme de captura d'aliment de la gambúsia a baixes temperatures. Es prediu que, amb l'actual canvi climàtic, la gambúsia pot ser capaç d'ampliar la seva distribució a mesura que els rius s'escalfin.



GENERAL INTRODUCTION

INVASIVE SPECIES AND MOSQUITOFISHES

Invasive species represent a growing threat to global biodiversity, causing great economic and ecological impacts and being part of global change (Vitousek et al. 1997, Pimentel et al. 2000). Invasive species introduced by human activities are a major environmental problem, and after habitat loss, invasive species are the most important threat to native species worldwide. Invasive species compete with the natives, alter and change the habitats and even cause extinctions of indigenous species (Clavero and García-Berthou 2005). Severe economic costs have also resulted from many invasions; for example, Pimentel et al. (2000) estimated that the cost of invasive species to the U.S. economy was approximately \$137 billion every year and the Spanish Ministry of Environment spent around 9 million of euros to monitor and control the expansion of *Eichhornia crassipes* (<http://www.magrama.gob.es/>). The number of invasions has been correlated with economic growth, and since the industrial revolution, invasions have been increasing interruptedly. As a result of globalization and the expansion of world trade, the species composition of the different ecosystems is being homogenized (Levine and d'Antonio 2003).

This problem affects specially freshwater ecosystems, which are affected by many invasive species that cause serious ecological and economic impacts. The introduction of exotic species have caused the decline and, in some instances, the extinction of many native freshwater species (Williams et al. 1989, García-Berthou et al. 2005). Introduced species are nowadays considered a major threat to aquatic biodiversity. Freshwater fish are one of the most frequently introduced vertebrates, and survival and genetic integrity of native fishes are now in serious danger worldwide (e.g. Gido and Brown, 1999). Moyle et al. (1987) named as 'The Frankenstein Effect' to the impacts of introduced fishes on native species since the consequences of introductions

are harmful and unpredictable. The aquatic ecosystems altered by anthropogenic activities seem to be especially vulnerable to fish invasions. The higher rates of invasion in aquatic ecosystems are partly due to their increased ecological degradation (pollution, water abstraction, channelization, etc.), and also to the properties of the rivers which act as natural corridors of energy and material (Moyle and Light 1996).

Two of the most successful species spread intentionally worldwide have been the mosquitofishes (namely *Gambusia holbrooki* and *G. affinis*), introduced to more than 50 countries, and now present in all continents except Antarctica (García-Berthou et al. 2005). *Gambusia holbrooki* (see Fig. 1) is native from southern New Jersey to Florida and to the eastern Mobile Bay (Alabama); and *G. affinis* native range extends from the Gulf Slope drainage in central Indiana and southern Illinois to eastern Mexico,



Figure 1. The top picture is a female *G. holbrooki*, and the bottom picture is a male, both captured in the Ter River (Catalonia).

and from the western Mobile Bay (Alabama) to Texas and into eastern Mexico (Page and Burr, 1991).

In the beginning of the 1900s, the two species were introduced to temperate and tropical countries to be used for mosquito control and to help to prevent the transmission of diseases such as malaria and yellow fever; for example mosquitofish was thus transported to Hawaii in 1905 and to Taiwan in 1911 (Walton et al. 2012). Although often confused and wrongly cited, *G. affinis* has been more translocated within the United States, and mostly *G. holbrooki* seems to be present in Europe, Australia and probably other regions (Vidal et al. 2009). *Gambusia holbrooki* was introduced to Spain from North Carolina (United States) in 1921, and from there to Italy in 1922. From Italy, mosquitofish was introduced elsewhere in Europe and to other countries (Vidal et al. 2009, Fig. 2). Nowadays, some local and regional projects still encourage the introduction of exotic fish as effective mosquito control agents whereas the scientific literature either questions this ability or shows that native fish are similar if not more effective and that the negative impacts of introducing exotic fish outweigh any positives (Lloyd 1986).



Figure 2. Current distribution of mosquitofish introduced to the western Mediterranean region (modified from Krumholz, 1948).

THE ECOLOGICAL IMPACTS OF *GAMBUSIA HOLBROOKI*

The success of mosquitofish as control agents and the reputation as a problem species accused of impacting other aquatic species may be attributed to several reasons: fast reproduction that allows a quick population to grow from introductions of small number of fish; the capacity to thrive in habitats with extreme environmental conditions; omnivorous feeding habits that permit them to live on a wide range of food items including algae when invertebrates are scarce; good adaptability to novel and human altered habitats usually free from predators; and lack of difficulty to produce them in captivity (Moyle 2002, Pyke 2008).

Mosquitofish are generalist predators that cause trophic cascades and produce shifts in ecosystem functioning. For example, predation by mosquitofish can act in a top-down manner by causing a reduction in zooplankton densities, which in turn release phytoplankton from zooplankton grazing, contributing to an increase of eutrophy (Hurlbert *et al.* 1972, Margaritora *et al.* 2001). Both experimental and field studies have provided evidence that the introduction of mosquitofish has contributed to the decline, and in some instances extinction, of many populations of native amphibians (Hamer *et al.* 2002, Kats & Ferrer 2003). Predation over amphibian eggs and larvae is often identified as the principal effect, influencing amphibian composition and diversity. For example, Gamradt and Kats (1996) showed 87% mortality of California newt larvae (*Taricha torosa*) in stream experiments where three mosquitofish were put together with four newt larvae for one day.

There are numerous examples of decline and extinction of native and endemic fishes worldwide due to mosquitofish introductions (Courtenay & Meffe 1989, Pyke 2008, Stockwell and Henkanaththegedara 2011, see Table 1). Lloyd (1990) already

listed at least thirty-five fish species worldwide which had declined in abundance or range as a result of interactions with *Gambusia*. Moreover, studies have clearly illustrated the negative effects and replacement of the American cyprinodontiforms *Cyprinodon tularosa*, *Heterandria formosa* and *Poeciliopsis occidentalis* due to mosquitofish impact (Meffe 1985, Meffe & Snelson 1989, Belk and Lydeard 1994, Rogowski and Stockwell 2006). Mosquitofish predation on the early stages of fish, antagonistic behaviour, competition for food and space resources or behavioural interference have been suggested as the main causal mechanisms of impact to native species (Rincón *et al.* 2002, Mills *et al.* 2003, Alcaraz *et al.* 2008).

Table 1 Experimental evidence of negative impacts of *Gambusia affinis* and *G. holbrooki* (adapted from Stockwell and Henkanathgedara 2011)

Taxa	Impact	Reference
Impacts on invertebrates		
<i>G. affinis</i>	Reduced zooplankton and insect populations; high algal densities	Hurlbert <i>et al.</i> (1972)
<i>G. affinis</i>	Reduced aquatic macroinvertebrate abundance in rice fields	Farley & Younce (1977)
<i>G. affinis</i>	Reduced pelagic aquatic invertebrates; increased algae and some benthic invertebrates	Hurlbert & Mulla (1981)
<i>G. affinis</i>	Reduction of aquatic macroinvertebrates in rice fields	Bence (1988)
Impacts on fish		
<i>G. affinis</i>	Replacement of <i>Poeciliopsis occidentalis</i> by predation	Meffe (1985b)
<i>G. holbrooki</i>	Reduced population growth of <i>Heterandria formosa</i>	Lydeard & Belk (1993)

Taxa	Impact	Reference
<i>G. holbrooki</i>	Size-selective predation on small <i>Heterandria formosa</i> in experimental mesocosms	Belk & Lydeard (1994)
<i>G. holbrooki</i>	Reduced growth and lack of egg survival of <i>Pseudomugil signifer</i>	Howe et al. (1997)
<i>G. holbrooki</i>	Caudal fin damage and mortality of <i>Edelia vittata</i>	Gill et al. (1999)
<i>G. holbrooki</i>	Heavy predation on <i>Aphanius iberus</i> and <i>Valencia hispanica</i> juveniles	Rincón et al. (2002)
<i>G. holbrooki</i>	Food competition and antagonistic behaviour on <i>Aphanius fasciatus</i>	Alcaraz et al. (2008)
<i>G. holbrooki</i>	Food competition and antagonistic behaviour on <i>Aphanius iberus</i>	Carmona-Catot et al. (2013)
<i>G. affinis</i>	Reduced growth and survival of <i>Lotichthys phlegethontis</i> young of year	Mills et al. (2004)
<i>G. affinis</i>	Reduced population size and biomass of <i>Cyprinodon tularosa</i>	Rogowski & Stockwell (2006a)
<i>G. affinis</i>	No impact on larval survival; increased body growth of <i>Siphateles bicolor mohavensis</i>	Henkanaththegedara & Stockwell, unpublished data

Impacts on amphibians

<i>G. affinis</i>	Elimination of <i>Hyla regilla</i> tadpoles	Hurlbert & Mulla (1981)
<i>G. affinis</i>	Predation on <i>Taricha torosa</i> larvae	Gamradt & Kats (1996)
<i>G. affinis</i>	Predation on <i>Hyla regilla</i> tadpoles	Goodsell & Kats (1999)
<i>G. affinis</i>	Delayed metamorphosis and reduced growth rates of <i>Rana aurora draytonii</i>	Lawler et al. (1999)
<i>G. holbrooki</i>	Reduced survival of endangered <i>Litoria aurea</i> tadpoles	Hamer et al. (2002)

The establishment rate of *Gambusia holbrooki* in Europe has been estimated as high as 97%, the greatest amongst the top ten most commonly introduced aquatic species worldwide (García-Berthou *et al.* 2005). In Europe, the species more likely

affected by mosquitofish are the Iberian endemic cyprinodontiforms, such as *Aphanius iberus* (Fig. 3), *A. baeticus*, *Valencia hispanica* and also the Mediterranean endemic *Aphanius fasciatus* (Bianco 1995, Rincón *et al.* 2002, Caiola and De Sostoa 2005, Alcaraz *et al.* 2008). Both *Aphanius iberus* and *Valencia hispanica* are listed by the National Catalogue of Endangered Species as in danger of extinction (Doadrio 2002). These two species with *A. fasciatus* are also listed in the Annex II (strictly protected fauna species) of the Convention on the Conservation of European Wildlife and Natural Habitats (Bern Convention) and in the Annex II of the European Council Directive (1992/43/EEC) “Habitat Directive”.



Figure 3. The top picture is a female *Aphanius iberus*, and the bottom picture is a male, both captured at the Fra Ramon Lagoon (Catalonia).

TEMPERATURE EFFECTS ON FISH INTERACTIONS

Temperature is frequently considered the most controlling abiotic factor regulating the outcome of interspecific competition in freshwater ecosystems (Lapointe and Light 2012; Oyugi et al. 2012). Fluctuating ambient temperatures affect body temperature of organisms, and since most aquatic organisms are poikilotherms, temperature is often considered a fundamental factor in stream ecosystems. In freshwater fish, the effects of temperature on predation and competition have been well studied (e.g. Cunjak and Green 1986; Rowe et al. 2007). Condition-specific competition may be particularly important when considering invasive species in relation to variation in temperature. Changed regimes in water temperatures could alter the impacts of invasive species on the natives due to changes in competitive dominance between species.

Eastern mosquitofish is a warmwater species that since its introduction in Europe has settled populations in most Mediterranean regions; however, it has not established populations in northern Europe (Benejam et al. 2009). Mosquitofish has little tolerance to low temperatures, and prefers warm and slow-flowing habitats (Fig. 4) to which their physiology is adapted and in which they display the maximum invasive potential (e.g. Laha and Mattingly 2006, Priddis et al. 2009). Some aspects of the effects of temperature on mosquitofish life history, biology and antagonistic behaviour towards native species have been studied (Table 2). For example in the Iberian peninsula, *G. holbrooki* aggression towards two native toothcarp species *Aphanius iberus* and *Valencia hispanica* was studied by Rincon et al. (2002) which conducted trials in January, April and July with mean water temperature of 14, 22 and 26 °C, respectively. In this study, aggressive behavior by mosquitofish seemed to be mediated by water

temperature, and also by other factors such as reproductive condition and body size. Other behavioural studies in relation with temperature have been undertaken with *G. affinis* which is closely related and similar to *G. holbrooki*. Thus, *G. affinis* also displayed increased aggression with rising temperatures towards *Galaxias maculatus* in New Zealand (Rowe et al. 2007) and the least chub, *Iothichthys phlegethontis* in the USA (Priddis et al. 2009), with deleterious impacts on these species. As water temperature can mediate interspecific interactions in fishes (e.g. Cunjak and Green 1986, Taniguchi et al. 1998) and in face of climate change, the main goal of the experiment in the third chapter is to further elucidate the extent and manner in which temperature mediates the interaction between toothcarp and mosquitofish, and help to predict the interactive effects and establish recovery plans."

Table 2 Literature review of previous research that studied the biology and behavior of *Gambusia affinis* or *G. holbrooki* in relation to temperature.

References	Mosquitofish spp.	Temperatures used (°C)	Study topic
Al-Habbib & Yacoob (1993)	<i>G. affinis</i>	10 - 41	Temperature tolerance
Baker <i>et al.</i> (2004)	<i>G. affinis</i>	10, 15, 20, 25	Antagonistic behaviour
Beaudouin <i>et al.</i> 2008	<i>G. holbrooki</i>	14 - 32	Growth
Carveth <i>et al.</i> (2006)	<i>G. affinis</i>	39 - 42	Upper thermal tolerance
Cech <i>et al.</i> (1985)	<i>G. affinis</i>	10, 15, 20, 25, 30, 35	Respiratory metabolic rates
Chipps & Wahl (2004)	<i>G. affinis</i>	5, 15, 25, 32, 40	Bioenergetics model
Fraile <i>et al.</i> (1994)	<i>G. affinis</i>	8, 18	Spermatogenesis
Hammill <i>et al.</i> (2004)	<i>G. holbrooki</i>	18, 30	Swimming performance
Knipprath & Mead (1966)	<i>G. affinis</i>	14, 15, 26, 27	Fatty acids
Koya and Kamiya (2000)	<i>G. affinis</i>	10 - 26	Reproduction
Laha & Mattingly (2006)	<i>G. affinis</i>	10, 15, 20, 25	Specific growth rate
Medlen (1951)	<i>G. affinis</i>	9, 27	Reproduction
Meffe (1991)	<i>G. holbrooki</i>	0 - 35	Life history
Meffe (1992)	<i>G. holbrooki</i>	25, 32	Life history
Otto (1973)	<i>G. affinis</i>	5 - 43	Temperature tolerance
Otto (1974)	<i>G. affinis</i>	25 - 44	Temperature tolerance
Rincón <i>et al.</i> (2002)	<i>G. holbrooki</i>	14 - 26	Antagonistic behaviour
Rowe <i>et al.</i> (2007)	<i>G. affinis</i>	10, 15, 20, 25	Antagonistic behaviour
Specziár (2004)	<i>G. holbrooki</i>	11 - 38	Life history and feeding ecology
Vondracek <i>et al.</i> (1988)	<i>G. affinis</i>	20, 25, 30	Growth and reproduction
Wilson (2005)	<i>G. holbrooki</i>	14 - 38	Reproduction and swimming performance
Wilson and Johnston (2004)	<i>G. holbrooki</i>	18 - 34	Reproduction
Wilson <i>et al.</i> (2007)	<i>G. holbrooki</i>	18, 30	Reproduction and swimming performance
Wurtsbaug and Cech (1983)	<i>G. affinis</i>	10, 15, 20, 25, 30, 35	Growth rate

VARIATION ALONG LATITUDINAL GRADIENTS

One of the most prominent large-scale environmental gradients is latitude, along which seasonality, temperature, and photoperiod vary, which in turn affect organisms. A very well studied variation along the latitudinal gradient is the tendency for body size to increase with latitude, mostly due to temperature, known as the Bergmann's Rule or its intraspecific equivalent (Gaston et al. 2007). This pattern has been demonstrated many times in homeothermic animals, such as mammals and birds (Ashton 2002). More recently, it has also been documented that some poikilothermic taxa, such as several reptiles, also follow Bergmann's rule. Thus, for example, the spiny lizards *Sceloporus undulatus*, *S. jarrovi* and *S. virgatus* follow the intraspecific equivalent of Bergmann's rule (Sears and Angilletta 2004). However, this rule has many exceptions, such as the lizard *Sceloporus graciosus* and several freshwater fishes in North America (Belk and Houston, 2002).

The latitudinal gradient in species diversity is another of the most studied patterns in biogeography. For most taxonomic groups, species richness shows the highest numbers at equatorial regions, and declines with increasing latitude. This variation in species richness also applies to organisms inhabiting marine or freshwater ecosystems, and both ectotherms and endotherms (Hillebrand 2004). Much research effort has been devoted to demonstrate that biotic interactions play a key role in the diversity of species. In a review of a wide range of interactions, Schemske et al. (2009) showed that biotic interactions are more important in tropical regions than at high latitudes, with examples of higher importance of herbivory, insect predation and mutualisms such as ant-plant interactions in tropical regions. At mediterranean-climate regions, Puig et al. (1991) observed a latitudinal diversity gradient among the temporal

systems of the Iberian Peninsula with increasing diversity of the communities towards the south.



Figure 4. Typical habitat of *G. holbrooki* near the mouth of the Daro River (Catalonia).

LATITUDINAL VARIATION IN THE PHYSICAL PROPERTIES OF WATER

Most areas of the world experience seasonal climates, with monthly variation in temperature, photoperiod, precipitation, and other weather-related variables, which consecutively entails numerous changes in the ecosystem functioning and organisms (i.e. life history, abundance, distribution, etc). Seasonality is a widespread factor affecting most temperate environments but it is still not well understood how it varies across large geographical and latitudinal gradients. Moreover, it is not clear how the seasonality effects are transmitted between levels of organization, and especially how seasonal effects are transmitted from air temperatures to water temperatures, and how these affect the aquatic organisms since river temperature is a major control on aquatic life (Allan 2007). The temporal and spatial variations in the thermal regime of lotic systems are the outcome of numerous factors that interact among them, and whose effects and extents are difficult to differentiate individually (Poole and Berman 2001).

Temperature and photoperiod are the main factors affecting seasonal growth of poikilothermic organisms (i.e. organisms whose body temperature varies with the environment) in temperate regions (Fry 1947). In certain species, photoperiod seems to be an earlier signal for temperature rise and helps to match enzyme production with more favorable temperatures (e.g. Villarreal et al. 1988). Although the temperature cycle follows the photoperiod with a short time lag, seasonal curves for temperature and photoperiod are similar, which adds difficulty to single out the effects of each factor on growth rate, despite temperature often being the leading factor for most poikilotherms organisms (Moreau 1987, Fontoura and Agostinho 1996). Conover and Present (1990) suggested that organisms embrace a combined strategy by adjusting to differences in

both mean temperature and length of the growing season which often covary across latitudes. In temperate regions, growth is often highly seasonal, and there is not a significant growth for a large proportion of the year. Evidence has demonstrated that populations of certain species display differential growth rates for different latitudes and seasons (Rhymer 1992, Yamahira and Conover 2002).

The role of seasonality in ecosystem functioning and structure has long been appreciated. However, how seasonality varies across large geographic and latitudinal gradients is less understood for freshwater ecosystems. In particular, it is not well known how water temperature and ecosystem functioning vary in lowland freshwater ecosystems along latitudinal gradients, despite its enormous importance to predict the effects of climate change.

LATITUDINAL VARIATION IN LIFE HISTORY TRAITS

Variation in population abundances, life history traits, and other biological features along spatial gradients are also prominent subjects of ecology and biogeography. However, variation of life history traits along the latitudinal gradient is not well known for freshwater fishes (Blanck and Lamouroux 2007). Latitudinal variation in critical environmental factors (such as temperature) might resemble the changes triggered by climate on biological traits; therefore, further latitudinal research is key to foresee the potential effects of climate change (Power et al. 2005).

Growth rate is an important variable that reflects the relative ecological success of a species in its natural environment. Growth rate is generally positively related to temperature; thus in colder climates we often find a decrease in growth. In freshwater

fishes, growth rate usually decreases with latitude due to low water temperatures (Belk and Houston 2002). For example, L'Abée-Lund et al. (1989) studied migratory brown trout (*Salmo trutta*) in a latitudinal gradient from 58 to 70 °N in Norway and found that annual length increment was negatively correlated with latitude but positively correlated with river temperature and growing season length. However, longevity has been often demonstrated to have a positive relationship with latitude, thus fish at high latitudes reach older ages than fish at lower latitudes and therefore mortality is lower towards the poles (i.e. Jonsson & L'Abée-Lund 1993).

As a widespread invasive fish, the eastern mosquitofish is an ideal organism to study variation in life history traits and population features along spatial gradients, including latitude. Benejam et al. (2009) studied mosquitofish from France to southern Spain in eight Mediterranean rivers and found latitudinal clines in many life history traits along the latitudinal gradient. Thus, southern mosquitofish populations had higher abundance and allocated more energy to reproduction but had lower body condition than in France (Benejam et al. 2009). Further research on the biology of the invasive species along spatial gradients is fundamental to understand how these new species may continue to spread and impact native species.



OBJECTIVES

OBJECTIVES

The main objective of this thesis is to assess the variation of life history traits of the eastern mosquitofish (*Gambusia holbrooki*) along spatial gradients in which there is a shift in seasonality, temperature and other variables. We also experimentally studied the temperature-specific competition between the eastern mosquitofish and an endemic, endangered cyprinodontid fish of the Iberian Peninsula (*Aphanius iberus*).

Chapter I. Comparing latitudinal and upstream–downstream gradients: life history traits of invasive mosquitofish. We examine life history variation in a highly invasive mosquitofish along latitudinal and upstream-downstream river gradients and assess the effects of age on this variation. We hypothesized similar responses in populations inhabiting environments more favorable to this species (lower latitudes and lower reaches of rivers). We sampled mosquitofish at the lowest reaches of ten river basins along 6° of latitude in the Mediterranean region and seven sites along the upstream-downstream gradient in three of the rivers. We examined abundance, population structure, size-at-age, and other life-history traits along these gradients.

Chapter II. Seasonality along latitudinal gradients: from stream temperature to growth of invasive mosquitofish. The objectives of this paper are: (i) to quantify the degree of seasonality along a latitudinal gradient in a range of variables from air and water temperatures, to limnological features and growth of an invasive mosquitofish; and (ii) to analyze the relationship among these variables along a latitudinal gradient. We studied water features and mosquitofish growth at ten river mouths in the Mediterranean coast during a ten-month period, along a gradient of more

than six degrees in latitude. In chapter one, invasive mosquitofish from many of the same sites displayed weak effects of latitude on life-history traits. We therefore predicted that although air temperature would be strongly related to latitude and season, water features and fish growth would display less latitudinal variation in seasonality and would depend more on local factors.

Chapter III. Temperature-specific competition between invasive mosquitofish and an endangered cyprinodontid fish. We examine the role of water temperature in determining the outcome of interspecific competition between invasive mosquitofish and native toothcarp (*Aphanius iberus*). As mosquitofish are known to be aggressive, we predicted that they would be more aggressive and also would further restrict toothcarps access to food. However, as mosquitofish are a warmwater species 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 mosquitofish's reduced competitive ability.



CHAPTER I

COMPARING LATITUDINAL AND UPSTREAM– DOWNSTREAM GRADIENTS: LIFE HISTORY TRAITS OF INVASIVE MOSQUITOFISH

Carmona-Catot G., Benito J. & García-Berthou E.
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<http://onlinelibrary.wiley.com/doi/10.1111/j.1472-4642.2011.00743.x/abstract>

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Abstract

Aim: Variation of life history traits along spatial gradients is poorly understood in invasive species and particularly in freshwater fish. We aimed to examine life history variation in a highly invasive fish (*Gambusia holbrooki*) along latitudinal and upstream–downstream river gradients and to assess the effects of age on this variation. We hypothesized similar responses in populations inhabiting environments more favourable to this species (lower latitudes and lower reaches of rivers).

Location: European rivers from southern Spain to southern France.

Methods: We sampled mosquitofish from the lowest reaches of ten river basins along 6° of latitude in the Mediterranean region and seven sites along the upstream–downstream gradient in three of the rivers. We examined abundance, population structure, size-at-age and other life history traits along these gradients.

Results: As hypothesized, lower reaches and lower latitudes both resulted in higher reproductive effort and lower body condition of mosquitofish. However, these patterns explained low per cent variation, were nonlinear and strongly depended on fish age. Independently of fish size, age groups differed in reproductive effort, in the gonadal weight–size relationship and its variation along spatial gradients. Mean size-at-age (or overall body size) did not vary with latitude (so the intra-specific version of Bergmann's rule or its converse does not apply) and in contrast increased upstream in rivers.

Main conclusions: Our findings suggest that for life history traits of freshwater organisms, river longitudinal variation plays a role as important as climate, with often differential effects. Our results also illustrate the poor knowledge of spatial variation of many life history traits, which precludes the understanding and prediction of biological invasions in a rapidly changing world.

Keywords

Biological invasions; growth; invasive species; latitude; life history; Poeciliidae; reproductive investment; temperature

Appendix S1 Size, age and growth of mosquitofish *Gambusia holbrooki*.

Age estimation in fish is always prone to various sources of error (e.g. Campana 2001). To support our aging estimates and to facilitate aging of mosquitofish in future studies (photographs of mosquitofish scales are very rare in the literature), we provide them below with some guidelines on annuli interpretation (Fig. S1). Most mosquitofish were aged 0 but a few males were age 1 and a few females were age 1 or 2 years old. Although six to eight scales per fish were mounted on slides, they always suggested the same age, with the exception of a few scales, often smaller, that had a very large focus and fewer circuli than the rest of scales (as in Fig. 2 of Sire and Géraudie 1984), which we interpreted as regenerating scales and discarded for aging estimates. Similarly to another cyprinodontiform fish (Fernández *et al.*, 1988; García-Berthou & Moreno-Amich, 1992), annuli were easily distinguished by increased separation of circuli in the oral part of the scale (top in Fig. S1) but more clearly because of several circuli cut across several others (cutting-over) in the dorsal and ventral parts (right and left in Fig. S1) of the scales. When present, the first annuli is quite easy to distinguish, the second is more difficult. Different shape (allometry) of the scales of the three age groups was generally observed (and is apparent in Fig. S1) but this has not been statistically evaluated. Ongoing length-frequency analysis of a subsequent monthly sampling of almost the same mosquitofish populations has provided evidence of the validity of these aging estimates.

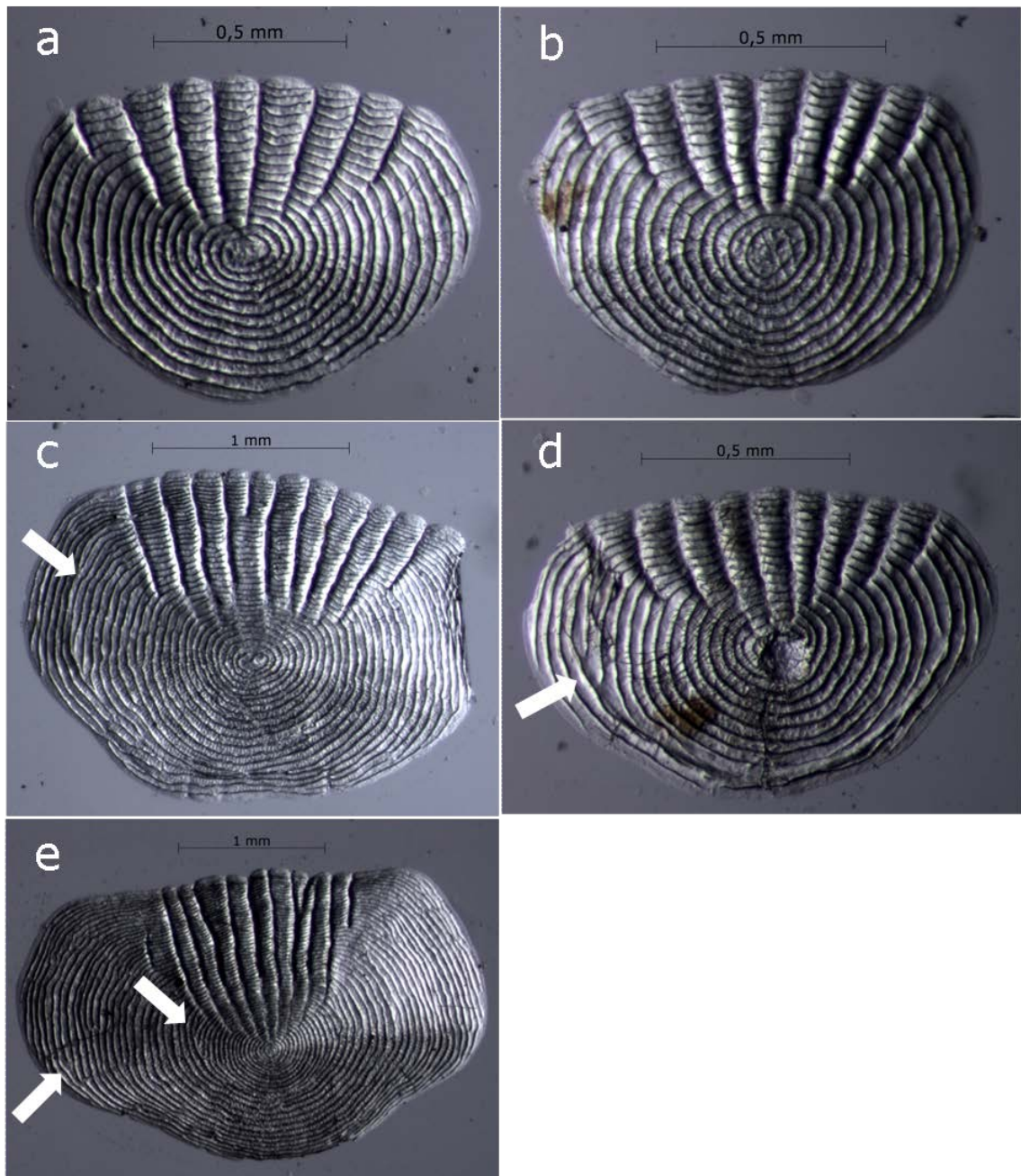


Fig. S1 Photographs of scales of mosquitofish aged 0 (photographs *a* and *b*), 1 (*c* and *d*), and 2 years old (*e*). The arrows denote the annuli. *a*) 19.1 mm SL female captured on 27 June 2005 at site S1 of Segura River; *b*) 19.0 mm SL male captured on 28 June 2005 at site S3 of Segura River; *c*) female 33.98 mm SL captured on 28 June 2005 at Almanzora River; *d*) 21.5 mm SL male captured on 1 July 2005 at site F1 of Fluvià River; and *e*) 41.2 mm SL female captured on 28 June 2005 at site S3 of Segura River.

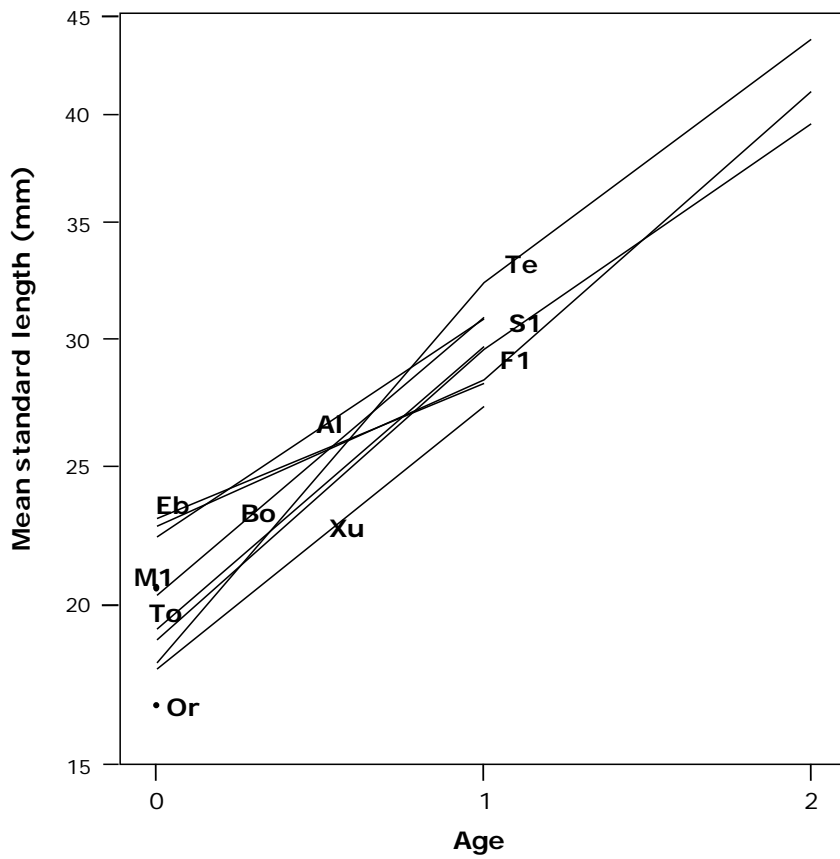


Fig. S2 Mean standard length at age 0-2 years for the female mosquitofish captured at the most downstream sites of each of the ten river basins studied. See Table 1 for the site codes.

Table S1 Mean standard length of male mosquitofish by age and sampling site. See

Table 1 for Site codes and location.

Site code	River zonation	Age	SL (mm)
Or	1	0	16.94
Bo	1	0	15.85
M4	4	0	16.34
M4	4	1	23.05
M	3	0	17.43
M3	3	1	21.56
M1	1	0	17.29
M1	1	1	21.07
F3	3	0	17.8
F3	3	1	23.84
F1	1	0	17.14
F1	1	1	22.43
Te	1	0	17.21
To	1	0	17.27
Eb	1	0	18.3
Eb	1	1	23.71
Xú	1	0	16.27
Xú	1	1	19.91
S3	3	0	17.6
S2	2	0	17.84
S2	2	1	23.69
S1	1	0	16.68
Al	1	0	17.75
Al	1	1	21.38

Table S2 Mean standard length of female mosquitofish by age and sampling site. See

Table 1 for Site codes and location.

Site code	River zonation	Age	SL (mm)
Or	1	0	16.77
Bo	1	0	20.35
M4	4	0	23.19
M4	4	2	38.97
M3	3	0	19.76
M3	3	1	29.55
M3	3	2	38.1
M2	2	1	30.9
M1	1	0	20.35
M1	1	1	30.91
F3	3	0	19
F3	3	1	30.05
F3	3	2	45.08
F2	2	0	22.77
F2	2	2	38.1
F1	1	0	25.12
F1	1	1	28.97
F1	1	2	41.91
Te	1	0	22.09
Te	1	1	34.32
Te	1	2	43.67
To	1	0	19.23
To	1	1	29.72
Eb	1	0	23.03
Eb	1	1	28.23
Xu	1	0	17.9
Xu	1	1	27.25
S3	3	0	23.45
S3	3	1	29.48
S3	3	2	41.23
S2	2	0	19.61
S2	2	1	29.55
S2	2	2	39.05
S1	1	0	17.56
S1	1	1	33.05
Al	1	0	22.37
Al	1	1	30.89

References for the Appendix S1

- Campana, S.E. (2001) Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. *Journal of Fish Biology*, **59**, 197-242.
- Fernández-Delgado, C., Hernando, J.A., Herrera, M. & Bellido, M. (1988) Age, growth and reproduction of *Aphanius iberus* (Cuv. & Val., 1846) in the lower reaches of the Guadalquivir river (south-west Spain). *Freshwater Biology*, **20**, 227-234.
- García-Berthou, E. & Moreno-Amich, R. (1992) Age and growth of an Iberian cyprinodont, *Aphanius iberus* (Cuv. & Val.), in its most northerly population. *Journal of Fish Biology*, **40**, 929-937.
- Sire, J.-Y. & Géraudie, J., (1984) Fine structure of regenerating scales and their associated cells in the cichlid *Hemichromis bimaculatus* (Gill). *Cell Tissue Research*, **237**, 537-547.



CHAPTER II

SEASONALITY ALONG LATITUDINAL GRADIENTS: FROM STREAM TEMPERATURE TO GROWTH OF INVASIVE MOSQUITOFISH.

Carmona-Catot G., Santos A., Tedesco P. & García-Berthou E.
Under review in *Oecologia*

**Seasonality along latitudinal gradients: from stream temperature
to growth of invasive mosquitofish**

**Gerard Carmona-Catot · Alejandra F.G.N. Santos · Pablo A. Tedesco · Emili García-
Berthou**

Gerard Carmona-Catot · Emili García-Berthou

Institute of Aquatic Ecology, University of Girona, E-17071 Girona, Catalonia

Alejandra Santos

*Universidade Federal Fluminense, Departamento de Zootecnia e Desenvolvimento Sócio-
Ambiental Sustentável, Niterói, RJ, Brazil*

Pablo A. Tedesco

Muséum National d'Histoire Naturelle, UMR BOREA IRD 207, 75005 Paris, France

Abstract Most ecosystems undergo substantial variation over the seasons, ranging from changes in abiotic features, such as temperature and precipitation, to changes in species abundance and composition. How seasonality varies along latitudinal gradients is not well known in freshwater ecosystems, despite being very important in predicting the effects of climate change and in helping to advance ecological understanding. We evaluated the degree of seasonality in ten river mouths along a latitudinal gradient for a set of variables, ranging from air and water temperatures, to physical and chemical properties of water and growth of an invasive fish species (eastern mosquitofish, *Gambusia holbrooki*). Our results show that although most of the variation in air temperature was explained by latitude and season, this was not the case for water features, including temperature, in lowland Mediterranean streams, which depended less on season and much more on local factors. Similarly, although there was evidence of latitude-dependent seasonality in fish growth, the relationship was nonlinear and weak and the significant latitudinal differences in growth rates observed during winter were compensated later in the year and did not result in overall differences in size and growth. Our results suggest that although latitudinal differences in air temperature cascade through properties of freshwater ecosystems, local factors and complex interactions often override this variation with latitude and might therefore hinder projections of species distribution models and effects of climate change.

Key words latitude · season · river · invasive fish · climate change · Europe · Iberian Peninsula

Additional Supporting Information

Fig. S1 Study area with the location of the sampling sites (full circles) at the ten Mediterranean river basins studied. The empty diamonds indicate large cities. The inset map shows the current distribution of mosquitofish in the western Mediterranean region (modified from Krumholz, 1948)

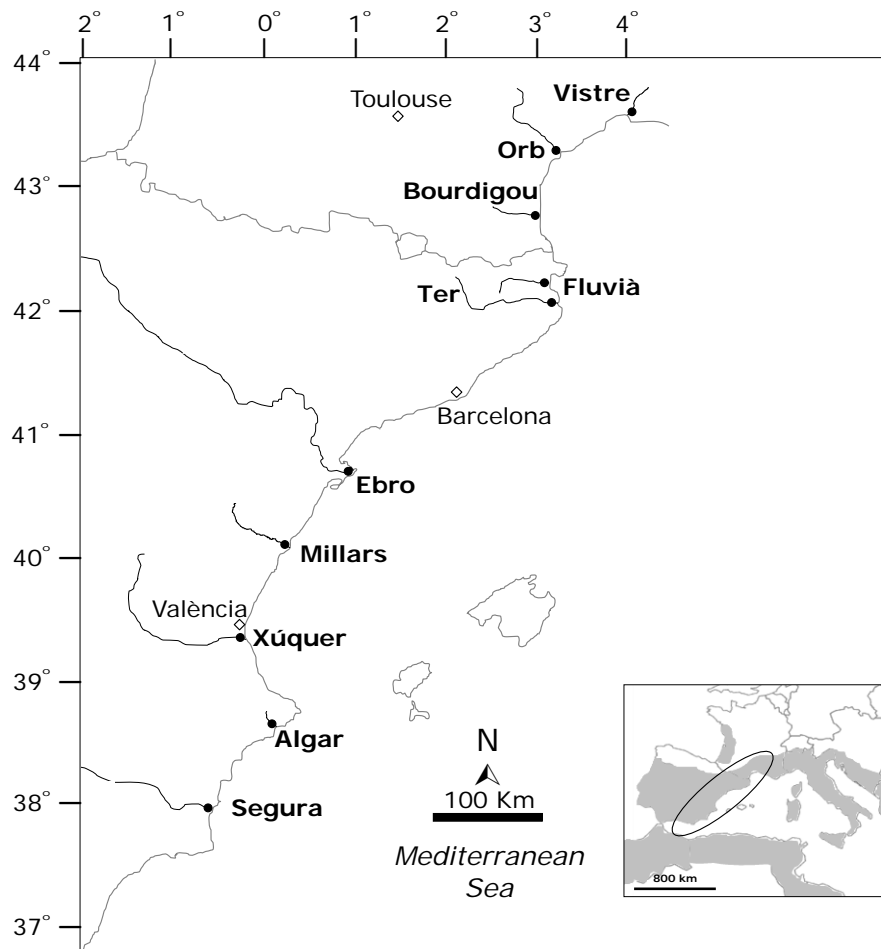


Table S1. Correlation matrix (Spearman's coefficient below the diagonal and n above) of the environmental variables along the latitudinal gradient. (*, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$).

Variables	1	2	3	4	5	6	7	8	9	10
1. Latitude (°N)		113	29	93	93	93	93	85	85	85
2. Air temperature (°C)	-0.25**		27	89	89	89	89	81	81	81
3. Water temperature (°C)	-0.50**	0.89***		29	29	29	29	29	29	29
4. Conductivity ($\mu\text{S cm}^{-1}$)	-0.33**	0.03	0.25		93	93	93	85	85	85
5. pH	-0.09	-0.17	0.30	0.0		93	93	85	85	85
6. O ₂ %	0.05	-0.23*	-0.34	-0.16	0.64***		93	85	85	85
7. O ₂ (mg L ⁻¹)	0.05	-0.42***	-0.47*	-0.19	0.60***	0.96***		85	85	85
8. Ammonium (mg L ⁻¹)	-0.28*	0.11	0.50**	0.28**	-0.22*	-0.57***	-0.56***		85	85
9. Nitrate (mg L ⁻¹)	-0.78***	0.00	0.13	0.25	-0.03	-0.13	-0.07	0.33**		85
10. Nitrite (mg L ⁻¹)	-0.60***	-0.05	0.37*	0.18	0.01	-0.17	-0.15	0.51***	0.76***	
11. Phosphate (mg L ⁻¹)	-0.50***	0.37***	0.41*	0.31**	-0.10	-0.34**	-0.41***	0.44***	0.47***	0.42***

Fig. S2 Principal component analysis of the environmental variables along the latitudinal gradient. The black dots correspond to the site scores and continuous arrows to the loadings of variables. Latitude and months were added as supplementary variables after the PCA to help interpretation; both projected variables were significant (permutation tests, $P < 0.05$). The first PCA axis explains 46.5% of the variation and the second 21.0%.

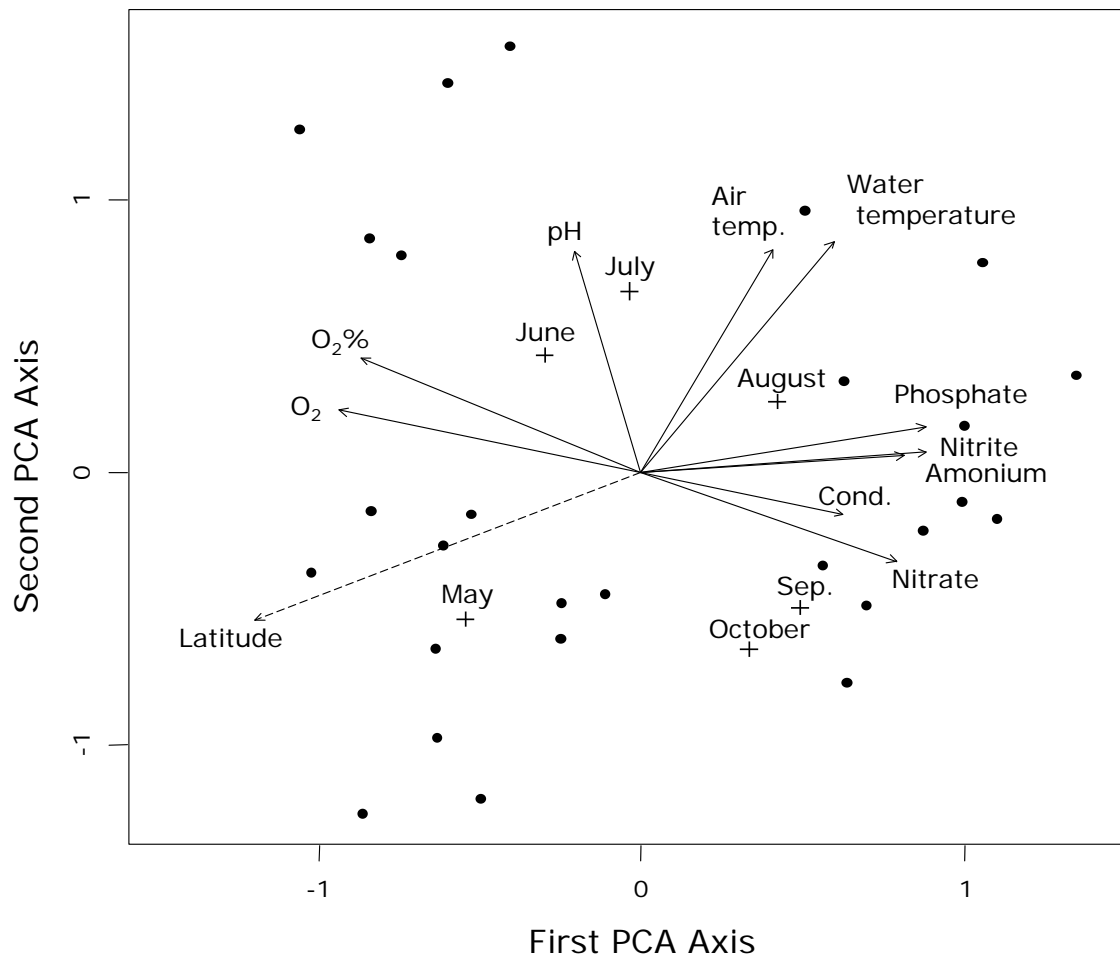


Fig. S3 Partial RDA of the environmental variables with latitude (arrow) as explanatory variable, after accounting for the effects of season (covariate or conditioning variable). The black dots correspond to the site scores and crosses to response variables. The first axis (RDA) explains 23.7% of the variation and the second axis (PCA) explains 37.3%.

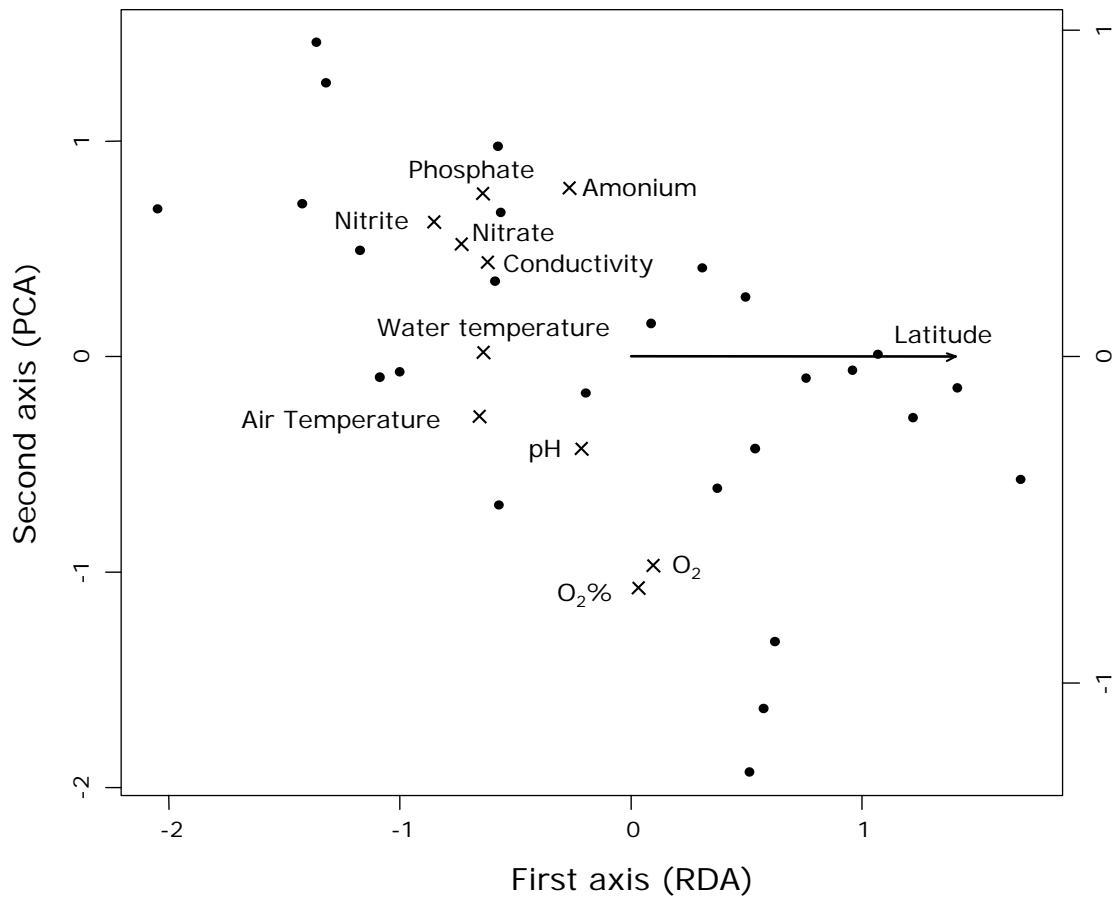


Fig. S4 Monthly length–frequency distributions of female mosquitofish by site. The numbers at the left-bottom corners are the site codes (see Table 1). The distribution for Segura (bottom-right, site code = 10) is used as an example to show the mosquitofish cohorts according to aging from scales.

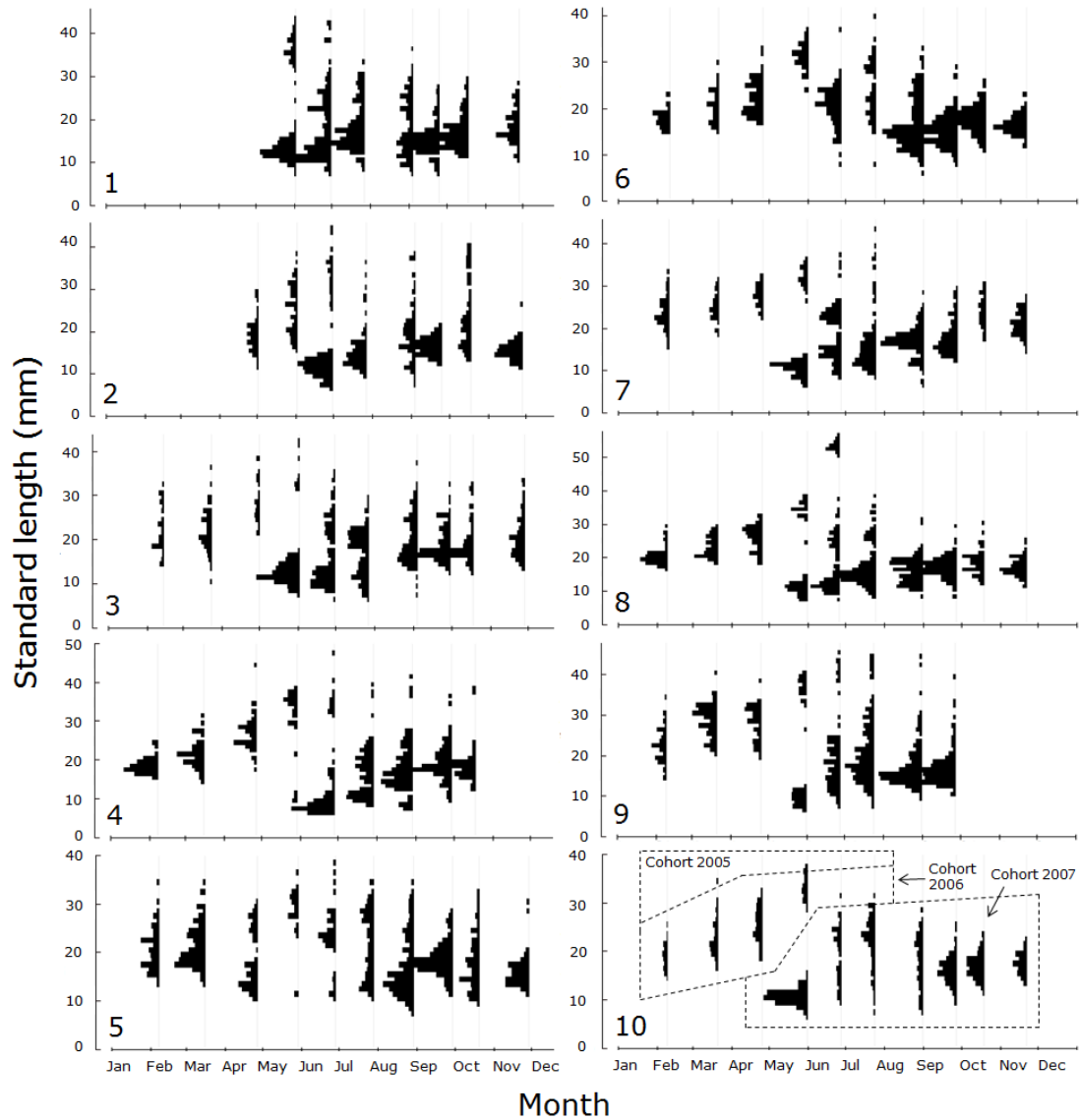


Fig. S5 Monthly length–frequency distributions of male mosquitofish by site. The numbers at the left-bottom corner are the site codes (see Table 1).

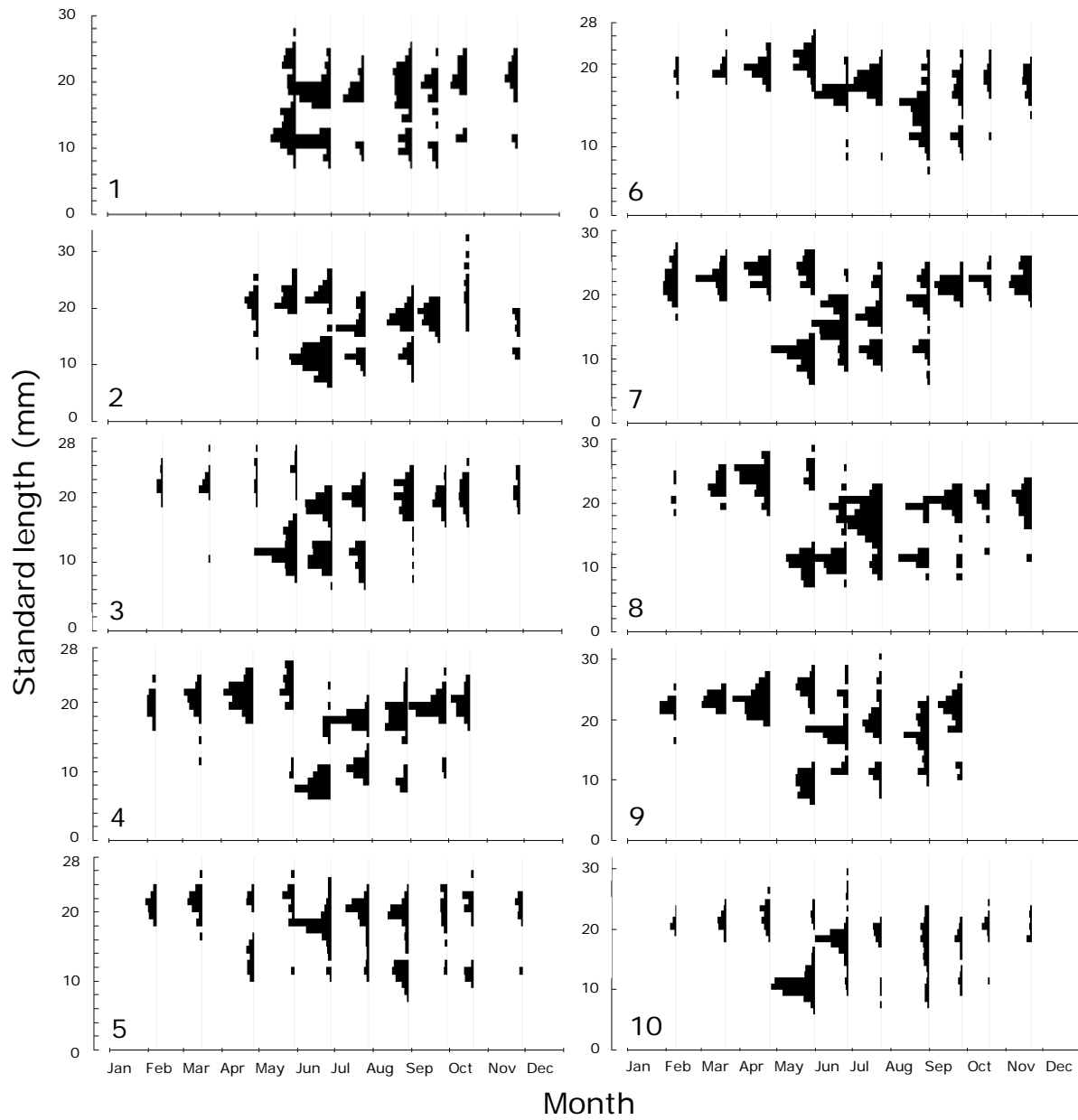


Fig. S6 Growth (length-age relationship) of female mosquitofish by site (figures on top of each scatterplot, see site codes in Table 1). The red line is the additive model (“loess” smoother with the “xyplot” function in *R*) and the circles the observed data.

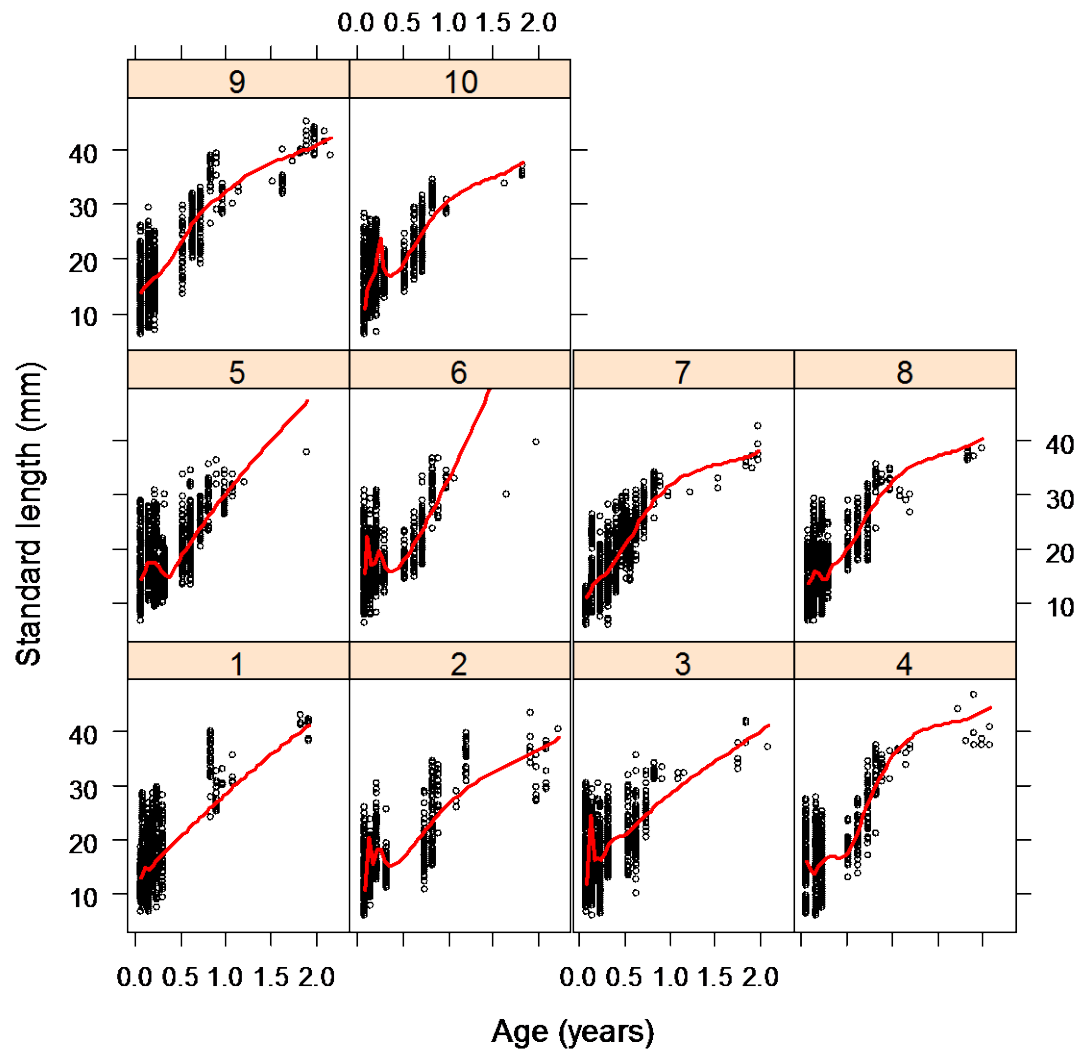


Fig. S7 Growth (length-age relationship) of male mosquitofish by site (figures on top of each scatterplot, see site codes in Table 1). The red line is the additive model (“loess” smoother with the “xyplot” function in *R*) and the circles the observed data.

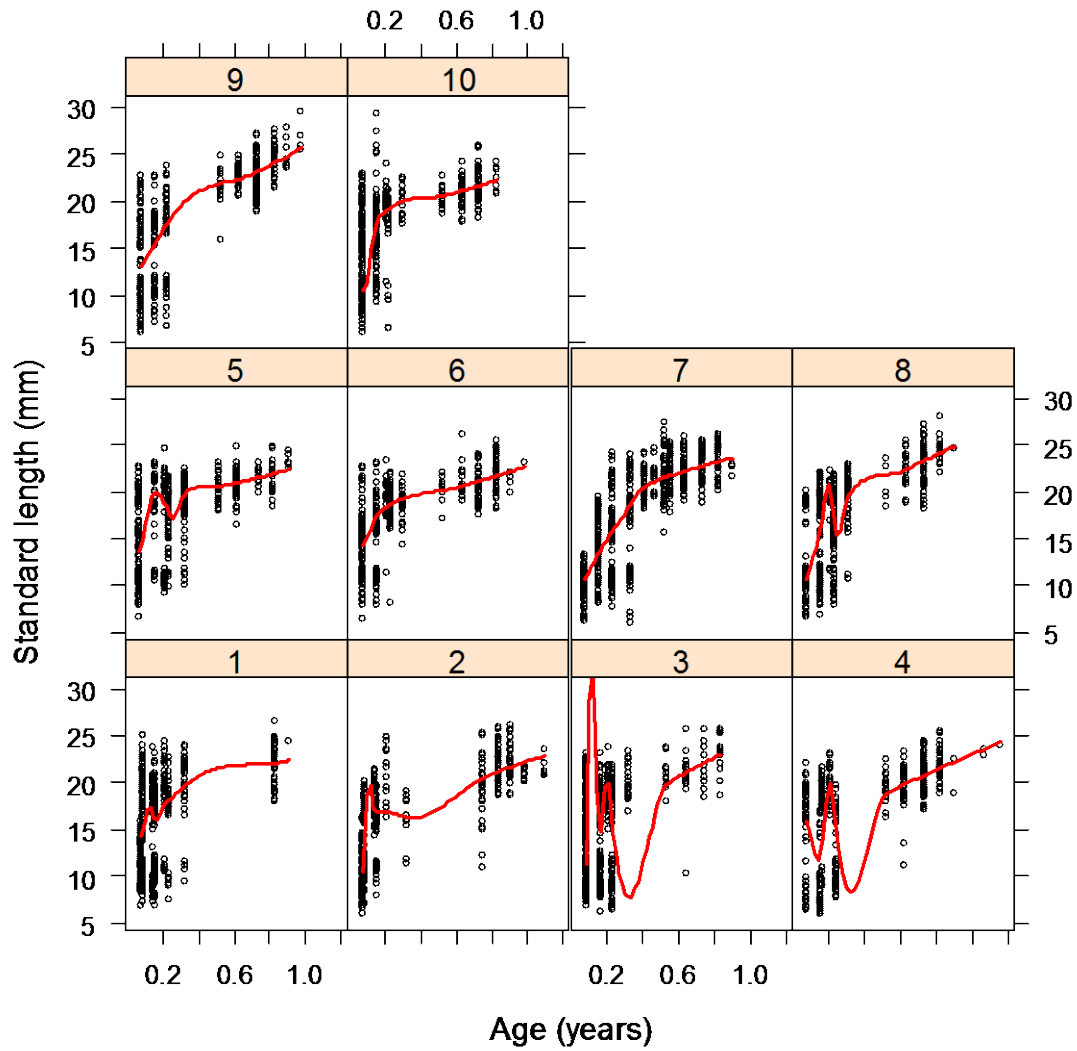


Fig. S8 Growth rates of mosquitofish between February and March along the latitudinal gradient. The numbers indicate the sampling sites (see Table 1). The top plot is for males and the bottom plot for females. *P* values of the regression analyses respectively are 0.12 and 0.05; note that the r^2 are considerable but sample size, and so statistical power, for this analyses is very low ($n = 8$)

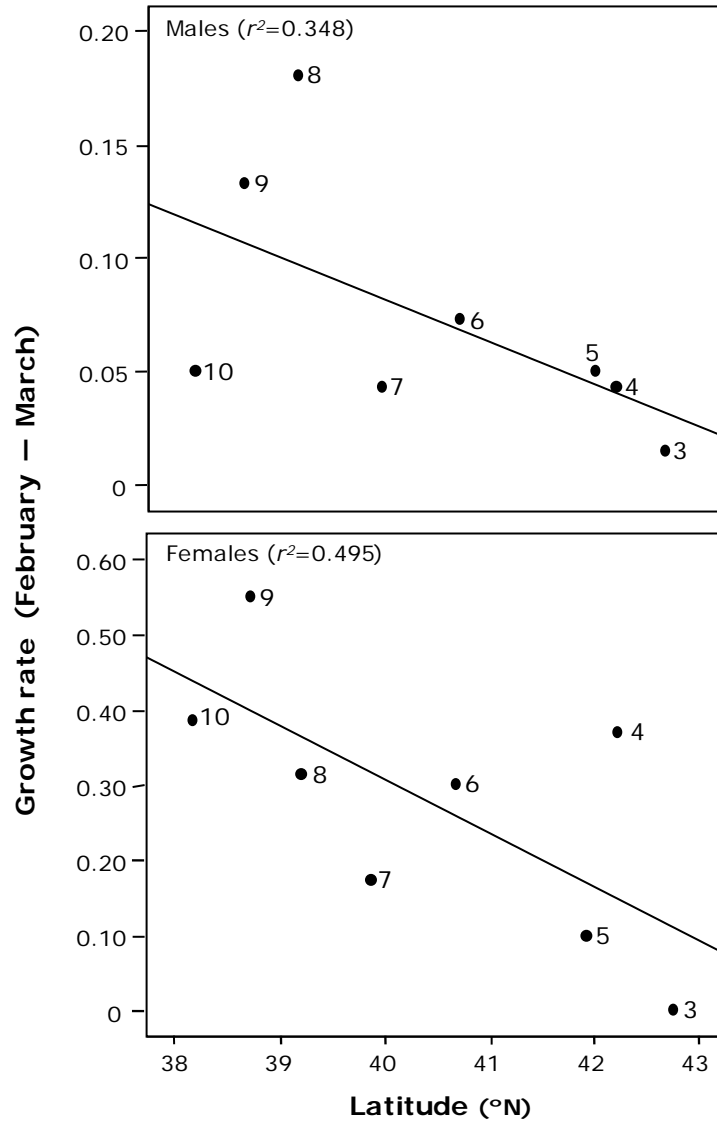
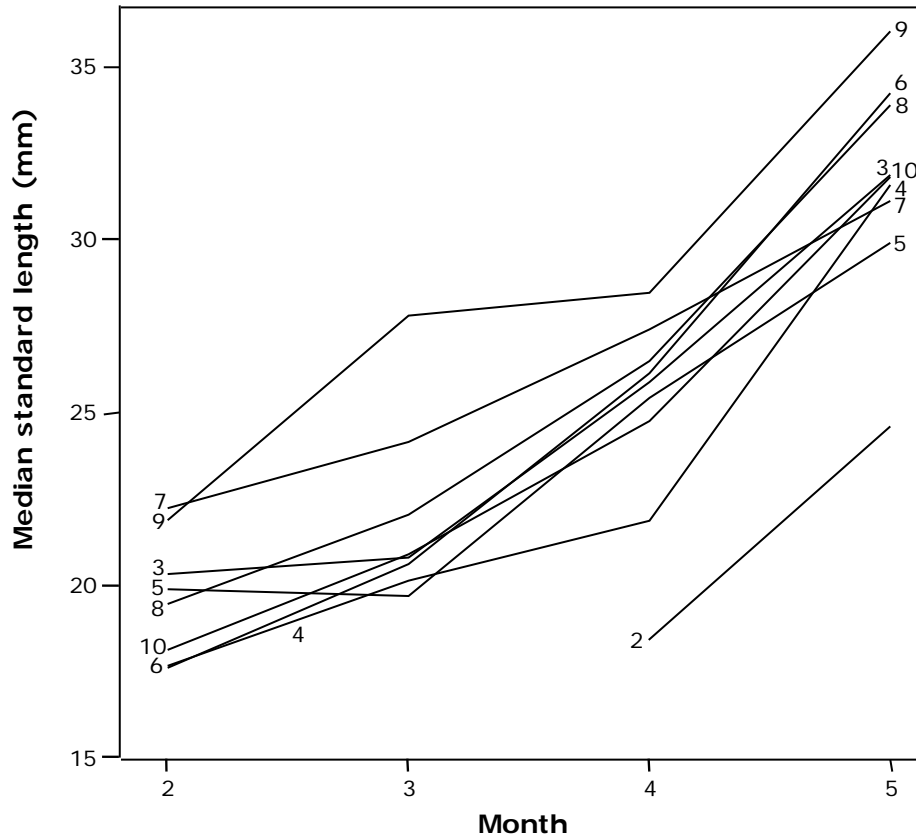


Fig. S9 Median standard length of female mosquitofish captured along the latitudinal gradient by month during late winter and early spring. The numbers are the site codes (see Table 1). Note that median size is greatest in the southern sites 7 and 9 and smallest in the northern sites 2 and 4 in winter and early spring but these differences are less clear in May (month 5).





CHAPTER III

TEMPERATURE-SPECIFIC COMPETITION BETWEEN INVASIVE MOSQUITOFISH AND AN ENDANGERED CYPRINODONTID FISH

Carmona-Catot G., Magellan K. & García-Berthou E.

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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.

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

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 mosquitoes

[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].

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 Empordà 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, Fluvià and Muga rivers near Girona, Spain, and toothcarp from Fra Ramon lagoon, Baix Empordà 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 (61 × 31 × 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 allowed to adapt 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 × 28 × 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 χ^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 χ^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 first more often at 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:8 mosquitofish, 24°C, 15:15, 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

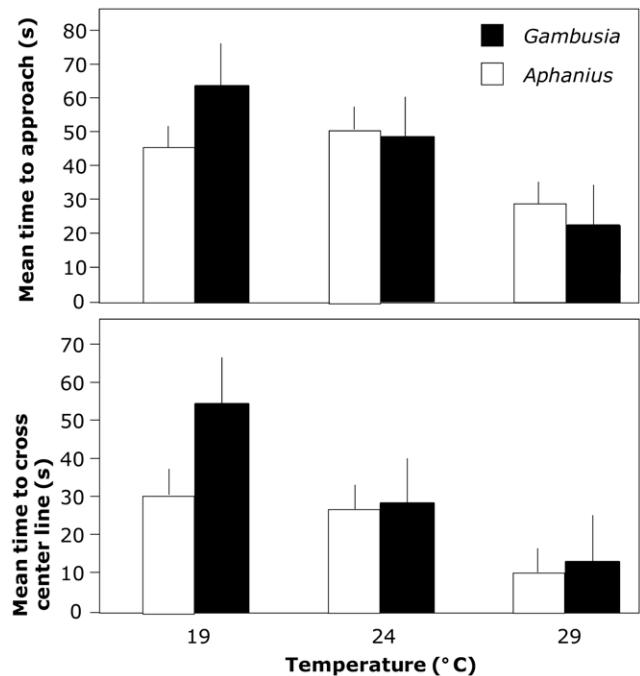


Figure 1. Mean time to a) cross the center line and b) approach a heterospecific for *G. holbrooki* and *A. iberus* as a function of temperature. Means and SE (error bar) are shown.
doi:10.1371/journal.pone.0054734.g001

equaled or exceeded that of the other behaviors (Figure 2). Temperature \times 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 \pm 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 \pm 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, 13:6:11; 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, $P = 0.11$).

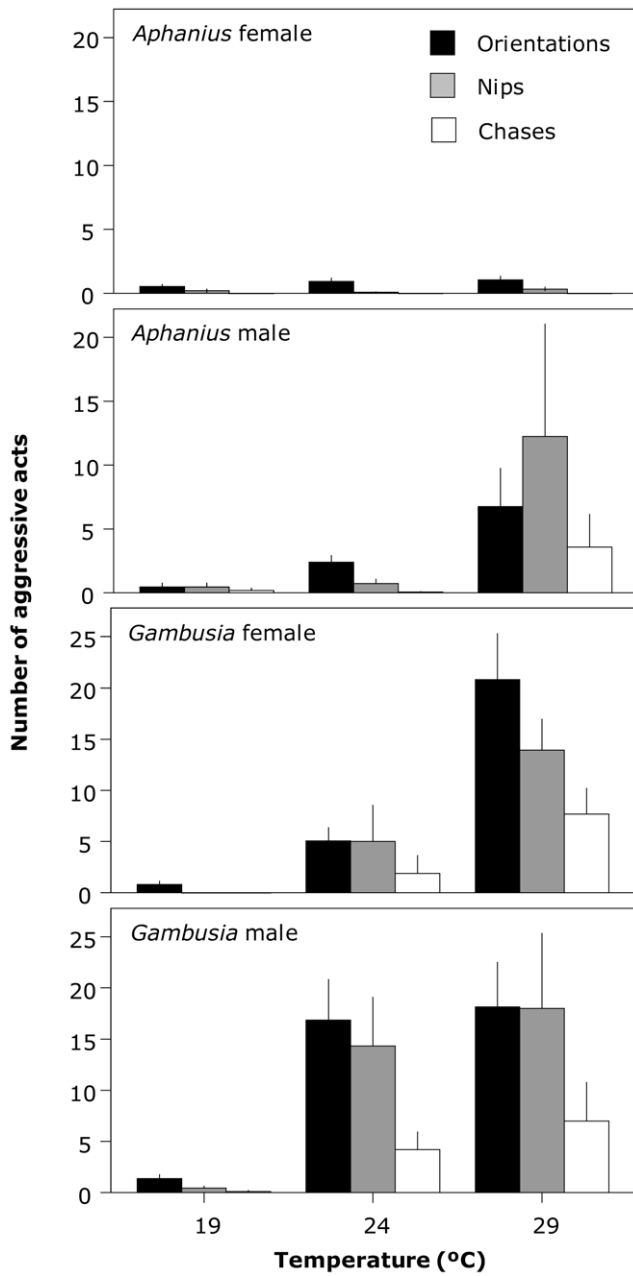


Figure 2. Aggressive acts (orientations, nips and chases) performed by *Gambusia holbrooki* towards *Aphanis iberus* and vice versa under the different temperature treatments and species combinations. Means and SE (error bar) are shown. Note the different scales on the y-axis for each species. doi:10.1371/journal.pone.0054734.g002

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

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

Species	Orientations	Nips	Chases
Source of variation			
<i>Gambusia</i>			
Temperature	300.29***	149.72***	82.31***
Sex	16.46***	64.81***	7.36 **
Temperature × Sex	75.33 ***	24.05***	11.70**
<i>Aphanis</i>			
Temperature	35.47***	31.56***	37.46***
Sex	16.46***	26.95***	-
Temperature × Sex	13.69**	11.88**	-

Values are χ^2 .
 *** indicates $P < 0.01$;
 **** indicates $P < 0.001$; d.f. are 2 for temperature, 1 for sex, and 2 for their interaction.
 doi:10.1371/journal.pone.0054734.t001

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,

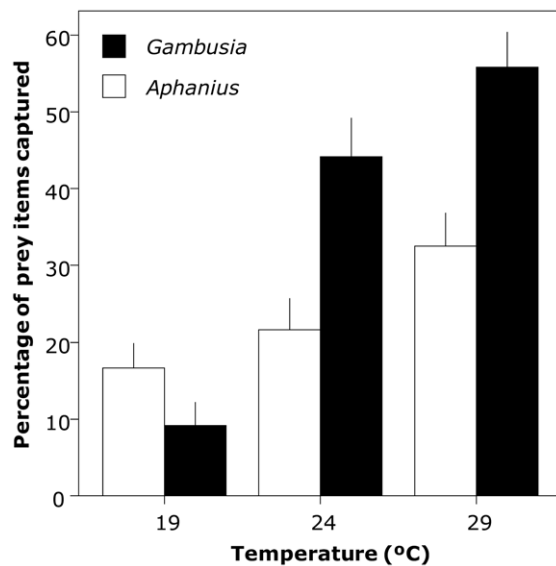


Figure 3. Effects of temperature on the number of prey items captured by *Gambusia holbrooki* and *Aphanis iberus*. Means and SE (error bar) are shown. Note that the totals for both species for 19 and 24°C do not equal 100%. This reflects the prey that remained uneaten. doi:10.1371/journal.pone.0054734.g003

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 suscepti-

bility 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 [56] 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.

We have shown here that temperature-specific competition may facilitate coexistence of native species with invasive mosquitofish. 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

References

- Dunson WA, Travis J (1991) The role of abiotic factors in community organization. *Am Nat* 138: 1067–1091.
- Taniguchi Y, Rahel FJ, Novinger DC, Gerow KG (1998) Temperature mediation of competitive interactions among three fish species that replace each other along longitudinal stream gradients. *Can J Fish Aquat Sci* 55: 1894–1901.
- Warner S, Travis J, Dunson WA (1993) Effect of pH variation on interspecific competition between two species of hyliid tadpoles. *Ecology* 74: 183–194.
- De Staso J, Rahel FJ (1994) Influence of water temperature on interactions between juvenile Colorado River cutthroat trout and brook trout in a laboratory stream. *T Am Fish Soc* 123: 289–297.
- Krassoi FR, Brown KR, Bishop MJ, Kelaher BP, Summerhayes S (2008) Condition-specific competition allows coexistence of competitively superior exotic oysters with native oysters. *J Anim Ecol* 77: 5–15.
- Greiner La Peyre 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.
- Costanzo KS, Kesavaraju B, Juliano SA (2005) Condition-specific competition in container mosquitoes: the role of noncompeting life-history stages. *Ecology* 86: 3289–3295.
- Dunson WA, Travis J (1994) Patterns in the evolution of physiological specialization in salt-marsh animals. *Estuaries* 17: 102–110.
- Taniguchi Y, Nakano S (2000) Condition-specific competition: implications for the altitudinal distribution of stream fishes. *Ecology* 81: 2027–2039.
- Holway DA, Suarez AV, Case TJ (2002) Role of abiotic factors in governing susceptibility to invasion: a test with Argentine ants. *Ecology* 83: 1610–1619.
- Fobert E, Fox MG, Ridgway M, Copp GH (2011) Heated competition: how climate change will affect non-native pumpkinseed *Lepomis gibbosus* and native perch *Perca fluviatilis* interactions in the U.K. *J Fish Biol* 79: 1592–1607.
- Wenger SJ, Isaak DJ, Luce CH, Neville HM, Fausch KD, et al. (2011) Flow regime, temperature, and biotic interactions drive differential declines of trout species under climate change. *Proc Natl Acad Sci U S A* 108: 14175–14180.
- Côte IM, Green SJ (2012) Potential effects of climate change on a marine invasion: The importance of current context. *Acta Zool Sinica* 58: 1–8.
- Lapointe NWR, Light T (2012) Landscape-scale determinants of non-native fish communities. *Divers Distrib* 18: 282–293.
- Oyugi DO, Cucherousset J, Robert Britton J (2012) Temperature-dependent feeding interactions between two invasive fishes competing through interference and exploitation. *Rev Fish Biol Fisher* 22: 499–508.
- Giorgi F, Lionello P (2008) Climate change projections for the Mediterranean region. *Global Planet Change* 63: 90–104.
- Caissie D (2006) The thermal regime of rivers: a review. *Freshwater Biol* 51: 1389–1406.
- Rahel FJ, Olden JD (2008) Assessing the effects of climate change on aquatic invasive species. *Conserv Biol* 22: 521–533.
- Meffe GK, FFS (1989) Ecology and evolution of livebearing fishes (Poeciliidae). New York: Prentice Hall.
- Benejam L, Alcaraz C, Sasal P, Simon-Levert G, Garcia-Berthou E (2009) Life history and parasites of the invasive mosquitofish (*Gambusia holbrooki*) along a latitudinal gradient. *Biol Invasions* 11: 2265–2277.
- Vidal O, Garcia-Berthou E, Tedesco PA, Garcia-Marin J-L (2009) Origin and genetic diversity of mosquitofish (*Gambusia holbrooki*) introduced to Europe. *Biol Invasions* 12: 841–851.
- Carmona-Catot G, Benito J, Garcia-Berthou E (2011) Comparing latitudinal and upstream-downstream gradients: life history traits of invasive mosquitofish. *Divers Distrib* 17: 214–224.
- Rincón PA, Correas AM, Morcillo F, Risueno P, Lobón-Cerviá J (2002) Interaction between the introduced eastern mosquitofish and two autochthonous Spanish toothcarps. *J Fish Biol* 61: 1560–1585.
- Caiola N, de Sostoa A (2005) Possible reasons for the decline of two native toothcarps in the Iberian Peninsula: evidence of competition with the introduced *Eastern mosquitofish*. *J Appl Ichthyol* 21: 358–363.
- Alcaraz C, Bisazza A, Garcia-Berthou E (2008) Salinity mediates the competitive interactions between invasive mosquitofish and an endangered fish. *Oecologia* 155: 205–213.
- Moreno-Amich R, Pou Q, Quintana X, Garcia-Berthou E (1999) Monografía sobre los peces ciprinodontidos ibéricos fartet y samaruc. In: Planelles-Gomis M, editor. Efecto de la regulación hídrica en la conservación del fartet (*Lebias iberica*) en Aiguamolls de l'Empordà: importancia de los refugios de población. València, Spain: Generalitat Valenciana 115–131.
- Doadrio I (2002) Atlas y libro rojo de los peces continentales de España; Ambiente MdM, editor. Madrid, Spain.
- Freyhof J, Brooks E (2011) European Red List of Freshwater Fishes. Luxembourg: Publications office of the European Union.
- Oliva-Paterna FJ, Torralva M, Fernández-Delgado C (2006) Threatened fishes of the world: *Aphanius iberus* (Cuvier & Valenciennes, 1846) (Cyprinodontidae). *Environ Biol Fish* 75: 307–309.
- Andersson M (1994) Sexual selection. Princeton, New Jersey: Princeton University Press.
- Priddis E, Rader R, Belk M, Schaalje B, Merkley S (2009) Can separation along the temperature niche axis promote coexistence between native and invasive species? *Divers Distrib* 15: 682–691.

Supporting Information

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

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

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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.

32. Oliva Paterna FJ, Torralva 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*): ontogenetic diet shift and prey electivity. *Environ Biol Fish* 78: 193–207.
35. Botham MS, Kerfoot CJ, Louca 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, Childress MJ, Ptacek MB (2012) Individual variation in male size and behavioral repertoire in the sailfin molly *Poecilia latipinna*. *Ethology* 118: 411–421.
37. Ward A, Axford S, Krause J (2002) Mixed-species shoaling in fish: the sensory mechanisms and costs of shoal choice. *Behav Ecol Sociobiol* 52: 182–187.
38. Karplus I, Zion B, Rosenfeld L, Grinshpun Y, Slosman 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. Magellan K, Kaiser H (2010) Male aggression and mating opportunity in a poeciliid fish. *Afr Zool* 45: 18–23.
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 *Gambusia affinis* and *Galaxias maculatus*: implications for whitebait fisheries in New Zealand rivers. *J Appl Ichthyol* 23: 668–674.
44. Sinclair ELE, Ward AJW, Seebacher F (2011) Aggression-induced fin damage modulates trade-offs in burst and endurance swimming performance of mosquitofish. *J Zool* 283: 243–248.
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. Ashley PJ (2007) Fish welfare: Current issues in aquaculture. *Appl Anim Behav Sci* 104: 199–235.
47. Laha M, Mattingly 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, Elwood 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) Rules of engagement for resource contests in a social fish. *Anim Behav* 82: 93–99.
50. Magurran A, Maciás 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. Oltra R, Todolí R (2000) Reproduction of the endangered killifish *Aphanius iberus* at different salinities. *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, Bückle LF, Espina S (2002) Temperature preference and acclimation in *Poecilia sphenops* (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: 586–588.
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: 75–81.
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 (2000) 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.



GENERAL DISCUSSION

GENERAL DISCUSSION

The results of this thesis, besides helping to further understand the variation of life histories in freshwater fish, has provided a better understanding of how invasive mosquitofish has adapted to our ecosystems and displaced the native species. We have shown that mosquitofish presented similar life histories at lower latitudes and at the lower reaches of rivers (**first chapter**), and also that mosquitofish growth and water features of lowland streams depend more strongly on local factors than on latitude and season (**second chapter**). We also experimentally investigated the role of temperature on the agonistic behaviour and food competition between mosquitofish and the endangered *Aphanius iberus* (**third chapter**).

Comparing latitudinal and upstream–downstream gradients: life history traits of invasive mosquitofish.

The study in the **first chapter** is one of the few studies that has concurrently sampled along latitudinal and upstream-downstream gradients to compare differences in life-history traits of stream fish. We confirmed patterns shown in a previous latitudinal sampling of similar mosquitofish populations (Benejam et al. 2009), but we additionally compared them to upstream-downstream gradients. We found that environments more favorable to mosquitofish (i.e. lower latitudes and lower reaches) resulted in similar responses of life histories: more reproductive effort with less condition in the warmer environments of lower latitudes and downstream reaches. These patterns depended on

fish age that in addition to fish length improved our understanding of the effects of environmental gradients on mosquitofish life-history variation.

We showed that age is a fundamental factor to comprehend life-history variation in mosquitofish: independently of size, age groups differed in their reproductive effort, in the relationship between gonadal weight and size (also see Tedesco et al. 2008), and in the variation of reproductive effort along spatial gradients. Age has generally been ignored in life-history studies of mosquitofish (but see Fernandez-Delgado 1989; Fernandez-Delgado and Rossomanno 1997) probably because its estimation is laborious, because of their short longevity and because it was assumed that the effects of length and age are interchangeable. Thus, both Benejam et al. (2009) and the analysis conducted at the population level of the **first chapter** showed that gonadal weight was lower in the north after accounting for fish size, suggesting a decreased investment in reproduction at higher latitudes. Therefore, analyses considering age indicated that gonadal weight for the young-of-the-year and for the whole population (without separating for age classes) had a similar relationship with latitude. However, older mosquitofish (age-1) showed no relationship or increasing gonadal weight (age-2) with increasing latitudes. Consequently, analyses of life-history variation neglecting age only provide a partial picture and mostly reflect variation in the most abundant age group (young-of-the-year).

Most freshwater fishes appear to rather follow the converse of Bergmann's rule at the intra-specific level (Gaston et al. 2007). However, mean size-at-age or overall average size of the mosquitofish populations along the studied gradient did not vary with latitude, so the intra-specific equivalent of Bergmann's rule or its converse does not apply. This is the first study to confirm it with size-at-age data.

Seasonality along latitudinal gradients: from stream temperature to growth of invasive mosquitofish.

In the **second chapter**, we collected data every month to assess the effects of seasonality on abiotic factors and fish growth along a latitudinal gradient. We illustrated how seasonality in air temperature was stronger and similar across latitudes than for water temperatures, which was less seasonal. The proportion of unexplained variation for water temperature was larger than for air temperatures, suggesting a significant influence of the unique characteristics of each watershed and local factors, such as groundwater influences, discharge, channel form and orientation, riparian canopy and particular anthropogenic perturbations (Webb 1996, Hawkins et al. 1997, Poole and Berman 2001). Variation of nutrient concentrations was less explained by latitude and seasonality than for temperatures. Nutrient concentrations were negatively related to latitude (as in Dodds et al. 2002), possibly because sites at lower latitudes are more affected by water shortage, degraded stream flows and intensive agricultural land uses (Sabater and Barceló 2010). Oxygen concentrations and pH were more related to season, with higher diurnal values in spring and early summer, as expected from higher photosynthetic activity due to growth of macrophytes and algae (Cox 2003, Desmet et al. 2011).

Since most freshwater fish are ectotherms, water temperatures substantially affect their physiology and resultant growth rates (Magnuson et al. 1979, Wood and McDonald 1997). We found seasonal growth in all mosquitofish populations along the latitudinal gradient but stronger seasonality in the two northernmost populations. However, the relationship of growth with latitude was weak, as also observed in other

life-history traits studied in the **first chapter**. We also detected a weak relationship of winter growth rates and overall growth performance with latitude and that most differences in size at the onset of the growing season disappeared during spring. These patterns suggest countergradient variation in mosquitofish growth (i.e. northern populations have been selected to compensate the shorter growing season with higher growth rates) but experiments or transplanting studies are necessary to confirm it, since it is quite common in freshwater fish (Conover and Present 1990, Conover et al. 2009).

In summary, the **second chapter** shows that air temperature is strongly seasonal, dependent on latitude, and it is little dependent on local factors. However, stream properties (such as water temperature and nutrient concentrations) and fish growth display less seasonal variation and depend more on local factors (such as watershed or reach characteristics and anthropogenic perturbation).

Temperature-specific competition between invasive mosquitofish and an endangered cyprinodontid fish.

The **third chapter** shows indication for both condition-specific competition between mosquitofish and fartet, and a reversal of competitive hierarchies mediated by temperature. Mosquitofish conducted almost five times more aggressive acts than fartet, and while both species displayed increased aggression at higher temperatures, this increase was much larger for mosquitofish. Therefore, mosquitofish have the potential to competitively displace toothcarp by interference competition, and the strength of this interaction probably increases at warmer temperatures. Competitive exclusion through aggression at higher temperatures has been proposed as a significant factor to describe

the distribution of fish species along spatial gradients (Taniguchi et al. 1998, De Staso and Rahel 1994, Taniguchi and Nakano 2000).

Temperature also influences the potential for exploitation competition. While toothcarp captured the first prey item more often at 19 °C, this situation was reversed at 24 and 29 °C, providing an example of potential competitive reversal. In fact, rather than toothcarp being more dominant at lower temperatures, they benefited from reduced exploitation competition from mosquitofish at colder temperatures. Release from dominance by a competitively superior species appears to be a common factor in competitive reversal (e.g. De Staso and Rahel 1994, Taniguchi and Nakano 2000).

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 displayed much more aggression than females. Both species show a marked sexual dimorphism with larger females (Meffe 1989, Oliva-Paterna et al. 2006), so this may account for some of the observed differences. In addition, females are often more sociable and engage in more schooling than males, 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 (Magurran and Maciás Garcia 2000).

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. Thus, this thesis strengthens the interest of latitudinal research, which is key to further understand the effects of climate change at the different organization levels of aquatic ecosystems. The variation along the latitudinal gradient in important abiotic parameters (such as temperature) resembles climate-driven changes in

biological characteristics over time. This information is of significant importance to further understand the invasive potential of mosquitofish around the globe.

For a future perspective, the experiment in **third chapter** could be improved if the design was changed to a factorial design with different levels of temperature and salinity (see for example the design in Wurtsbaugh et al. 1983). Both results from the **third chapter** and those from Alcaraz et al. (2008) showed evidences that temperature and salinity are two important parameters that mediate the competition outcome between *G. holbrooki* and *Aphanius* spp. Lower salinities and higher temperatures taken apart seemed to be the most favorable conditions for *G. holbrooki*, but it would be interesting to explore how temperature and salinity interact and probably change the competition outcomes. Furthermore, the **first and second chapter** would benefit if air and water temperature recordings would be spatially and temporally increased to provide a more precise picture (see Arismendi et al. 2012). We also suggest that stream flow data would provide further insights on how water temperature and other stream variables vary seasonally and latitudinally, and how they also affect fish growth. One of the main human impacts on rivers is flow regulation and impoundment which greatly alter the natural thermal regimes of rivers (Webb et al. 2008). Additionally, we consider that mark-recapture techniques would be useful in the future to study the seasonal growth of individual mosquitofish at the different sampling locations, rather than using population averages. Reznick and Bryant (2007) and Reznick et al. (1996) successfully used mark-recapture techniques with *Poecilia reticulata* in Trinidad to study the evolution of life histories of these species in different habitats.

GENERAL CONCLUSIONS

GENERAL CONCLUSIONS

- 1) Mosquitofish has similar responses of life-history traits in more favorable environments: more reproductive effort often with less condition in the warmer environments of lower latitudes and downstream reaches. However, these patterns explained low percent variation and strongly depended on fish age.
- 2) Mean size-at-age or overall average size of mosquitofish did not vary with latitude, so the intra-specific equivalent of Bergmann's rule or its converse does not apply.
- 3) River longitudinal variation plays a role as important as climate, with often differential effects, for life-history traits of mosquitofish and probably other freshwater organisms. There is poor knowledge of spatial variation of many life-history traits of freshwater fishes.
- 4) In lowland streams, most of the variation in air temperature is explained by latitude and season, but this is not the case for water features which depend less on season and latitude, and much more on local factors, even for water temperature.
- 5) There is evidence of latitude-dependent seasonality in fish growth, although the relationship is nonlinear and weak. The significant latitudinal differences in mosquitofish growth rates during winter are compensated later in the season and

do not result in overall differences in size and growth along the latitudinal gradient.

- 6) Mosquitofish exhibit much more aggression than toothcarp. Mosquitofish have the potential 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.

- 7) Males of both mosquitofish and the Iberian tothcarp carry out more overtly aggressive acts than females; this fact is probably related to the marked sexual dimorphism and associated mating systems of these two species.

References

- Alcaraz C, Bisazza A & García-Berthou E (2008) Salinity mediates the competitive interactions between invasive mosquitofish and an endangered fish. *Oecologia* 155: 205-213.
- Allan JD, Castillo MA (2007) Stream ecology: structure and function of running waters. pp. 388. Springer, Dordrecht, The Netherlands.
- Arismendi I, Johnson SL, Dunham JB, Haggerty R & Hockman-Wert D (2012) The paradox of cooling streams in a warming world: Regional climate trends do not parallel variable local trends in stream temperature in the Pacific continental United States. *Geophysical Research Letters* 39.
- Ashton KG (2002) Patterns of within-species body size variation of birds: strong evidence for Bergmann's rule. *Global Ecology & Biogeography* 11: 505-523.
- Belk MC & Lydeard C (1994) Effect of *Gambusia holbrooki* on a similar-sized, syntopic Poeciliid, *Heterandria formosa*: Competitor or predator? *Copeia* 1994: 296-302.
- Belk MC & Houston DD (2002) Bergmann's rule in ectotherms: A test using freshwater fishes. *The American Naturalist* 160: 803-808.
- Benejam 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. *Biological Invasions* 11: 2265-2277.
- Bianco PG (1995) Mediterranean endemic freshwater fishes of Italy. *Biological Conservation* 72: 159-170.
- Blanck A & Lamouroux N (2007) Large-scale intraspecific variation in life-history traits of European freshwater fish. *Journal of Biogeography* 34: 862-875.
- Caiola N & de Sostoa A (2005) Possible reasons for the decline of two native toothcarps in the Iberian Peninsula: evidence of competition with the introduced Eastern mosquitofish. *Journal of Applied Ichthyology* 21: 358-363.
- Clavero M & Garcia-Berthou E (2005) Invasive species are a leading cause of animal extinctions. *Trends in Ecology & Evolution* 20: 110.

- Conover DO, Duffy TA & Hice LA (2009) The covariance between genetic and environmental influences across ecological gradients. Reassessing the evolutionary significance of countergradient and cogradient variation. *Annals of the New York Academy of Sciences* 1168: 100-129.
- Conover DO & Present TMC (1990) Countergradient variation in growth rate: compensation for length of the growing season among Atlantic silversides from different latitudes. *Oecologia* 83: 316-324.
- Courtenay WRJ, Meffe GK (1989) Small fishes in strange places: a review of introduced poeciliids. *Ecology and evolution of livebearing fishes.* pp. 319-331. In: G.K. Meffe, F.F. Snelson Jr, (ed). Prentice Hall, Englewood Cliffs, NJ.
- Cox B (2003) A review of dissolved oxygen modelling techniques for lowland rivers. *The science of the total environment* 314-316: 303-334.
- Cunjak RA & Green JM (1986) Influence of water temperature on behavioural interactions between juvenile brook charr, *Salvelinus fontinalis*, and rainbow trout, *Salmo gairdneri*. *Canadian Journal of Zoology* 64: 1288-1291.
- De Staso J & Rahel FJ (1994) Influence of water temperature on interactions between juvenile Colorado river cutthroat trout and brook trout in a laboratory stream. *Transactions of the American Fisheries Society* 123: 289-297.
- Desmet NJS, Van Belleghem S, Seuntjens P, Bouma TJ, Buis K & Meire P (2011) Quantification of the impact of macrophytes on oxygen dynamics and nitrogen retention in a vegetated lowland river. *Physics and Chemistry of the Earth, Parts A/B/C* 36: 479-489.
- Doadrio I (2002) Atlas y libro rojo de los peces continentales de España. In: Ministerio de Medio Ambiente, (ed). Madrid, España.
- Dodds WK, Smith VH & Lohman K (2002) Nitrogen and phosphorus relationships to benthic algal biomass in temperate streams. *Canadian Journal of Fisheries and Aquatic Sciences* 59: 865-874.

- Fernández-Delgado C (1989) Life-history patterns of the mosquito-fish, *Gambusia affinis*, in the estuary of the Guadalquivir river of south-west Spain. *Freshwater Biology* 22: 395-404.
- Fernández-Delgado C & Rossomanno S (1997) Reproductive biology of the mosquitofish in a permanent natural lagoon in south-west Spain: two tactics for one species. *Journal of Fish Biology* 51: 80-92.
- Fontoura NF & Agostinho AA (1996) Growth with seasonally varying temperatures: an expansion of the von Bertalanffy growth model. *Journal of Fish Biology* 48: 569-584.
- Fry FEJ (1947) Effects of the environment on animal activity. pp. 1-62. No. 68. In: Ont. Fish. Research Lab, (ed). The University of Toronto press, Toronto.
- Gamradt EC & Kats LB (1996) Effect of introduced Crayfish and Mosquitofish on California newts. *Conservation Biology* 10: 1155-1162.
- García-Berthou E, Alcaraz C, Pou-Rovira Q, Zamora L, Coenders G & Feo C (2005) Introduction pathways and establishment rates of invasive aquatic species in Europe. *Canadian Journal of Fisheries and Aquatic Sciences* 62: 453-463.
- Gaston KJ, Chown SL & Evans KL (2007) Ecogeographical rules: elements of a synthesis. *Journal of Biogeography* 35: 483-500.
- Gido KB & Brown JH (1999) Invasion of North American drainages by alien fish species. *Freshwater Biology* 42: 387-399.
- Hamer A, Lane S & Mahony M (2002) The role of introduced mosquitofish (*Gambusia holbrooki*) in excluding the native green and golden bell frog (*Litoria aurea*) from original habitats in south-eastern Australia. *Oecologia* 132: 445-452.
- Hawkins C, P. & Hogue J, N. (1997) Channel morphology, water temperature, and assemblage structure of stream insects. *Journal of the North American Benthological Society* 16: 728-749.
- Hillebrand H (2004) On the generality of the latitudinal diversity gradient. *The American Naturalist* 163: 192-211.

- Hurlbert SH, Zedler J & Fairbanks D (1972) Ecosystem alteration by mosquitofish (*Gambusia affinis*) predation. *Science* 175: 639-641.
- Jonsson B & L'Abée-Lund JH (1993) Latitudinal clines in life-history variables of anadromous brown trout in Europe. *Journal of Fish Biology* 43: 1-16.
- Kats LB & Ferrer RP (2003) Alien predators and amphibian declines: review of two decades of science and the transition to conservation. *Diversity and Distributions* 9: 99-110.
- L'Abée-Lund JH, Jonsson B, Jensen AJ, Sættem L, Heggberget TG, Johnsen B & Naesje TF (1989) Latitudinal variation in life-history characteristics of sea-run migrant brown trout *Salmo trutta*. *The Journal of Animal Ecology* 58: 525-542.
- Laha M & Mattingly HT (2006) Identifying environmental conditions to promote species coexistence: An example with the native Barrens topminnow and invasive western mosquitofish. *Biological Invasions* 8: 719-725.
- Lapointe NWR & Light T (2012) Landscape-scale determinants of non-native fish communities. *Diversity and Distributions* 18: 282-293.
- Levine JM & D'Antonio CM (2003) Forecasting biological invasions with increasing international trade. *Conservation Biology* 17: 322-326.
- Lloyd L (1986) An Alternative to insect control by "Mosquitofish", *Gambusia affinis*. *Arbovirus research in Australia* 156-163.
- Lloyd LN (1990) Native fish as alternatives to the exotic fish, *Gambusia*, for insect control. In: D.A. Pollard, (ed). *ASFB Workshop on introduced and translocated fishes and their ecological effects Bureau of Rural Resources Proceedings No. 8*, AGPS. Canberra.
- Magnuson JJ, Crowder L, B. & Medvick P, A. (1979) Temperature as an ecological resource. *American Zoologist* 19: 331-343.
- Magurran A (2000) Sex differences in behaviour as an indirect consequence of mating system. *Journal of Fish Biology* 57: 839-857.

- Margaritora FG, Ferrara O & Vagaggini D (2001) Predatory impact of the mosquitofish (*Gambusia holbrooki* Girard) on zooplanktonic populations in a pond at Tenuta di Castelporziano (Rome, Central Italy). *Journal of Limnology* 60: 189-193.
- Meffe GK (1985) Predation and species replacement in American southwestern fishes: A case study. *The Southwestern Naturalist* 30: 173-187.
- Meffe GK, F.F. S (1989) Ecology and evolution of livebearing fishes (Poeciliidae). New York: Prentice Hall.
- Mills MD, Rader RB & Belk MC (2004) Complex interactions between native and invasive fish: the simultaneous effects of multiple negative interactions. *Oecologia* 141: 713-721.
- Moreau (1987) Mathematical and biological expression of growth in fishes: recent trends and further developments. In: Summer- Felt RC, Hall GE. (eds.), *Age and Growth of Fish*. Iowa State University Press, Ames, Iowa, pp. 81–113.
- Moyle PB (2002) *Inland fishes of California*. University of California Press, Berkeley.
- Moyle PB & Light T (1996) Biological invasions of fresh water: Empirical rules and assembly theory. *Biological Conservation* 78: 149-161.
- Moyle PB, Li HW, Barton B (1987) The Frankenstein effect: impact of introduced fishes on native fishes of North America. pp. 415–426. In: R. H. Stroud, (ed). *The role of fish culture in fisheries management*. Bethesda: American Fisheries Society.
- Oliva-Paterna FJ, Torralva M & Fernández-Delgado C (2006) Threatened fishes of the world: *Aphanius iberus* (Cuvier & Valenciennes, 1846) (Cyprinodontidae). *Environmental Biology of Fishes* 75: 307-309.
- Oyugi DO, Cucherousset J & Robert Britton J (2012) Temperature-dependent feeding interactions between two invasive fishes competing through interference and exploitation. *Reviews in Fish Biology and Fisheries* 22: 499-508.
- Page LM, Burr BM (1991) *A field guide to freshwater fishes: North America north of Mexico*. Houghton Mifflin Harcourt.

- Pimentel D, Lach L, Zuniga R & Morrison D (2000) Environmental and economic costs of nonindigenous species in the United States. *BioScience* 50: 53-65.
- Poole GC & Berman CH (2001) An ecological perspective on in-stream temperature: natural heat dynamics and mechanisms of human-caused thermal degradation. *Environmental Management* 27: 787-802.
- Power M, Dempson JB, Reist JD, Schwarz CJ & Power G (2005) Latitudinal variation in fecundity among Arctic charr populations in eastern North America. *Journal of Fish Biology* 67: 255-273.
- Priddis E, Rader R, Belk M, Schaalje B & Merkley S (2009) Can separation along the temperature niche axis promote coexistence between native and invasive species? *Diversity and Distributions* 15: 682-691.
- Puig MA, Aboal M & de Sostoa A (1991) New approaches to Mediterranean fluvial communities. *Oecologia Aquatica* 10: 13-20.
- Pyke GH (2008) Plague minnow or Mosquito fish? A review of the biology and impacts of introduced *Gambusia* species. *Annual Review of Ecology, Evolution, and Systematics* 39: 171-191.
- Reznick D & Bryant M (2007) Comparative long-term mark-recapture studies of guppies (*Poecilia reticulata*): differences among high and low predation localities in growth and survival. *Annales Zoologici Fennici* 44: 152-160.
- Reznick DN, Butler M, J., Rodd H, F. & Ross P (1996) Life-history evolution in guppies (*Poecilia reticulata*) 6. Differential mortality as a mechanism of natural selection. *Evolution* 50: 1651-1660.
- Rhymer JM (1992) An experimental study of geographic variation in avian growth and development. *Journal of Evolutionary Biology* 5: 289-306.
- Rincón PA, Correas AM, Morcillo F, Risueño P & Lobón-Cerviá J (2002) Interaction between the introduced eastern mosquitofish and two autochthonous Spanish toothcarps. *Journal of Fish Biology* 61: 1560-1585.

- Rogowski DL & Stockwell CA (2006) Assessment of potential impacts of exotic species on populations of a threatened species, white sands pupfish, *Cyprinodon tularosa*. *Biological Invasions* 8: 79-87.
- Rowe DK, Smith JP & Baker C (2007) Agonistic interactions between *Gambusia affinis* and *Galaxias maculatus*: implications for whitebait fisheries in New Zealand rivers. *Journal of Applied Ichthyology* 23: 668-674.
- Sabater S. & Barceló D. (eds.) (2010) *Water scarcity in the Mediterranean: Perspectives under global change*. Springer, Berlin.
- Schemske DW, Mittelbach GG, Cornell HV, Sobel JM & Roy K (2009) Is there a latitudinal gradient in the importance of biotic interactions? *Annual Review of Ecology, Evolution, and Systematics* 40: 245-269.
- Sears MW & Angilletta MJ (2004) Body size clines in *Sceloporus* Lizards: Proximate mechanisms and demographic constraints. *Integrative and Comparative Biology* 44: 433-442.
- Stockwell CA, Henkanaththegebara SM (2011) Evolutionary conservation biology of Poeciliids. In: J. Evan, A. Pilastro, I. Schlupp, (ed). *Ecology and evolution of Poeciliid fishes*. University of Chicago Press, Chicago.
- Taniguchi Y & Nakano S (2000) Condition-specific competition: implications for the altitudinal distribution of stream fishes. *Ecology* 81: 2027-2039.
- Taniguchi Y, Rahel FJ, Novinger DC & Gerow KG (1998) Temperature mediation of competitive interactions among three fish species that replace each other along longitudinal stream gradients. *Canadian Journal of Fisheries and Aquatic Sciences* 55: 1894-1901
- Tedesco PA, Benito J & García-Berthou E (2008) Size-independent age effects on reproductive effort in a small, short-lived fish. *Freshwater Biology* 53: 865-871.
- Vidal O, García-Berthou E, Tedesco PA & García-Marín J-L (2009) Origin and genetic diversity of mosquitofish (*Gambusia holbrooki*) introduced to Europe. *Biological Invasions* 12: 841-851.

- Villarreal CA, Thorpe JE & Miles MS (1988) Influence of photoperiod on growth changes in juvenile Atlantic salmon, *Salmo salar* L. J. Journal of Fish Biology 33: 15-30.
- Vitousek P, M., D'Antonio CM, Loope L, L., Rejmánek M & Westbrooks R (1997) Introduced species: A significant component of human-caused global change. New Zealand Journal of Ecology 21: 1-16.
- Walton WE, Henke JA, Why AM (2011) *Gambusia affinis* (Baird and Girard) and *Gambusia holbrooki* Girard (Mosquitofish). Chap. 22, pp. 261-273. In: R Francis, (ed). *A handbook of global freshwater invasive species*. Earthscan Co, London, United Kingdom.
- Webb BW (1996) Trends in stream and river temperature. Hydrological Processes 19: 205-226.
- Webb BW, Hannah DM, Moore RD, Brown LE & Nobilis F (2008) Recent advances in stream and river temperature research. Hydrological Processes. 22: 902-918.
- Williams JE, Johnson JE, Hendrickson DA, et al. (1989) Fishes of North America endangered, threatened, or of special concern. Fisheries 14: 2-20.
- Wood, C. M. & McDonald, D. G. (1997) Global warming: implications for freshwater and marine fish. Cambridge University Press, Cambridge, United Kingdom.
- Wurtsbaugh WA & Cech J (1983) Growth and activity of juvenile Mosquitofish: temperature and ration effects. Transaction of the American Fisheries Society. 112: 653-660.
- Yamahira K & Conover DO (2002) Intra- vs. interspecific latitudinal variation growth: Adaptation to temperature or seasonality? Ecology 83: 1252-1262.

