Universitat de Girona

ECOLOGICAL INTERACTIONS BETWEEN AN INVASIVE FISH (GAMBUSIA HOLBROOKI) AND NATIVE CYPRINODONTS: THE ROLE OF SALINITY

Carles ALCARAZ CAZORLA

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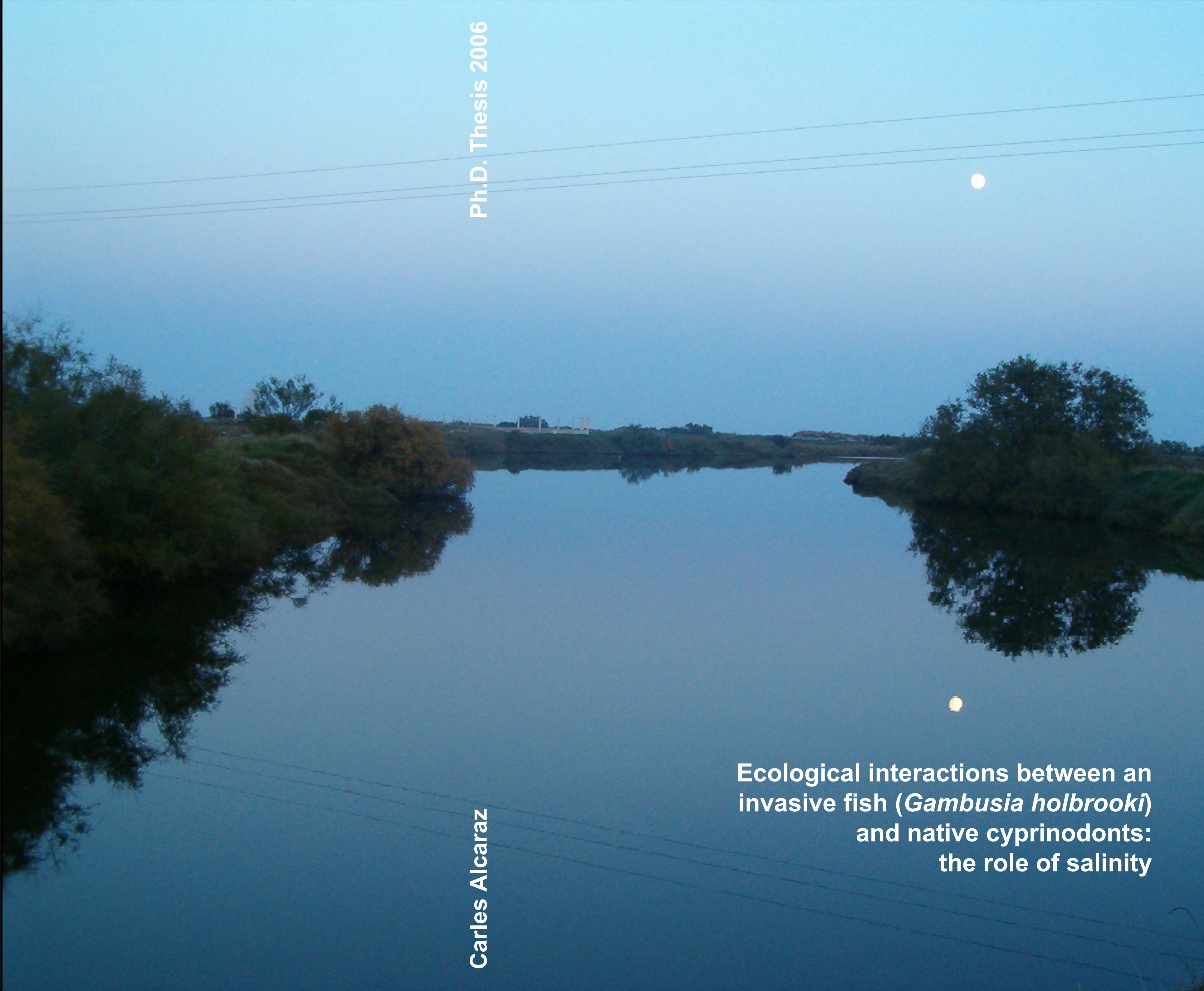

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Carles Alcaraz

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Memòria de la tesi doctoral

Carles Alcaraz Cazorla

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de Girona
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Aquàtica

Tesi doctoral

Ecological interactions between an invasive fish (*Gambusia holbrooki*) and native cyprinodonts: the role of salinity

Memòria presentada per Carles Alcaraz Cazorla
per a l'obtenció del títol de Doctor
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Carles Alcaraz Cazorla

Vist-i-plau del director

Dr. Emili García-Berthou
Prof. Titular d'Universitat, Ecologia
Dept. de Ciències Ambientals
i Institut d'Ecologia Aquàtica
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A les meves nebodetes,
la Júlia,
i el seu somriure que tot ho il·lumina,
l'Helena,
i el seu àngel que tot ho emplena

“Writing isn't writing, it's rewriting”

* P.Caputo, A rumor of war p.349 (1977/96). Pimlico, London.

Foto de la portada, Carles Alcaraz

Agraïments

Abans de res m'agradaria començar dient que aquest treball no hauria estat possible sense la col·laboració i ajuda de molta gent que inclús de vegades hi ha participat sense saber-ho. A l'hora de buscar responsables del perquè d'aquest treball, i encara que se que començar pels caps és de pilotes..., és evident que el "culpable" de tot plegat és l'Emili. Ell és qui ara fa uns anys just abans de que acabés la carrera em va donar la oportunitat de col·laborar amb ell fent petites coses i posteriorment de fer aquesta Tesi. És per això que m'agradaria donar-li les gràcies, per obrir-me les portes de l'ecologia de peixos, per la confiança, pels comentaris, per haver-me fet veure l'estadística d'una forma diferent, per les correccions, les correccions de les correccions, les correccions de les correccions ja corregides... (i és que quan veig un boli vermell ja tremolo...), en fi per tot això i més moltes gràcies.

I bé un cop dins del grup, com en tots els principis suposo, vaig poder gaudir de diferents feinetes. Així és com vaig acabar mostrejant en una bassa que s'asseca quan fa Sol i s'emplena quan plou, d'aigua i d'uns estranys "éssers de tres ulls", els triops. Com suposo que tots heu endevinat, els primers mostrejos els vaig compartir amb en Dani, moltes gràcies pel temps al camp, per les basses i per les classes de taxonomia improvisada. Durant aquest inicis també es va posar en marxa el Life de l'Estartit i on també em va tocar ajudar, en aquest cas formant equip amb en Quim, segurament la persona amb la que més hores de camp he compartit, i és que els fartets encara fugen quan ens apropem per Fra Ramon. Va ser just durant un d'aquest mostrejos, concretament el que va coincidir amb un període "inusual", "estrany", "poc freqüent"... d'inundació de la salicòrnia que va començar aquesta tesi. Així que moltes gràcies Quim, pels temps de mostreig, per les hores traient peixos dels tremalls i per introduir-me a l'ecologia del fartet, desprès de tot resulta que va ser divertit.

Va ser durant aquesta primera part que vaig haver de resoldre els primers grans obstacles i és per això que vull agrair a en David les hores que vam dedicar a separar sediment i els temps dedicat a intentar buscar el sistema de fer menys feixuga la feina, i al grup de bitxos, en Dani, Stéphanie, Jordi S., Anna B. i Sandra B. (us hi heu fixat mai la quantitat de noms repetits que tenim a Ecologia???) per les hores de lupa, per les classificacions, i sobretot per la paciència que vau demostrar cada cop que entrava per la porta amb un perdoneu teniu un momentet és que he trobat un bitxo i no tinc ni idea de que pot ser...

Tampoc seria just oblidar-me de la resta de companys del grup de peixos, a en Quim (petit) li va tocar patir el mostreig del gradient de salinitat de la gambúsia, i a en Benito (que ja sé que no és el nom, però és com li diem tots) i l'Inma per les interminables hores de laboratori classificant peixos, comptant i pesant embrions. Amb en Lluís B. tot i que no forma part d'aquesta tesi vaig compartir un dels mostrejos més estressants que segurament hem fet i farem mai (ups... a partir de l'any que bé s'haurà de fer cada més) el del gradient latitudinal i és que en una setmana vam anar de França a Múrcia a molestar totes les gambúsies que vam poder. Moltes gràcies a tots nois, per les hores de camp compartides i evidentment per l'ajuda. També cal dir que amb vosaltres he fet el que segurament és el mostreig més espectacular que he fet fins ara, el dels túnels de Tortosa.

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Bé, fins aquí aquest diari de ruta, que més que uns agraïments semblen unes memòries, però arribats aquest punt també vull agrair a la resta de peixòlegs, l'Anna V., en Lluís Z. en Ramon i en Miguel (l'últim d'arribar) la seva participació en aquest treball. Sense els seus comentaris, diferents punt de vista i altres ajudes de tot tipus en diferents moments d'aquest treball el resultat no hauria estat aquest.

Als meus companys de seminari, que no són pocs i que es mereixerien una secció a part, ja que amb ells comparteixo el meu espai vital i per tant són la gent que hi tinc cada dia més propera. D'esquerra a dreta, Elisabet, Sandra S., Joan, Irene, Annita, Lluís, Anna B., Quim, Marta i Gemma U., i tots aquells que en un moment o altre hi heu compartit un tallet. Moltes gràcies, quan vulgueu tornem a fer una partida de "tetris-seminari". Especialment vull agrair a l'Anna B. les estones d'esbarjo i cafè a can Paco i sobretot compartint les penúries de la Tesi, ja queda poquet per acabar amb tot això així que ànims!!!

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A tots aquells amics que en un moment o altre s'han creuat en el meu camí per anar a compartir una cervesa i una estona d'esbarjo.

I per últim i per això no menys important, a la meva família, de fet a les meves dues famílies, la d'aquí de Girona per deixar-me fer i provar d'entendre que uns peixets tant petits puguin donar per tant, quan ni tan sols es mengen i menys encara que es pugui mirar que hi ha a dins de la panxa d'una cosa tant petita. I a la meva família de Carboneras, per introduir-me al món de la recollida d'olives, pels àpats, per les hores pescant calamars, i per servir-me de refugi on escapar-me de tant i ajudar-me a desconnectar una mica de tot plegat. I és en aquest punt, en el de desconnectar on haig de fer una menció especial a les meves dues nebodetes, la Júlia que va venir per veurem començar i l'Helena que ha va arribar per supervisar el final. Que ningú s'enfadi, però segurament elles són, sense saber-ho, les que més han fet per aquest treball, amb les seves ganes de gresca i jugar permanents. A tots moltes gràcies, l'únic que em sap greu és que estigui escrita en anglès i no us pugui ajudar una mica a entendre el perquè de tota aquesta feinada.

I ara si que ja acabo, a la Naza, per intentar posar la voluntat d'entendre-ho tot, per llegir un munt de coses que de segur li devien sonar a xino, pel seu optimisme infinit i posar llum en mig de la foscor i sobretot per estar sempre allà i no desesperar durant les interminables hores d'espera.

I a risc de fer-me pesat, que sé que ho sóc, moltes gràcies a tots, a valgut la pena, de veritat.

Contents

Summary

One of the main current threats to biodiversity and ecosystem function is the introduction of invasive species. We reviewed 26 variables on the life-history, ecology and human use of the 69 inland fish species of the Iberian Peninsula reporting that the phylogeny, variability (in addition to central tendency) and human use are needed to a better understanding of the differences between native and invasive species. The taxonomic distribution of invasive fish species deviated significantly from world freshwater richness and in contrast to native species, invasive fish belonged to only five taxonomic orders but to a wide spectrum of families not native to the Iberian Peninsula. The life-history traits were highly dependent on taxonomy since after accounting for phylogeny, invasive species displayed differences in reproductive season. Human use was also significantly different between native and invasive species and produced more variability in life-history traits of invasive species and uneven taxonomic distribution because of the high diversity of species introduced. We show that accounting for taxonomy and studying variability in addition to central tendency is important in the comparison of life-history traits between native and invasive species.

Some of the species most threatened by invasive fish species, mainly the mosquitofish (*Gambusia holbrooki*), are the Mediterranean native cyprinodontiforms. We report the first data on the use of occasionally-inundated habitats, the diet and prey electivity of the Spanish toothcarp (*Aphanius iberus*). During a flooding period, we sampled macroinvertebrates and fish in three contrasting habitats: mats of green macroalgae, open water, and glasswort (that is occasionally inundated). The three habitats differed in density and species composition, with algal mats showing higher invertebrate biomasses and glasswort higher organisms diversity. Glasswort presented the highest density of mature toothcarp, whilst immature fish were similarly abundant in the glasswort and algal mat habitats. Condition (weight-length relationship) and food biomass in the gut contents of immature toothcarp were higher in algal mats than in glasswort, whereas there were no differences for mature fish. Glasswort was positively selected by mature fish but seemed a disadvantageous habitat for immature toothcarp. In general, toothcarp’s diet was omnivorous, dominated by harpacticoid copepods, copepod nauplii and detritus. However, diet composition varied among habitats, depending on prey availability. Although a benthic feeding habitat has previously been suggested, in our study the diet was based rather on water column organisms for both glasswort and algal mat habitats. There was also an ontogenetic diet shift, with an increase of mean prey length with fish length, clearly linked to a microhabitat change. Smaller fish showed positive electivity on planktonic prey, while larger fish elected more benthic organisms.

Mosquitofish (*Gambusia holbrooki*) is one of the worst invasive species worldwide with

strong impacts on ecosystem functioning and native species. It has been previously hypothesized, based on field observations, that salinity limits the invasive success of mosquitofish and constitutes a refuge for native cyprinodontiforms. However, there are few studies on how salinity affects its ecology and biology and whether it mediates the behavioral interactions with native species is largely unknown. In the last part of this work we report information to understand the role of salinity in the invasive success of mosquitofish. We examined differences on mosquitofish life history traits inhabiting from fresh (near ca. 0.2‰ of salinity) to polysaline waters (ca. 23‰). Salinity affected density and life history traits of both male and female mosquitofish. Both mosquitofish sexes decreased they density and increased reproductive investment with salinity at the cost of somatic condition in females. Females from higher salinity waters reproduced earlier, since presented embryos on advanced developmental stages, and presented heavier embryos (independently of the maturation state, after correction for brood and fish size). Furthermore, our data showed that some mosquitofish can adopt a matrotrophic strategy although it has previously been considered a lecithotrophic species. On the other hand, we experimentally tested the role of salinity (0, 15, 25‰) on the agonistic behaviour and food competition between mosquitofish and an endangered cyprinodont, demonstrating that mosquitofish decreased its aggressive behaviour towards the toothcarp and captured less prey, reducing its efficiency with salinity increases. In contrast, toothcarp did not change its behaviour with different salinity treatments. Therefore, the effects of salinity on mosquitofish life history and behavioural traits confirm previous field observations that salinity limits mosquitofish invasive success.

Resumen

Actualmente una de las principales amenazas a la biodiversidad y funcionamiento de los ecosistemas, es la introducción de especies invasoras. A partir de la revisión de 26 variables sobre la biología reproductora, ecología y usos humanos de las 69 especies de peces continentales de la Península Ibérica concluimos que la filogenia, variabilidad (además de la tendencia central) y los usos humanos son necesarios para una mejor comprensión de las diferencias entre las especies nativas e invasoras. La distribución taxonómica de las especies invasores se desvió significativamente de la riqueza mundial de las aguas continentales, y en contraste a las nativas, las invasoras pertenecieron a sólo cinco órdenes taxonómicos aunque a un amplio rango de familias no nativas de la Península Ibérica. Las características de la biología reproductiva dependieron mucho de la taxonomía, ya que después de eliminar el efecto de la filogenia, las especies invasoras se diferenciaron en la época reproductiva. Los usos dados por el hombre también resultaron significativos en esta diferenciación, produciendo más variabilidad en las características de la biología reproductiva de las invasoras y una desigual distribución taxonómica debida a la alta diversidad de especies introducidas. Por tanto, considerar el efecto de la taxonomía y estudiar la variabilidad además de la tendencia central es importante en la comparación de las características referentes a la reproducción de las especies nativas e invasoras.

Dentro de las especies más afectadas por la introducción de peces, principalmente la gambusia (*Gambusia holbrooki*), se encuentran los ciprinodontiformes nativos del Mediterráneo. Aportamos los primeros datos sobre el uso de los hábitats ocasionalmente inundados, la dieta y selección de presas del fartet (*Aphanius iberus*). Durante un periodo de inundación, muestreamos los macroinvertebrados y los peces en tres hábitats próximos: matas de macroalgas verdes, agua libre y salicornia (sólo ocasionalmente inundado). Los tres hábitats se diferenciaron en la densidad y la composición de especies, con las algas verdes mostrando una mayor biomasa de invertebrados y la salicornia mayor diversidad de organismos. La salicornia presentó la densidad más alta de fartets adultos, aunque los juveniles presentaron abundancias similares en la salicornia y las algas verdes. La condición (relación longitud-peso) y la biomassa de los contenidos estomacales de los juveniles fueron superiores en las algas verdes, mientras que no existieron diferencias entre los adultos. Mientras los adultos seleccionaron positivamente la salicornia, éste pareció ser un hábitat desventajoso para los juveniles. En general, la dieta de los fartets fue omnívora, dominada por harpacticoides, nauplios y detritos. Sin embargo, la composición de la dieta varió entre los diferentes hábitat en relación a la disponibilidad de presas. A pesar de que previamente se ha sugerido un hábito alimentario bentónico, en nuestro estudio la dieta estaba preferentemente basada en organismos de la columna de agua tanto en la salicornia como en las algas verdes. Además, existió un cambio ontogenético de la dieta, con un incremento de la longitud media de las presas con la longitud de los fartets, claramente relacionado con un cambio en el microhábitat. Mientras los fartets más pequeños mostraron preferencia por presas planctónicas, los más grandes seleccionaron preferiblemente organismos más bentónicos.

La gambusia (*Gambusia holbrooki*) es una de las peores especies invasoras en todo el mundo, con graves impactos sobre el funcionamiento de los ecosistemas y las especies nativas. Previamente, ha sido hipotetizado, basándose en observaciones de campo, que la salinidad limita el éxito invasor de la gambusia y constituye un refugio para los cyprinodontiformes nativos. No obstante, sólo hay unos pocos estudios sobre como la salinidad afecta su ecología y biología y se desconoce si media en las relaciones de comportamiento con las especies nativas. En la última parte de esta tesis daremos información que permita entender el papel de la salinidad en el éxito invasor de la gambusia. Examinamos las diferencias en la biología reproductiva de la gambusia procedentes de agua dulce (aprox. 0,2‰) a polihalinas (aprox. 23‰). La salinidad afectó la densidad y la biología reproductiva de ambos sexos de gambusia. Ambos relucieron su densidad e incrementaron el esfuerzo reproductivo a costa de la condición somática en las hembras. Las hembras de salinidades superiores se reproducen antes, ya que presentaron embriones en estadios más avanzados de desarrollo y más grandes (independientemente del estadio de maduración, longitud del pez y tamaño de la puesta). Además, nuestros datos muestran que algunas poblaciones de gambusia pueden adoptar una estrategia matrotrófica, a pesar de que previamente se la ha considerado una especie lecitotrófica. Por otro lado, experimentalmente hemos testado el papel de la salinidad (0, 15, 25‰) en el comportamiento agresivo y la competencia por el alimento entre la gambusia y un cyprinodóntido amenazado, demostrando que con el incremento de salinidad la gambusia disminuyó su agresividad y capturo menos presas, reduciendo su eficiencia. Por el contrario, el fartet, no cambió su comportamiento con la salinidad. Por tanto, los efectos de la salinidad en su biología reproductiva y comportamiento confirman la hipótesis previa de que la salinidad limita el éxito invasor de la gambusia.

Resum

Actualment una de les principals amenaces a la biodiversitat i funcionament dels ecosistemes és la introducció d'espècies invasores. Mitjançant la revisió de 26 variables sobre l'ecologia reproductiva, ecologia i els usos de l'home de les 69 espècies de peixos continental de la Península Ibèrica concloem que la filogènia, variabilitat (a més de la tendència central) y els usos de l'home són necessaris per entendre millor les diferencies entre les espècies natives i invasores. La distribució taxonòmica de les espècies invasores es va desviar significativament de la riquesa mundial de les aigües continental, i en contrast a les natives, les invasores van pertànyer a tan sols cincs ordres taxonòmics encara que a un ampli rang de famílies no natives de la Península Ibèrica. Les característiques de la biologia reproductiva van ser molt dependents de la taxonomia, ja que desprès d'eliminar l'efecte de la filogènia, les espècies invasores van diferenciar-se en l'època reproductora. Els usos de l'home també van resultar significatius en aquesta diferenciació, produint més variabilitat en les característiques de la biologia reproductora de les invasores i una desigual distribució taxonòmica deguda a l'elevada diversitat d'espècies introduïdes. Per tant, eliminar l'efecte de la taxonomia i estudiar la variabilitat a més de la tendència central és important en la comparació de les característiques referents a la reproducció de les espècies natives i introduïdes.

Entre les especies més afectades per la introducció de peixos, principalment la gambúsia (*Gambusia holbrooki*), es troben els cyprinodontiforms natius del Mediterrani. Aportem les primeres dades sobre l'ús dels hàbitats ocasionalment disponibles, la dieta i la selecció de preses del fartet (*Aphanius iberus*). Durant un període d'inundació, vàrem mostrejar els macroinvertebrats i els peixos en tres hàbitats propers: mates de macroalgues verdes, aigua lliure i salicòrnia (només ocasionalment inundats). Els tres hàbitats van diferenciar-se en la densitat i la composició d'espècies, amb les algues verdes presentant una major biomassa d'invertebrats i la salicòrnia major diversitat d'organismes. La salicòrnia va presentar la densitat més alta de fartets adults, encara que els juvenils van presentar abundàncies similars a la salicòrnia i les algues verdes. La condició (relació pes-longitud) i la biomassa dels continguts estomacals dels juvenils van ser superiors a les algues verdes, mentre que no van existir diferencies entre els adults. Mentre els adults seleccionaren positivament la salicòrnia, aquest va semblar un hàbitat desavantatjós pels juvenils. En general, la dieta dels fartets va ser omnívora, dominada per harpacticoids, nauplis i detritus. No obstant, la composició de la dieta va variar entre els diferents hàbitats en relació a la disponibilitat de preses. Encara que prèviament se li ha suggerit un hàbit alimentari bentònic, en el nostre estudi la dieta estava preferiblement basada en organismes de la columna d'aigua tant per la salicòrnia com a les algues verdes. A més, es va donar un canvi ontogenètic d'aquesta, amb un increment de la longitud mitjana de les preses amb la longitud dels fartets, clarament relacionada amb el microhàbitat. Mentre els fartets més petits mostraren preferència per preses més planctòniques, els més grans seleccionaren preferiblement organismes més bentònics.

La gambúsia (*Gambusia holbrooki*) és una de les pitjors espècies invasores en tot el món, amb greus impactes sobre el funcionament dels ecosistemes i les espècies natives. Prèviament ha estat hipotetitzat, basant-se en observacions de camp, que la salinitat limita l'èxit invasor de la gambúsia y constitueix un refugi pels cyprinodontiforms natius. No obstant, només hi ha uns pocs estudis sobre com la salinitat afecta a la seva ecologia i biologia i és completament desconegut si regula les relacions de comportament amb les especies natives. En la última part d'aquesta tesis donarem informació que permeti entendre el paper de la salinitat en l'èxit invasor de la gambúsia. Vam examinar les diferències en la biologia reproductiva de la gambúsia procedents d'aigua dolça (aprox. 0,2‰) a polihalines (aprox. 23‰). La salinitat va afectar la densitat i la biologia reproductiva d'ambdós sexes de gambúsia. Tot dos van reduir la seva densitat i van incrementar l'esforç reproductiu a costa de la condició somàtica en les femelles. Les femelles de salinitats superiors es reprodueixen abans, ja que presentaren embrions en estadis més avançats de desenvolupament y més grossos (independentment del estadi de maduració, longitud del peix i mida de la posta). A més, les nostres dades mostren que algunes poblacions de gambúsia poden adoptar una estratègia matrotròfica , encara que prèviament se l'ha considerat una espècie lecitotròfica . Per una altra banda, experimentalment hem testat el paper de la salinitat (0, 15, 25‰) en el comportament agressiu i la competència per l'aliment entre la gambúsia i un ciprinodòntid amenaçat, demostrant que amb l'increment de salinitat la gambúsia va disminuir la seva agressivitat y va capturar menys preses, reduint la seva eficàcia. Pel contrari, el fartet, no va canviar el seu comportament amb la salinitat. Per tant, els efectes de la salinitat en la seva biologia reproductora i comportament confirmen la hipòtesi prèvia que la salinitat limita l'èxit invasor de la gambúsia.

Introduction

BIOLOGICAL INVASIONS

Invasive species

After habitat degradation, invasive species are the second leading cause of biodiversity loss, particularly in freshwater ecosystems (Moyle *et al.* 1986, Vitousek *et al.* 1997, Mack *et al.* 2000). Besides imposing huge economic costs, invasive species cause extinction of native species, reduction of genetic diversity, and biotic homogenization through a variety of mechanisms (Rahel 2000). Human activities have recently accelerated the intentional and accidental spread of species across their natural dispersal barriers (e.g. Kolar & Lodge 2001, Alcaraz *et al.* 2005, García-Berthou *et al.* 2005). Invasive species can present huge, and not always predictable, effects on ecosystem processes and the soil communities presenting high economical consequences (Mack *et al.* 2000, Ehrenfeld & Scott 2001). Thus, nowadays biological invasions are considered second leading threat to biodiversity loss after habitat destruction, and an important element of global change (Vitousek *et al.* 1996, Chapin *et al.* 2000). Mediterranean biomes and grassland ecosystems are the most sensitive to species introductions (Sala *et al.* 2000, Gritti *et al.* 2006). Biotic invasions constitute only one outcome −indeed, the least likely outcome− of a multistage process that begins when organisms are transported from their native ranges to new localities (Mack *et al.* 2000). There are three main stages in the invasion process (Richardson *et al.* 2000, Kolar & Lodge 2001):

- Introduction: the intentional or unintentional introduction to the wild of a species imported into a region beyond its native range.
- Establishment: the establishment of self-sustaining, reproductive populations.
- Invasion: population growth and spreading of the species.

The impacts of invasive species

At a local scale, the strong impacts of introduced species are wide and different, affecting at different levels. For instance, invasive species alter and modify ecosystem structure by altering composition and community structure of invader areas (Vitousek 1990, Ehrenfeld & Scott 2001). The change in species composition of communities can have high effects on ecosystem process. Some works have demonstrated changes in species richness (Juliano 1998, Sanders *et al.* 2003), in the type of species present (“functional groups”), differing from native species in resource acquisition and/or resource use efficiency (Vitousek 1990, Tompkins *et al.* 2003) and in the presence of a ‘‘keystone’’ species change food-web architecture, altering the trophic structure of overall species of the area invaded (Crooks 2002, Beisner *et al.* 2003, McCarthy *et al.* 2006). Therefore, invasive species by additions of new ones, substitution of one or more native species, or loss of native diversity, alter ecosystem processes. These changes on ecosystem dynamics may have cascading effects on other species and may affect the potential for restoration and the methods used (Ehrenfeld & Scott 2001).

In a similar way, direct relationships among invaders and native species, at a population level, are diverse and overall present negative effects for latter (Mack *et al.* 2000). A vast literature exists on harmful effects of invasive species on native ones. For example there are a lot of examples of direct interaction as depredation of native species by invaders (Perry *et al.* 1997, MacNeil *et al.* 2003, Whitlow *et al.* 2003) or competition, e.g. by competitive exclusion (Human & Gordon 1997, Juliano *et al.* 2004, Minchinton *et al.* 2006) or resource competition (Hrabik *et al.* 1998, Westman *et al.* 2002, Badano & Pugnaire 2004). In a similar way, exist a few studies appointing on indirect effects as the introductions of new parasites or pathogens (Manchester & Bullock 2000, Prenter *et al.* 2004), and sometimes this new parasites and pathogens allow and facilitate the arrival of new invasive species (Van Riper *et al.* 1986). Finally, invasive species can present genetic impacts through hybridization and introgression with related native species (Mooney & Cleland 2001). These changes in genetic constitution and in phenotype suppose a loss in biodiversity, while hybridization may affect the adaptedness of native species to the local environment (Manchester & Bullock 2000).

Freshwater invasions

Freshwater ecosystems are especially prone to biological invasions, in part because of habitat alteration and degradation (Moyle & Light 1996, Rahel, 2002). The impacts of invasive freshwater fish are variable and poorly understood but include some of the most dramatic cases (Drake *et al* . 1989, Moyle & Light 1996) and the Iberian Peninsula is no exception to this (García-Berthou & Moreno-Amich 2000, Elvira & Almodóvar 2001). However, there are few studies that try to assess the distinctive features of successful invasive freshwater fish (Kolar & Lodge 2001, Marchetti *et al.* 2004, Vila-Gispert *et al.* 2005). Mediterranean streams have strong seasonal patterns of flow: low flow in the hot summer drought and flash floods during autumn and spring storms. Interannual variability in precipitation is high while lengthy periods of drought are common. As a consequence, the native fish fauna is depauperate and highly endemic (Doadrio *et al.* 1991). The natural variability in environmental conditions of many Iberian streams has been greatly reduced by water regulation. In addition, industrial waste and sewage effluents cause water quality to deteriorate. These profound modifications to the fluvial systems of this region directly threaten the native fish fauna and favor the invasion of non-native species (Elvira 1995, 1998).

The case of invasive fish species

According to the United Nations Food and Agriculture Organization’s (FAO’s) Database of Invasive Aquatic Species (DIAS), (http://www.fao.org/waicent/faoinfo/fishery/statist/fisoft/dias/mainpage.htm) the ten world’s most introduced aquatic species are all freshwater fish, which have been introduced to over 50 countries (Table 1).

Table 1. Invasion transitions of the 10 most frequently introduced aquatic species in the world plus 2 additional species (obtained from the Food and Agriculture Organization’s Database on Introductions of Aquatic Species). The number of introductions refers to introductions to different countries. Several introductions of the same species into the same country are not considered. The *P* values correspond to *G* tests of independence of establishment or presence of ecological effects (yes/no) in different continents (From García-Berthou *et al.* 2005).

Species	Number of introductions	% established	*P*	% with ecological effects	*P*
Oreochromis mossambicus	172	85.9	< 0.0005	81	0.26
Cyprinus carpio	124	82	0.91	86	0.47
Oncorhynchus mykiss	99	53.8	0.38	88	0.51
Ctenopharyngodon idella	91	11.3	0.06	60	0.71
Hypophthalmichthys molitrix	79	26.8	0.2	75	0.24
Oreochromis niloticus	78	70.2	< 0.0005	75	0.25
Gambusia spp.	67	96.8	0.43	50	0.15
Micropterus salmoides	64	72.9	0.78	86	0.19
Aristichthys nobilis	55	19.6	0.27	80	0.48
Carassius auratus	54	92.3	0.63	75	0.37
Lepomis gibbosus	25	91.3	0.01	71	–
Procambarus clarkii	24	88.9	0.62	86	0.74

The introduction pathways of invasive fish were investigated by García-Berthou and collaborators (2005). They have shown that exist significant differences among continents in the frequency of introductions of different species. For instance, the pumpkinseed sunfish has been introduced mostly to Europe, whereas the mosquitofish mosquitofish (*Gambusia* spp.) are widespread in Oceania, and tilapias (*Oreochromis* spp.) mostly to America and Oceania, the largemouth bass (*Micropterus salmoides*) mostly to Africa, and the bighead carp (*Aristichthys nobilis*) mostly to Asia. There are also some significant intercontinental differences in establishment success but only for a few species (Table 1) (e.g. Over 65% of introduced tilapias successfully established, except in Europe, where all introductions have failed), and establishment success is generally high and less important than the introduction effort. Therefore, the differential introduction frequency is mediated by historical and sociological factors rather than ecological factors, e.g. mosquitofish are relatively less introduced in Africa and South America but may establish successfully in many countries. Over 50% of the successful introductions of the 12 species had ecological effects, and the occurrence of ecological effects does not depend significantly on the destination continent (Table 1) or year of introduction. On the other hand, these 12 species did not show significant relationship between the number of introductions and the percentage established or the percentage causing ecological effects.

Invasive fish in Europe

Most of the introduced aquatic species in Europe are fish of freshwater habitats and of intentional introduction. This suggests two opposite introduction pathways in Europe for intentional (freshwater fish) and accidental introduction (euryhaline invertebrate species). García-Berthou *et al.* (2005) distinguished three regions as introduction origin:

- Northwestern European countries, dominated by the UK, where Ireland was the main destination. The UK gave 14 of the total of 26 introductions to Ireland.
- Northern countries, dominated by Germany, that provide species to many different countries.
- Southwestern countries, with France as the main donor of species to Spain and Portugal.

On the other hand, large countries such as the UK, Germany, and France were distinguished as origin for fish introductions to other countries but not as destination species providing for other European countries. This reflects the strong asymmetry in the exchange of non-indigenous species (NIS), with strong unidirectional pathways. In contrast to other taxa such as plants and terrestrial vertebrates, freshwater species introduced to Europe come mostly from North America (Welcomme 1991, Vilà *et al.* 2001) and enter through mid-latitude countries in western Europe (France, UK, and Germany) and from here go mostly to southern Europe (Fig. 1).

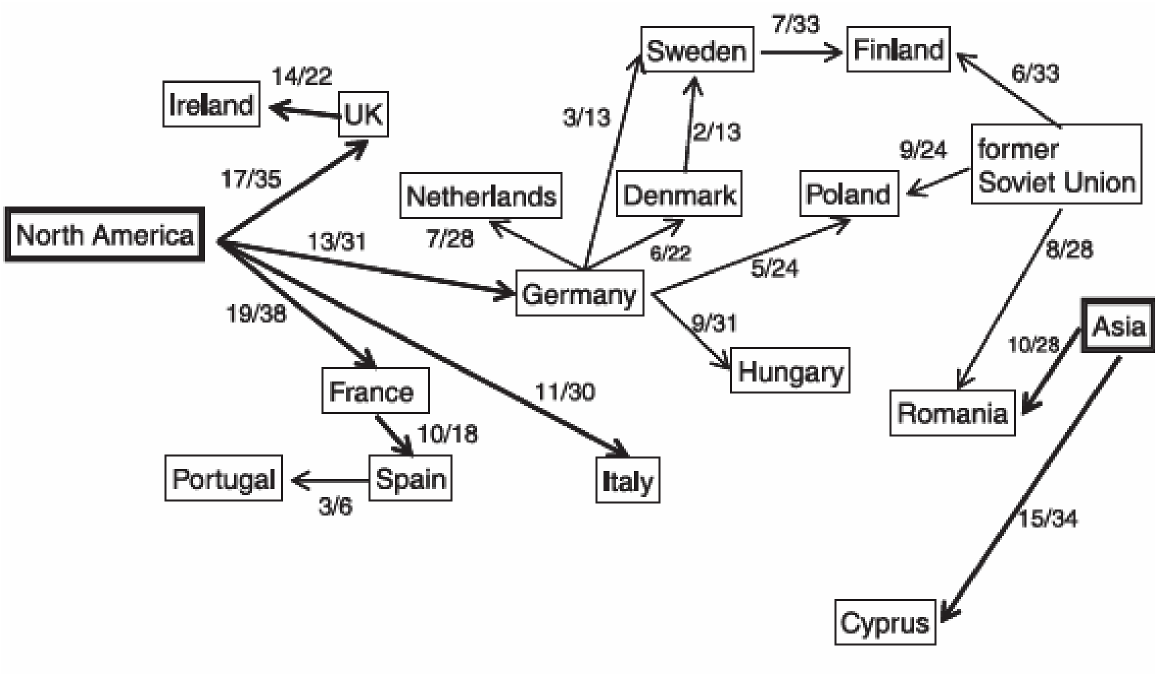

Figure 1. The main introduction pathways of aquatic species within Europe. The fractions denote the number of species introduced from one country to another (numerator) followed by the total number of introductions received, excluding those of unknown origin (denominator). For instance, of the 22 species of known origin introduced to Ireland, 14 (i.e., 64%) came from the United Kingdom. Relationships involving 10 or more introductions are indicated by thick arrows. Less important relationships have been omitted (From García-Berthou *et al.* 2005).

ONE OF THE WORST, THE MOSQUITOFISH

Description

The mosquitofish (*Gambusia holbrooki*) (Fig. 2) is a cyprinodontiform fish species of the Poeciliidae family. The head is large with a flattened upper surface. The mouth is small, upturned, protrusible with lower jaw a little longer than upper presenting bands of minute teeth on both jaws. The mouth is not reaching as far back as the front of the eyes. The eyes are very large relative to the body and positioned near dorsal profile. The dorsal fin is single and soft rayed (6-8 rays, usually 7) placed well back, above or posterior to anal fin (9-11 rays, usually 10) that is rounded or elongate and pointed. Pectoral fins are short, rounded and positioned high on sides near the top of gill openings. Whereas, pelvic fins are in abdominal position, are tiny rounded and present bases close together (see NSW 2003). The caudal peduncle is long, deep and compressed, and the caudal fin is rounded. The head and body are covered with large cycloid scales and there is no lateral line, with between 26 and 30 scales in a maximum longitudinal line (Doadrio 2002). Mosquitofish fish present between 31-33 vertebras and gill present 13-15 stout gill rakes of moderate length (NSW 2003).

Figure 2. Mosquitofish photo (top) and sexually dimorphic differences (bottom). (Photo: Carles Alcaraz).

The mosquitofish (Fig. 2) is a small dimorphic fish species (Fig. 2), males grow to 35 mm in length, while females reach 80 mm long (Doadrio 2002). Males cease growing when they reach maturity, but females continue to grow until they die (Vargas & De Sostoa 1996). Gambusia are generally green olive to brown on the back, the sides are grey with a bluish sheen, and the belly is silvery-white (see NSW 2003). The ventral surface of the head is steel blue and often has a dark, diagonal stripe below the eyes. The eyes are grayish to olive. Fins are colourless, except for the dorsal and caudal fins, which may bear numerous fine black spots, sometimes forming indistinct rows. A well defined black spot on the upper rear abdomen is surrounded by a golden patch just above the vent. In mature females there is also a black patch above and somewhat forward of the vent (see NSW 2003). Mature males present a highly modified anal fin by an enlargement of the third, fourth and fifth rays to form a copulatory organ or gonopodium (Maitland 2000, Doadrio 2002) used to facilitate internal fertilization of eggs in the female.

Mosquitofish is a freshwater fish species that inhabits lentic and vegetated waters preferably below the 1000 m of altitude that usually don’t live more than 2 years. Mosquitofish is eurythermal fish that can inhabit much polluted waters with high temperature and low oxygen concentrations (Doadrio 2002). It is euryhaline fish inhabiting waters from fresh to polysaline waters below 30‰ (Nordlie & Mirandi 1996). The sex ratio is skewed in favour of females due to different survival taxa between both sexes (Snelson 1989, Vargas & De Sostoa 1996). Mosquitofish is viviparous and precocious. It grows rapidly, becoming sexually mature in less than two months (Doadrio 2002). The reproductive cycle is primarily governed by photoperiod, with reproduction ceasing once day length falls below 12.5-13 hours, with water temperature being favourable. The gestation period is between 21-28 days, after, females can give birth to 30-50 offspring, although broods may often exceed 100 offspring (Meffe & Snelson 1989). Immature male are sexually active well before their gonopodium has completely developed and before they are able to transfer sperm (Bisazza *et al* 1996).

Mosquitofish is an opportunistic omnivore, with preference for animal food (Meffe & Snelson 1989) such as aquatic invertebrates including bugs, beetles, fly larvae and also zooplankton. However, it is a generalist predator that adapts its diet according to prey availability, therefore terrestrial insects such ants and flies that fall onto the water surface can be an important part of its diet (Arthington 1989, García-Berthou 1999).

Therefore, mosquitofish are characterized by small size, viviparity, early sexual maturity (in a few weeks), fast growth, a high level of reproductive effort, and a short life-span. These are typical of unstable environments, where mortality is high, variable or unpredictable (Fernández-Delgado 1989, Poizat & Crivelli 1997). Mosquitofish are resistant to harsh conditions and very prolific. Viviparity and a high reproductive rate may give such an exotic species an advantage over native oviparous species (Vargas & De Sostoa 1996).

European introduction and dispersal

The mosquitofish (in fact two very close species: *G. holbrooki* and *G. affinis*) is one of the most widespread vertebrates, originally introduced in order to control mosquito populations and hence malaria (Krumholz 1948). Mosquitofish, considered as one of the world 100 worst invasive alien species by the GISP (Global Invasive Species Programme, http://www.issg.org/database/), has been introduced into more than 50 countries, being the 9th most frequently freshwater fish introduced (García-Berthou *et al.* 2005) and probably the most widely distributed fish in area occupied (Krumholz 1948, Murdoch & Bence 1987), although it is only native to the southern Atlantic coast of the United States and the Gulf of Mexico.

Therefore, mosquitofish were introduced to many countries to control mosquitoes at the beginning of the 20th century. In Southern Europe the mosquitofish was first introduced to the Iberian Peninsula in 1921 (Nájera 1944) and in 1922 was introduced to Italy from Spanish populations (see Krumholz 1948). From here, mosquitofish were introduced in 1924 to the Transcaucasic regions and from there to areas in the south and centre of the former USSR (see Grapputo *et al.* 2006). Later, mosquitofish were introduced to other malaric areas of the world (other European countries, East Asia, Australia and New Zealand) and its use as ornamental fish in aquarium have increased its dispersal over the world, nowadays we found it in all the continents except the Antarctica. In Europe, because of its temperature requirements, mosquitofish is present mainly in the Mediterranean area (Specziár 2004) (Fig. 3), where is very abundant.

Although mosquitofish is presently distributed only in southern Europe, it has had enormous success in invading different habitats within its distribution range, with climates varying from truly Mediterranean with mild winters, similar to its original habitat, to temperate. Thermal ponds, also, have been colonized (Specziár 2004).

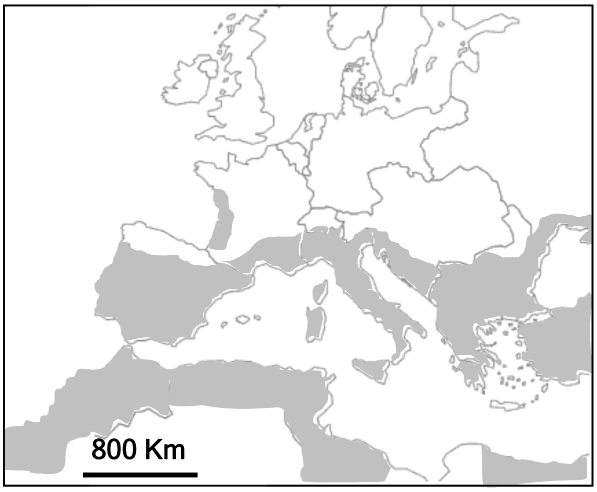

Figure 3. Mosquitofish distribution in the Mediterranean zone (Modified from Lloyd *et al.* 1986).

Ecological impacts

During the 20th century mosquitofish has been introduced extensively because of their reputation as mosquito-control agents (Krumholz 1948, Courtenay & Meffe 1989). Nevertheless, recent reviews question the effectiveness of the mosquitofish in controlling mosquito populations and mosquito-borne diseases (Arthington & Lloyd 1989, Courtenay & Meffe 1989, Rupp 1996), but its strong ecological impacts on native communities are quite well known. Mosquitofish aggressiveness and its great adaptability to new and human-disturbed habitat have facilitated its diffusion (Smith *et al.* 1989), becoming a serious predator and competitor for many native species.

Mosquitofish profoundly alter ecosystem functioning through trophic cascades (Hulbert *et al.* 1972, Hulbert & Mulla 1981, Cabral *et al.* 1998). For instance, the intense predatory activity on zooplankton may cause an increase in phytoplankton populations (top-down effect), altering the biological equilibrium of the water systems and contributing to an increase of eutrophy (Hurlbert *et al.* 1972, Margaritora *et al.* 2001). Many studies have been widely documented the negative effects of mosquitofish on native amphibians, demonstrating its role on the decline and local extinction of latter (Lawler *et al.* 1999, Hamer *et al.* 2002, Kats & Ferrer 2003). Predation by mosquitofish over amphibian eggs and larvae is generally reconognised as the main impact factor, determining amphibian composition and diversity. Many cases of decline and extinction of native fishes have also been described, especially fish of similar size (Courtenay & Meffe 1989, Schaefer *et al.* 1994, Mills *et al.* 2004). Arthington and Lloyd (1989) considered that mosquitofish have been implicated in the extinction of small fish species in the USA, Asia and Africa and in the reduction in range or abundance of twenty-five species in the worldwide, and nowadays is considered that some thirty-five fish species worldwide have declined in abundance or range as a result of interactions with mosquitofish (NSW 2003). The effects and replacement on the American cyprinodontiforms *Heterandria formosa* or *Poeciliopsis occidentalis* by the mosquitofish are well documented (Meffe *et al.* 1983, Meffe & Snelson 1989, Galat & Robertson 1992, Belk & Lydeard 1994, Schaefer *et al.* 1994). Predation by mosquitofish on eggs or larvae, aggressive behaviour, food competition or behavioural interference have been proposed as the causal mechanisms (Arthington & Lloyd 1989, Rincón *et al.* 2002, Mills *et al.* 2004).

In Europe, the fishes more affected by mosquitofish, probably, are the native endemic cyprinodontiforms such as the Spanish endemic cyprinodontiforms *Aphanius iberus*, *A. baeticus* and *Valencia hispanica* (Rincón *et al.* 2002, Caiola & De Sostoa 2005) or the Mediterranean endemic fish *Aphanius fasciatus* (Bianco 1995). Both *Aphanius iberus* and *Valencia hispanica* are considered as species in danger of extinction by the National Catalogue of Endangered Species (Doadrio 2002), and joint with the other cyprinodontid considered in this work (chapter V), the *Aphanius fasciatus* are listed into the Annexes II (strictly protected fauna species) and III (protected fauna species) of the Convention on the Conservation of European Wildlife and Natural Habitats (1979) “Bern Convention” and in the Annex II (Animal and plant species of community interest whose conservation requires the designation of special areas of conservation) of the European Council Directive (1992/43/EEC) “Habitat Directive”.

THE MEDITERRANEAN CYPRINODONTIFORM FISHES

The Spanish toothcarp

The Spanish toothcarp (*Aphanius iberus*) (Fig. 4) is a cyprinodontiform fish species of the Cyprinodontidae family. It is a small fish with oblong body and rounded fins. Its mouth is superior directed upwards with maxillary teeth with three apexes and disposed in only one row (Maitland 2000, Doadrio 2002). Relatively big scales, existing from 20-26 on average longitudinal line (Doadrio 2002). The dorsal fin is single (with 9 – 10 branched rays) placed well back and almost immediately above of the anal fin (with 8 – 9 branched rays) (Maitland 2000, Oliva-Paterna *et al.* 2006). Caudal peduncle is short and high, and the caudal fin is rounded with 18 rays. The Atlantic populations of the Spanish toothcarp have recently been distinguished as a new species (*A. baeticus*) because of differences in morphometrics and genetics (Doadrio *et al.* 2002).

Aphanius iberus shows an external sexual dimorphism (Fig. 4), adult male coloration pattern is characterized by narrow blue and silver vertical bars along the body size, coloration pattern is continued on the fins, mainly in the anal and caudal fins, which are crossed by wide dark vertical lines. Females are generally brown-greenish, showing several small dark spots over their body wich tend to form rows, one of them along the lateral line, their fins are transparent without any marks. However, usually females are larger (max. total length ≤ 60 mm) than males (max. total length ≤ 45 mm) of the same age (Doadrio 2002, Oliva-Paterna *et al.* 2006).

Figure 4. Female (top) and male (bottom) of Spanish toothcarp (Photo: Carles Alcaraz)**.**

A. iberus inhabits ponds, coastal lagoons, salt marshes, river-mouths and ditches (Doadrio 2002). Therefore, it is a strongly euryhaline and eurythermic fish (Fernández-Delgado *et al.* 1988, García-Berthou & Moreno-Amich 1992, Vargas & De Sostoa 1997), tolerating salinity over several times the sea salinity and temperatures higher than 30ºC. Its biology is characterized by fast growth and reduced longevity (aged up to 2+) (García-Berthou & Moreno-Amich 1992, Vargas & De Sostoa 1997, García-Berthou *et al.* 1999). It is a multiple spawner from May to August in the Ebro Delta (Vargas & De Sostoa 1997), although the spawning period is longer in southern localities (from April to October) (Oliva-Paterna *et al.* 2006). In each successive spawn up to 900 eggs are spawned and reaches sexual maturity in the first few months of life (at a total length of less than 20 mm). Therefore, its shows a reproductive strategy adapted to unstable environments with a high reproductive effort (Oliva-Paterna *et al.* 2006). *A. iberus* present an omnivorous diet, composed by animal preys, plant debris and detritus. Nevertheless, its diets show seasonal (Vargas & De Sostoa 1999), habitat and ontogenetic variations (Alcaraz & García-Berthou 2006). Animal preys are mainly micro crustaceans, and insects are not really important (Doadrio 2002).

The Mediterranean toothcarp

The Mediterranean toothcarp (*Aphanius fasciatus*) is a cyprinodontiform fish species of the Cyprinodontidae family. As for the Spanish toothcarp, it is a small fish with oblong body and rounded fins. Its mouth is superior with 12-16 maxillary teeth with three apex disposed in only one row (Maitland 2000, Doadrio 2002). It presents between 25-29 big scales on average longitudinal line (Doadrio 2002). The dorsal fin is single (with 10-13 branched rays) positioned well back and almost immediately above anal fin (with 9-13 branched rays) (Maitland 2000). Caudal peduncle is short and the caudal fin is rounded with 18 rays.

As *A. iberus*, *A. fasciatus* shows an external sexual dimorphism, adult male is characterized by broad golden vertical bars with blue reflexs alternated with narrow silvers bars, the dorsal fin present in its superior border a black line as the caudal fin that is traversed by a black line. Female coloration is less evident, with 10-14 vertical lines and showing small dark spots in the caudal peduncle, fins are transparent with any marks. Females usually are larger (max. total length ≤ 70 mm) than males (≤ 60 mm) and the sex ratio is skewed in favour of females (Leonardos & Sinis 1998, 1999, Maitland 2000, Doadrio 2002,).

A. fasciatus is found along the coastal regions of much of the eastern Mediterranean area, occurring in a variety of fresh and brackish waters including small weedy ponds and ditches (Maitland 2000), the most common habitat are transitional environments (Bianco

1995). It is a strongly eurythermic and euryhaline fish, that tolerates salinities around four to five that of the sea, and reduced longevity (aged up to 2+) (Bianco 1995, Doadrio 2002). Reproduction take place from April to September, life history traits of *A. fasciatus* include external fertilisation, large demersal eggs that are deposited on benthic vegetation, short generation time, high reproductive rate and rapid population turnover (Leonardos & Sinis 1998). Diet is composed by invertebrates, especially crustaceans and insect larvae (Maitland 2000).

Distribution and threats

The Spanish toothcarp is endemic of the Mediterranean coast of the Iberian Peninsula. Its distribution range, as well as for the other Spanish endemic cyprinodontiforms (Fig. 5), and in general for all Mediterranean native cyprinodontiforms, is characterized by a high degree of isolation among its populations, the originally continuous distribution have been fragmented into small isolated areas.

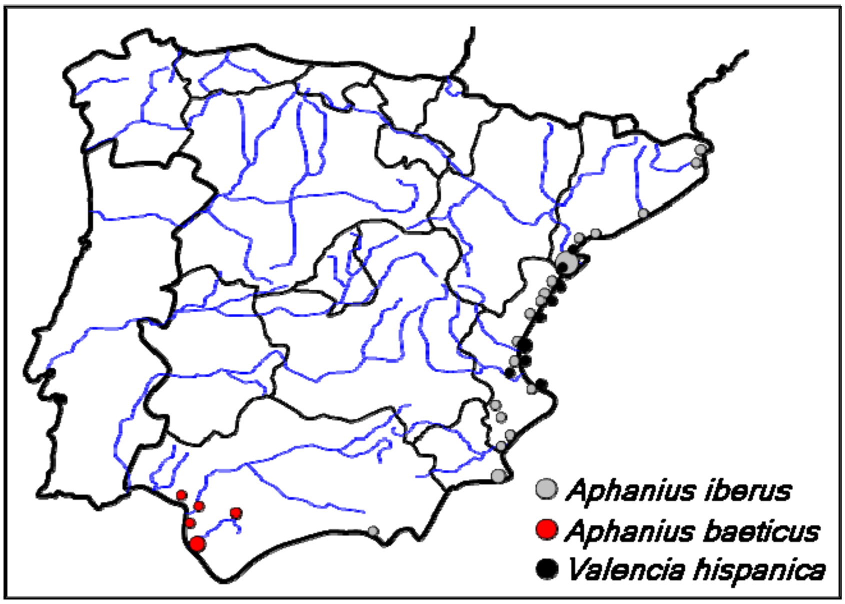

Figure 5. Current distribution of the three endemic Spanish cyprinodontiform species (Modified from Doadrio 2002).

Their populations are sparce and some of them are continuously decreasing in abundance (Doadrio 2002, Oliva-Paterna *et al.* 2006). 12 of the know 28 populations of *Aphanius iberus* have disappeared and one is extinct in the wild with only a few individuals still preserved in captivity, occupying nowadays less than 450 km^2 (Doadrio 2002). The

Aphanius baeticus situation is not better, since have been extinguished 10 know populations and nowadays only still 9 populations with an occupied area less than 200 km^2 (Doadrio 2002). Whereas *Valencia hispanica* only survive in 8 populations, but only 4 of these include enough individual to allow its conservation (Doadrio 2002).

Although the Mediterranean toohcarp is present along all the Mediterranean coast except the Iberian Peninsula (Changeux & Pont 1995) (Fig. 6), its populations are very fragmented and isolated (Maltagliati 1998, Maltagliati *et al.* 2003) showing a dramatic decline and in a few cases even extinction (Bianco 1995, Changeux & Pont 1995, Maltagliati 1998). Possibly, under this wide distribution range there are in fact several species, as suggested by genetic studies and by the bio-ecological properties of the species (Smith & William 2006).

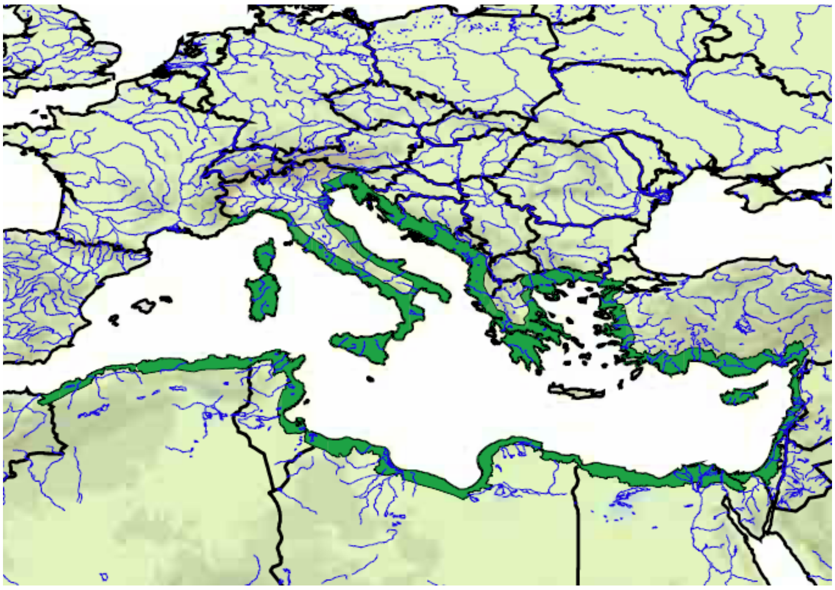

Figure 5. *Aphanius fasciatus* current distribution (Obtained from IUCN, http://www.iucn.org/places/medoffice/cd_fwfish/ materials/maps/Aphanius_fasciatus.pdf).

Because their limited and isolated distribution endemic cyprinodontiforms are highly threatened species considered in danger of extinction, during the last three decades there has been a progressive loss and alteration of its habitat especially as a result of an intensive agriculture and tourism development (Bianco 1995, Changeux & Pont 1995, Doadrio 2002, Oliva-Paterna *et al.* 2006). The Spanish toothcarp (*Aphanius iberus*) originally inhabited a wide range of lowland waters and although *A. fasciatus* tolerates a wide range of salinities and sometimes occurs in rivers (Tigano & Parenti 1988, Parenti & Tigano 1993), nowadays its distributions is also reduced to polysaline, eusaline and hypersaline waters. Several factors have been proposed to explain its decline including current threat to this species as the destruction of habitats, water pollution… but the introduction of exotic species and mainly the invasive and mosquitofish (*Gambusia holbrooki*) plays a dominant role, appearing to have displaced endemic cypridonotiform from its habitat (García-Berthou & Moreno-Amich 1992, Bianco 1995, Changeux & Pont 1995, Elvira 1995, Rincón *et al.* 2002).

Toothcarps, salinity and mosquitofish

Because the alarming threatened status of Mediterranean native cyprinodontiforms more studies on its biology and ecology are needed to improve and enhance better management and recovery plans. For instance, the role of resource availability and prey electivity is largely unknown for these fish species. In general, resource availability can help to understand fish distribution, structure and density. Resource availability and electivity is often not considered in fish feeding studies, although without its measurement little can be said about niche overlap and niche breadth (Hurlbert 1978). The study of electivity is important in order to understand the response of the invertebrate community and the patterns of intraspecific and interspecific competition (Gerking 1994: 249). Knowledge of the specific prey types consumed at different sites also allows a more mechanistic understanding of habitat selection (Nemerson & Able 2004).

Nowadays, it is observed that for both Spanish and Mediterranean toothcarp distributions, as for other Mediterranean native cyprinodontiforms, is mostly reduced to polysaline, eusaline and hypersaline waters, whereas the mosquitofish is widely distributed and occupying practically all low lands waters of reduced salinity where have displaced native cyprinodontiforms (García-Berthou 1992, Bianco 1995, Changeux & Pont 1995). However, although often it is considered that salinity limits invasive impact and distribution of mosquitofish (Bianco 1995, Nordlie & Mirandi 1996, Kandl 2001) and to act as a refuge to native fauna from latter, its importance is unknown. Exist some previous works that have shown mosquitofish cohabiting in highly saline waters with other species that are displaced in fresh water (Al-Daham *et al.* 1977, Fernandez-Delgado *et al.* 1988), therefore it is largely unknown the ecological mechanism by which salinity limits the invasive success of mosquitofish, and how it mediates the harmful ecological interactions between this and native fish species. Although the importance given to salinity as a constrainer factor of mosquitofish dispersal there are only a few studies about the effects of it on mosquitofish biology, namely metabolism (Chervinski 1983, Nordlie & Mirandi 1996). Furthermore, although is considered that life history traits are important to understand biological invasions (Woods 1993) and the high diversity of life history strategies previously presented by the mosquitofish, considered as a leading factor to understand its success trough the world (Haynes & Cashner 1995), the only studies on the salinity effects on mosquitofish life history traits are Stearns & Sage (1980) and Brown-Peterson & Peterson (1990), who studied short salinity gradients (two populations from fresh (0‰) to mesosaline (10‰) and oligosaline (2.5‰) waters respectively) in North America. Thus, the response of mosquitofish to a wide salinity range is unknown.

On the other hand, a detailed understanding of the mechanisms that allows the invasion success is required to improve successful control effort (Holway & Suarez 1999, Sol *et al.* 2002, Levine *et al.* 2003). Therefore, although behavioural interactions between invasive and native species and how these contribute to invasive success is needed, behvioural traits have received little attention in the literature (Holway & Suarez 1999, Rehage *et al.* 2005). The competitive interactions between *Gambusia holbrooki* and the Spanish endemic cyprinodontiforms *Aphanius iberus* and *Valencia hispanica* have been investigated by Rincón *et al.* (2002) and Caiola & De Sostoa (2005), who have experimentally demonstrated that the endemic cyprinondonts decrease feeding rates and receive more aggressions in the presence of mosquitofish. The occurrence and intensity of these potentially harmful effects, however, seemed to be modulated by water temperature, reproductive condition, relative size of the interacting fishes and species-specific habitat preferences (Rincón *et al.* 2002). However, another time, whether salinity mediates these interactions, its importance in behavioral interactions with native species and in the invasive success of mosquitofish is unknown.

OBJECTIVES

The main objective of this thesis is to assess the previously hypothesized mediation of salinity on the invasive success of mosquitofish (*Gambusia holbrooki*) and on competition with cyprinodontiform fishes. With this aim, we combined field studies on the ecology of *Gambusia holbrooki* and *Aphanius iberus* with experimental tests on the role of salinity on the competitive interaction.

In **Chapter I**, we reviewed 26 variables on the life history traits, ecology and human uses of the 69 inland fish species from the Iberian Peninsula. We show that phylogeny, variability (in addition to the central tendency) and human uses are needed to better understanding of the differences observed between invasive and native species groups.

In **Chapters II** and **III** we report ecological data of the Spanish toothcarp (*Aphanius iberus*). We assess the use of an occasionally flooded habitat by the Spanish toothcarp and the relationship of resource availability with population density, structure and prey electivity. We show that different size groups of the toothcarp select different habitats, diet variation among habitats, dependent on prey availability, and an ontogenetic diet shift linked to a microhabitat change.

In **Chapter IV** we examine differences on mosquitofish (*Gambusia holbrooki*) life history traits inhabiting from fresh to polysaline waters. We demonstrate that salinity affects density and life history traits of both male and female mosquitofish, with both sexes showing a unimodal relationship with salinity, decreasing their density and increasing reproductive effort with salinity at the cost of somatic condition in females.

In **Chapter V** we experimentally examined the competitive interactions between mosquitofish and another endangered cyprinodont (*Aphanius fasciatus*) and the role of salinity as a mediator of these behavioural interactions. We show that mosquitofish decrease its aggressive behaviour towards the toothcarp and capture less prey, reducing its efficiency with increasing salinity.

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Chapter I

Profiling invasive fish species: the importance of phylogeny and human use

ABSTRACT

Understanding the ecological differences between native and invasive species is of considerable scientific and practical interest. We examined such differences between native and invasive inland fish species from the Iberian Peninsula in order to analyse the importance of phylogenetic correction and variability (in addition to central tendency). We collected 26 quantitative and qualitative variables on the ecology, life-history traits and human use of the 69 inland fish species of the Iberian Peninsula, including native, invasive and migratory species. The taxonomic distribution of invasive fish species deviated significantly from world freshwater richness and in contrast to native species, invasive fish belongs to only five taxonomic orders but to a wide spectrum of families not native to the Iberian Peninsula. Because the life-history traits were highly dependent on taxonomy, the results, with or without applying phylogenetic methods, differed and after accounting for phylogeny, invasive species displayed higher and wider latitude in general and a different reproductive season mainly among salmonids and cyprinids. Human use was also significantly different between native and invasive fish species and produced more variability in life-history traits of invasive species and uneven taxonomic distribution because of the high diversity of species introduced. We show that accounting for taxonomy and studying variability in addition to central tendency is important in the comparison of life-history traits between native and invasive species.

Keywords

Biological invasions, Iberian Peninsula, independent contrasts, introduced species, Levene test, life-history traits, variance.

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INTRODUCTION

Biotic homogenization through the introduction of invasive species and extinction of native species is now recognized as one of the main threats to biodiversity and ecosystem function (Mack *et al.* 2000, Rahel 2002, Clavero & García-Berthou 2005). Predicting future invader species and vulnerable ecosystems is of immense scientific and practical interest (Rejmánek & Richardson 1996, Ricciardi & Rasmussen 1998, Mack *et al.* 2000). Among several approaches (Mack 1996, Rejmánek 2000), many studies have attempted to identify the distinctive biological traits of invasive species, although the results have been often inconclusive (Pyšek 1998, Goodwin *et al.* 1999, Mack *et al.* 2000). In general, invasive species have been suggested to be of wide geographical range, abundant, generalists, tolerant to abiotic factors and human commensals (Williamson 1996, Ricciardi & Rasmussen 1998, Lockwood 1999).

One of the most common methods of identifying biological traits of invaders is by comparing two sets of species in a given region, e.g. successful vs. unsuccessful introductions (Forsyth *et al.* 2004, Marchetti *et al.* 2004), native species vs. established introductions (Williamson & Fitter 1996, Vila-Gispert *et al.* 2005) or invasive vs. noninvasive introduced species (Kolar & Lodge 2001). Such comparisons provide different information because different species characteristics may determine success in different invasion transitions (transport, establishment and invasion) (Kolar & Lodge 2001). Comparing native and established invasives is the information most widely available (because unsuccessful introductions and invasive potential are poorly known) and may help understand the overall success of invasive over native species (niche overlap, reproductive ability), whereas other types of comparisons may provide information on specific invasion transitions. Except in a few recent studies of introduced mammals and birds (Duncan *et al.* 2001, Cassey *et al.* 2004, Forsyth *et al.* 2004), all these comparisons have been usually performed without controlling for phylogenetic effects, particularly for fish (Fisher & Owens 2004). Taxonomy has actually hardly been tested as a predictor of invasiveness (Lockwood 1999). However, it is well appreciated in comparative studies that treating closely related species as independent data may violate the independence assumption of most statistical methods (Harvey & Pagel 1991, Garland *et al.* 1999). The distribution of invasive species among taxonomic families and higher taxa has been shown to be far from random in plants (Pyšek 1998, Richardson & Rejmánek 2004) and birds (Lockwood 1999, Blackburn & Duncan 2001), and certain families and taxa have more invasive species. In a review of life-history data for 301 fish species in general (Vila-Gispert *et al.* 2002), taxonomic order was found more important than latitude, habitat or geographical region in determining life-history traits. Therefore, the potential of confounding taxonomy with biological attributes as determinants of invasiveness is great.

Freshwater ecosystems are especially prone to biological invasions, in part because of habitat alteration and degradation (Moyle & Light 1996a; Rahel 2002). The impacts of invasive freshwater fish are variable and poorly understood but include some of the most dramatic cases (Drake *et al.* 1989, Moyle & Light 1996a) and the Iberian Peninsula is no exception to this (García-Berthou & Moreno-Amich 2000, Elvira & Almodóvar 2001). However, there are few studies that try to assess the distinctive features of successful invasive freshwater fish (Kolar & Lodge 2001, Marchetti *et al.* 2004, Vila-Gispert *et al.* 2005). The objectives of this paper are: (1) to test whether there are ecological or human use differences between native and invasive inland fish species from the Iberian Peninsula, (2) to assess whether phylogenetic correction is necessary to analyse such data and (3) to test whether there are differences in variability of characters in addition to central tendency.

METHODS

Data set

We assembled data for the 69 inland fish species of the Iberian Peninsula (Doadrio 2002), including native, invasive and migratory inland species but excluding fish of marine origin (see pp. 97–98 of Doadrio (2002) for a full list of species). Invasive species are considered in this paper as non-native species that have established self-sustaining wild populations (stages III to V of Colautti & MacIsaac 2004). Note, however, that a few of the fish species considered, both native (e.g. *Acipenser sturio*) and invasive (e.g. *Oncorhynchus kisutch*), have small populations in the Iberian Peninsula with uncertain status. Moreover, a few fish species introduced more recently have not been considered, whereas the introduced status and taxonomy of a few species (e.g. *Gobio gobio*) is being currently revised.

The reference sources for the compilation of the life-history, ecological and human use variables were Maitland (2000), Doadrio (2002), FishBase (Froese & Pauly 2003) and Vila-Gispert *et al.* (2005). We considered the following 14 qualitative variables: taxonomic order and family; use in aquaculture (yes/no); use in aquarium (yes/no); use as bait (yes/no); use as game fish (yes, occasionally, or no); fisheries exploitation (yes/no); feeding type classified as invertebrate, omnivore, piscivore, zooplanktivore, or other (including parasites and lampreys);

reproductive guild (phytophils, phytolithophils, lithophils, internal or marine) from Mann (1996); habitat (lentic, lotic or generalist); salinity tolerance (yes/no); marine (yes/no); microhabitat (benthic, neustonic, pelagic or generalist) and climatic region (polar, temperate, subtropical or tropical). We also considered the following 12 quantitative variables: maximum reported age for the species (years), mean and maximum fecundity (number of eggs), age at maturity (years), mean and maximum observed total length (to the nearest cm), minimum record and range of habitat temperature (°C), maximum record and range of latitude (°N), midpoint of the reproductive season (month) and reproductive season span (number of months).

Statistical analyses

To test for differences in the frequency of qualitative variables between native and invasive species we used *G*-tests of independence (Sokal & Rohlf 1995). A multiple binary logistic regression model using a stepwise selection procedure (based on the likelihood ratio) was also used to identify qualitative variables that showed the most important differences between invasive and native species (Tabachnick & Fidell 2001). Logistic regression is a generalized linear model with binomial distribution and a logit link function, suitable for categorical dependent variables (Quinn & Keough 2002).

To test for taxonomic selection, we also compared the number of Iberian invasive species with the world number of freshwater fish species by taxonomic order (Nelson 1994) using the binomial distribution as detailed in Lockwood (1999). For this particular test, esociform fish species (only introduced *Esox Lucius* in the Iberian Peninsula) were distinguished from Salmoniformes (cf. Doadrio 2002) to match Nelson's usage.

Quantitative variables were analysed with two-sample *t*-tests (Sokal & Rohlf 1995) and Levene tests. The former compares the means of two groups, whereas Levene tests compare the variances of two or more groups and are more robust than other homogeneity-of-variance tests (Quinn & Keough 2002). For midpoints of reproductive season and reproductive season span, the guppy (*Poecilia reticulate*) data were excluded from the analyses because of its distribution limited in the Iberian Peninsula to a single, peculiar locality.

When many statistical tests are performed on some data, there is an increased risk of type I errors (wrongly rejecting null hypotheses). To overcome this, we used the procedure of Benjamini and Hochberg (1995), as implemented in the R Package (R Development Core Team 2003), for both quantitative and qualitative variables. This procedure controls the false discovery rate, i.e. the expected proportion of false discoveries amongst the rejected hypotheses, instead of the probability of a single type I error in the familywise error rate procedures (e.g. the usual Bonferroni or Holm corrections). The Benjamini and Hochberg (1995) procedure has more statistical power and robustness than familywise error rate procedures among other advantages (García 2004).

We used discriminant function analysis (DFA) to determine which variables discriminate most between native and invasive species and try to predict group membership from the multivariate set of quantitative variables. DFA creates functions that are linear combinations of the independent variables so that they separate the groups as much as possible. The regular and stepwise procedures of DFA (Tabachnick & Fidell 2001) were applied. To explore patterns of association among quantitative variables and to ordinate species, the principal component analysis (PCA) was applied to the correlation matrix. Kaiser-Meyer-Olkin’s (KMO) measure of sampling adequacy was used to assess the usefulness of a PCA. KMO ranges from 0 to 1 and should be well above 0.5 if variables are interdependent and a PCA is useful (Tabachnick & Fidell 2001).

Because closely related species may share a similar suite of traits through common ancestry, treating species as independent data points in a comparative study may confound differences between groups (e.g. invasive vs. native) with phylogeny (e.g. differences between orders) (Harvey & Pagel 1991, Duncan *et al.* 1999, Garland *et al.* 1999). As a phylogenetic comparative method, we applied Felsenstein’s (1985) method of independent contrasts, as implemented in the COMPARE 4.4 package (Martins 2003). The independent contrast method calculates the standardized difference between the values of the traits of sister taxa in a phylogeny. A ‘contrast’ quantifies the amount of evolutionary change that has occurred in a trait after the divergence of sister taxa from a common ancestor. Contrasts are calculated at all levels of a phylogeny by using reconstructions of ancestral states for internal nodes (Williams & Kay 2001). Pearson’s linear correlations are then used to test for correlations between the contrasts of the quantitative variables and the contrasts for invasive status. The construction of the phylogenetic tree was based on published phylogenies (Parenti 1981, Kendall 1988, Smith & Stearley 1989, Doadrio & Perdices 1997, Helfman & Collette 1997, Doadrio 2002). As we had several missing values for some variables, phylogenies were adapted for each quantitative variable and introduced in COMPARE 4.4.

To further understand the effect of phylogeny on selected quantitative variables, we also estimated the variance components explained by different taxonomic levels (orders, families, genera, and species) [see Jordano (1995) for a similar application]. Variance components analysis (Searle *et al.* 1992) was performed as a nested design (VARCOMP procedure in SPSS) with all factors treated as random-effects factors except the main one (taxonomic order in our case). All statistical analyses in this paper were performed with SPSS for Windows 11.5 (except when R or COMPARE are mentioned) with the default options (except when other are mentioned).

RESULTS

Univariate analyses

After correcting for multiple testing, native and invasive species significantly differed in the proportions of eight of the 14 qualitative variables: order, family, use in aquaculture, aquaria, as game fish or fisheries exploitation, reproductive guild and habitat (Table 1).

Table 1. Independence tests of qualitative variables of the inland fish in the Iberian Peninsula with species status (invasive or native). *P* values have been adjusted by the procedure of Benjamini and Hochber (1995).

Variable	*G*	d.f.	*P*
Order	32.71	13	0.009
Family	45.63	23	0.011
Aquaculture use	20.49	1	0.002
Aquarium use	27.15	1	0.002
Bait use	1.70	1	0.710
Gamefish use	5.30	1	0.037
Fisheries exploitation	6.28	1	0.024
Feeding type	5.93	4	0.250
Reproductive guild	15.60	4	0.011
Habitat	9.35	2	0.021
Salinity tolerance	4.49	1	0.053
Marine	0.29	1	0.690
Microhabitat	8.37	3	0.055
Climate	1.69	3	0.690

Of the 69 Iberian inland species, the 45 native fish belong to 13 different orders, whereas the 24 invasive species only belong to five different orders. One of these orders is only represented by invasive species (three siluriform species). Moreover, the proportion of world species that have been introduced depended on taxonomic order ($G = 41.1$, d.f. = 33, Monte Carlo $P < 0.0005$). Salmoniform fish significantly showed positive selection because the proportion of species that have been introduced (four of about 45) is much higher than for the rest of orders (Table 2). Characiform fish species are underrepresented and esociforms are overrepresented among invasives but this pattern was not significant after correcting for multiple comparisons.

The 45 native species belong to 16 families, whereas invasive species to 11 different families. Eight of these families (Centrarchidae, Cichlidae, Esocidae, Fundulidae, Ictaluridae, Percidae, Poeciliidae and Siluridae) are only represented by invasive species. The Cyprinidae is the most important family, with 24 native species (20 of the 25 Iberian endemic species are Cyprinidae) but only has seven invasive species.

With regard to the human use of these species, we found that invasive fish species are often used in aquaculture (67% of the invasive species), aquaria (88%), as game fish (67%) or commercially exploited (52%) in contrast to native species (respective percentages 13%, 24%, 38% and 21% of native species).

Referred to species biology, differences were found in the reproductive guild: 46% of the invasive species are phytofils (for only 14% of the native species), whereas native species are mainly lithophils (61% vs. 29% of the invasive species). Internal reproduction is only present in invasive poeciliids (*P. reticulate* and *Gambusia holbrooki*), and only two native species reproduce in deep sea (*Anguilla Anguilla* and *Platichthys flesus*). For the habitat, 79% of invasive vs. 47% of native species are lentic; 40% of native vs. 8% of invasive species are generalists (present in both lentic and lotic waters). We did not find significant differences in feeding type, salinity tolerance, marine habits, microhabitat and climatic region between invasive and native species.

As an indicative summary, a logistic regression analysis (stepwise procedure, $P < 0.05$) of all the qualitative variables selected aquarium ($P = 0.003$) and aquaculture ($P < 0.0005$) uses and reproductive guild ($P = 0.001$) as the most important variables distinguishing invasive and native species (Nagelkerke $R^2 = 0.78$).

Table 2. Number of native and invasive inland fish in the Iberian Peninsula by taxonomic order compared to the total number of freshwater fish species (Nelson 1994). *R* values are the binomial probabilities to the test for taxonomic selectivity of invasive species, comparing the Iberian invasives with the world pool of freshwater species (Lockwood 1999). *P* values are the *R* values adjusted for multiple comparisons by the procedure of Benjamini and Hochberg (1995).

Order	Number of freshwater species	Number of Iberian Natives	Number of Iberian invasives	*R*	*P*
Petromyzontiformes	32	3	0	0.926	0.998
Carcharhiniformes	1	0	0	0.998	0.998
Rajiformes	24	0	0	0.944	0.998
Ceratodontiformes	1	0	0	0.998	0.998
Lepidosireniformes	5	0	0	0.988	0.998
Polypteriformes	10	0	0	0.976	0.998
Acipenseriformes	14	1	0	0.967	0.998
Semionotiformes	6	0	0	0.986	0.998
Amiiformes	1	0	0	0.998	0.998
Osteoglossiformes	217	0	0	0.593	0.998
Anguilliformes	6	1	0	0.986	0.998
Clupeiformes	72	2	0	0.841	0.998
Gonorynchiformes	28	0	0	0.935	0.998
Cypriniformes	2662	28	7	0.145	0.822
Characiformes	1343	0	0	0.039	0.444
Siluriformes	2280	0	3	0.114	0.775
Gymnotiformes	62	0	0	0.861	0.998
Esociformes	10	0	1	0.024	0.401
Osmeriformes	42	0	0	0.904	0.998
Salmoniformes	45	2	4	$4.54\ 10^{-6}$	0.0002
Percopsiformes	9	0	0	0.979	0.998
Ophidiiformes	5	0	0	0.988	0.998
Gadiformes	1	0	0	0.998	0.998
Batrachoidiformes	5	0	0	0.988	0.998
Mugiliformes	1	0	0	0.998	0.998
Atheriniformes	146	1	0	0.703	0.998
Beloniformes	51	0	0	0.884	0.998
Cyprinodontiformes	794	2	4	0.082	0.700
Gasterosteiformes	19	2	0	0.955	0.998
Synbranchiformes	84	0	0	0.817	0.998
Scorpaeniformes	52	1	0	0.882	0.998
Perciformes	1922	1	5	0.173	0.840
Pleuronectiformes	4	1	0	0.990	0.998
Tetraodontiformes	12	0	0	0.971	0.998

Analyses of quantitative variables revealed that invasive and native species showed different variances in two of the variables analysed: reproductive season span and latitude range (Table 3). In contrast, no variable showed significant differences in central tendency between native and invasive species (*t*-test without correcting for phylogenetic effects).

Table 3. Differences in the quantitative variables between invasive and native species of freshwater fish in the Iberian Peninsula. Levene tests compare the variance of invasive and native species, whereas other tests compare the central tendency. The *t*-tests correspond to the separate variance formula (not assuming homoscedasticity) of the two independent sample test. *P* values have been adjusted by the procedure of Benjamini & Hochberg (1995). A positive correlation in the independent contrasts indicates a larger mean for invasive species (and vice versa).

Variable	**Levene test**		***t*-test**			**Independent contrasts**		
	F	***P***	***t***	**d.f.**	***P***	***r***	***N***	***P***
Maximum reported age	0.99	0.48	0.19	47.0	0.85	-0.011	50	0.94
Mean total length	0.29	0.71	0.95	46.5	0.35	0.069	62	0.59
Max. observed total length	3.83	0.15	-1.41	30.8	0.17	0.078	68	0.53
Age at maturity	5.01	0.12	-1.66	48.7	0.10	-0.123	55	0.37
Mean fecundity	2.69	0.26	-1.08	33.7	0.29	-0.038	54	0.78
Maximum fecundity	2.42	0.26	-0.97	38.7	0.34	-0.025	55	0.86
Midpoint of reproductive season	1.61	0.36	-0.47	36.8	0.74	-0.272	67	0.026
Reproductive season span	9.13	0.024	1.41	31.3	0.17	0.119	67	0.34
Mean habitat temperature	0.004	0.98	1.89	33.5	0.07	0.193	40	0.32
Temperature range	0.42	0.69	1.70	38.8	0.10	0.042	40	0.80
Mean latitude	0.001	0.98	1.28	36.8	0.21	0.318	46	0.031
Latitude range	29.88	0.003	0.75	36.7	0.46	0.313	46	0.034
PCA 1 score	0.37	0.85	1.26	45.4	0.22	0.130	65	0.30
PCA 2 score	5.30	0.025	1.44	30.7	0.16	-0.043	65	0.73

The central tendency of reproductive season span is similar for the two groups but invasive species display more variance than native ones (Fig. 1). Most species have a reproductive season of 2 to 3 months but several invasive species (poeciliids and some salmonids) have much longer seasons (≥ 5 months). The variability and distribution of latitudinal range is very different between native and invasive species. Native species are significantly more variable than invasive species and display a bimodal distribution with two groups, one with European species of wide distribution and the other with Iberian endemic species (Fig. 2).

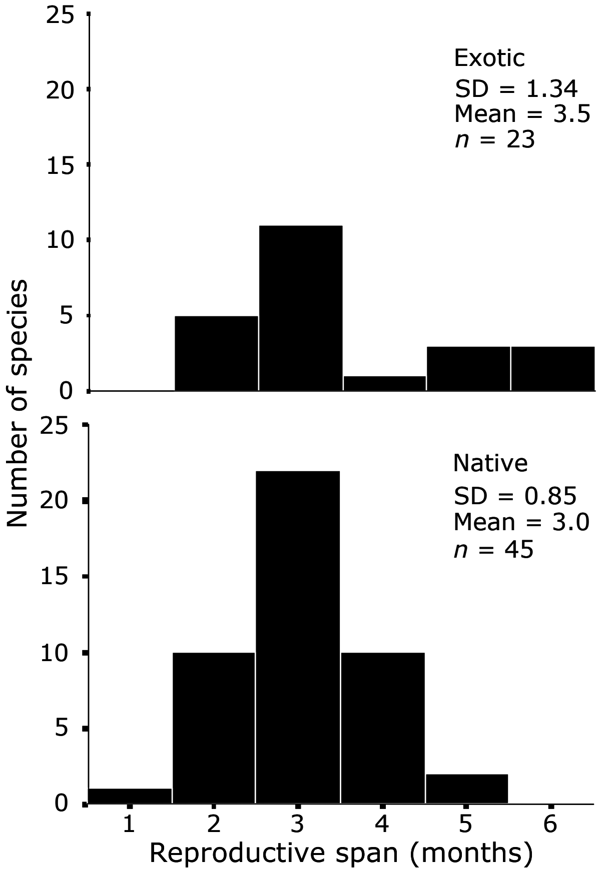

Figure 1. Length of the reproductive season span for the native and invasive inland fish species in the Iberian Peninsula. SD = standard deviation, n = number of fish with data.

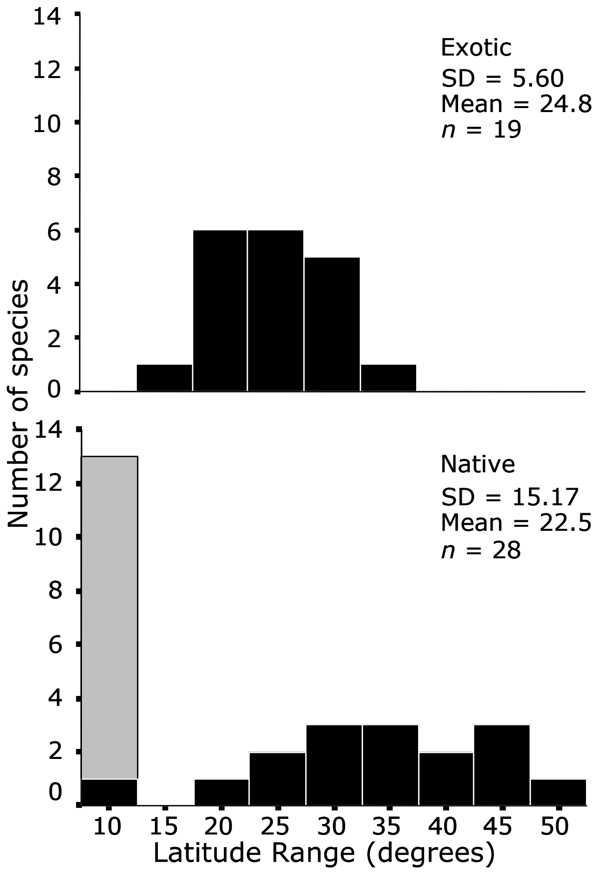

Figure 2. Latitude range for the native and invasive inland fish species in the Iberian Peninsula. The grey colour indicates species endemic to the Iberian Peninsula.

Multivariate analyses

Although a stepwise DFA suggested that age at maturity and maximum age significantly discriminated between native and invasive species (Wilks's $\lambda = 0.58$; $\chi 2 = 13.6$; $P = 0.001$), it only predicted correctly the origin status for 44.9% (cross-validated) of the species, and is therefore useless. A similar result was obtained with regular DFA. The seven species with highest age at maturity are all native but there is no significant difference in central tendency (Fig. 3).

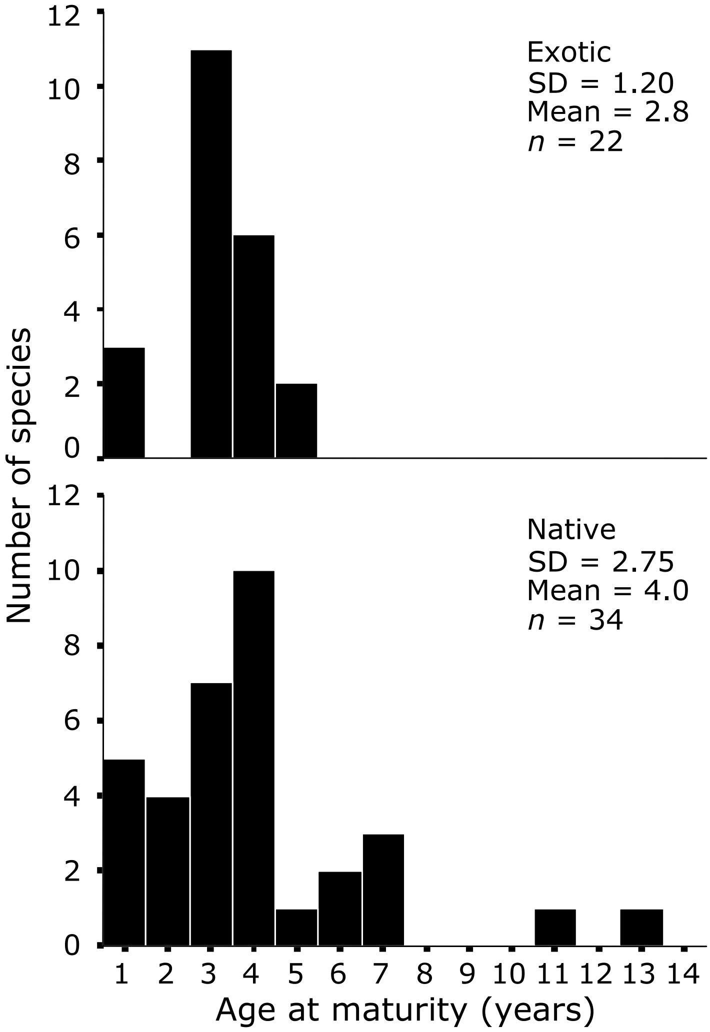

Figure 3. Age at maturity for the native and invasive inland fish species in the Iberian Peninsula.

Most of the variables were correlated and the KMO's measure of sampling adequacy (0.61) indicated the usefulness of a PCA, and the two first axes explained 24.6% and 20.2% of the variation, respectively. As also seen with the factor loadings (Fig. 4), the highest correlations were found between length, age at maturity, maximum latitude, and latitude range which were all positively correlated. The first PCA axis identified a dominant gradient of ecological and life-history traits that contrasts species with large size, late maturation, and from high and more diverse latitudes (such as *Salmo salar*, *Petromyzon marinus* and *Hucho hucho*) with small species, early maturation, and from low and narrower range of latitudes (such as *G. holbrooki* , *Aphanius iberus* and *Chondrostoma lemmingii*) (Fig. 4). The second axis contrasts species with higher fecundities, higher longevities, and from higher and wider with the opposite suite of traits (e.g. *Cottus gobio*, *Phoxinus phoxinus* and *Lampetra planeri*). There are no significant differences of mean PCA scores with species origin but invasive species are more variable in the PCA 2 scores than native ones (Fig. 4, Table 3).

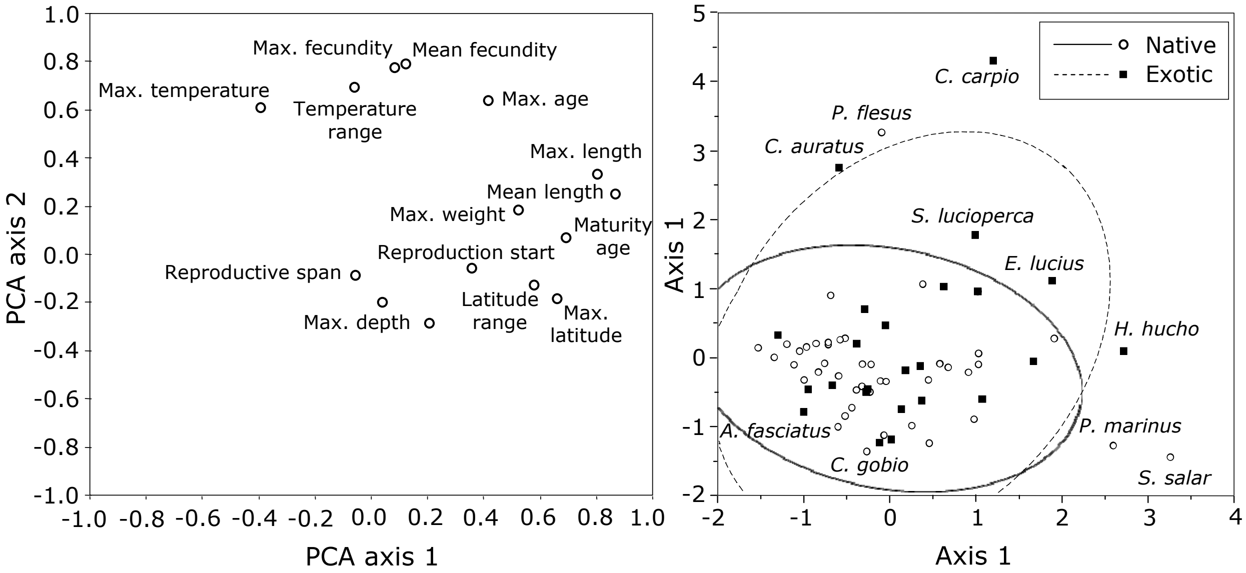

Figure 4. Principal components analysis of the 14 quantitative variables for native and invasive inland fish species of Iberian Peninsula. Left, factor loadings of the variables; right, species scores on the first two principal component axes (with 95% confidence ellipses). Symbols and ellipses identify the two groups (native and invasive).

The effect of phylogeny

Once the phylogenetic effect was eliminated, three variables (midpoint of reproductive season, mean latitude and latitude range) that were previously not significant (*t*-tests) now showed significant differences in central tendency between native and invasive species (independent contrasts, Table 3). Thus, although overall differences in latitude and reproductive season were small (Figs 2, 5 & 6), there were significant differences after accounting for phylogeny (i.e. within the same order or family) because the differences within families were larger and sometimes opposite. Among the families with enough information (i.e. with larger number of species), invasive salmonids reproduced on average 1.6 months before than the two native species, whereas the invasive cyprinids reproduced 0.8 month later than native cyprinids. Many of the invasive cyprinids (e.g. *Rutilus rutilus*, *Scardinius erythrophthalmus*, *Alburnus alburnus*) are central European species with wide distributions in contrast to native species, many of which are endemic to the Iberian Peninsula (so with smaller latitudinal range).

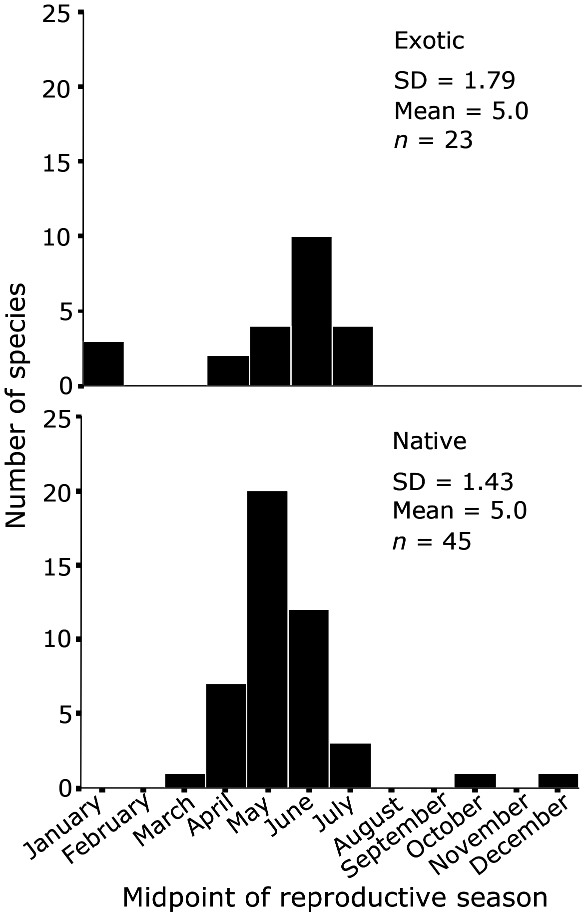

Figure 5. Midpoint of the reproductive season span for the native and invasive inland fish species in the Iberian Peninsula.

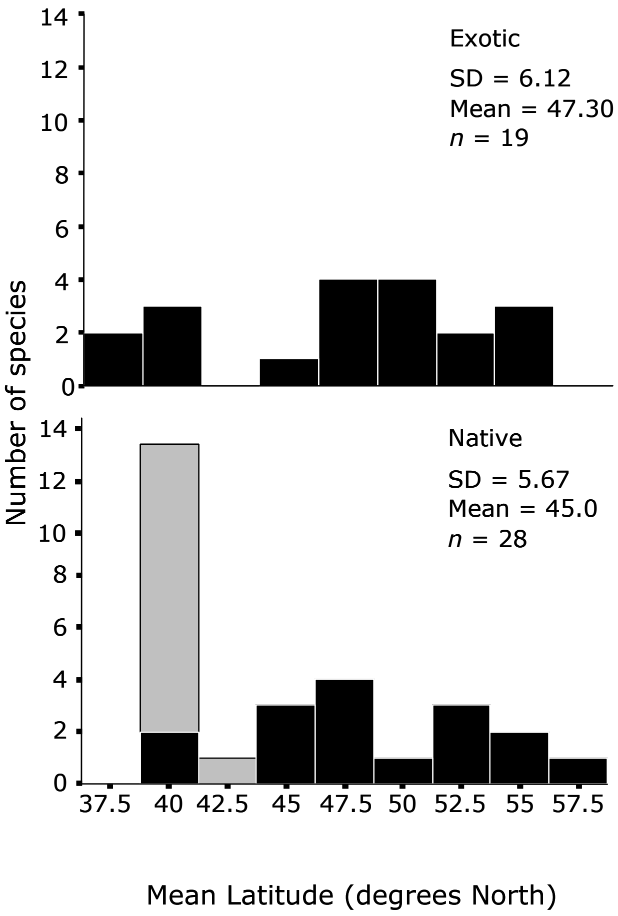

Figure 6. Mean latitude for the native and invasive inland fish species in the Iberian Peninsula. The grey colour indicates the Iberian endemic species.

Partitioning of trait variation among categories in the taxonomic hierarchy (Fig. 7) suggests high similarity in latitude range, age at maturity, length and fecundity among close phylogenetic relatives (i.e. among species of a certain genus or family). On average, phylogenetic effects at the ordinal and familial levels explain about 80% of the total variation in age at maturity, length and fecundity. This outcome suggests that age at maturity, length and fecundity are largely intrinsic and relatively stable characters of higher taxonomic levels (order and family). For instance, salmoniforms are usually large-sized with late maturation and high fecundities, whereas cyprinodontiforms display the opposite suite of traits. This pattern contrasts with the greater generic and specific effects exhibited by midpoint of reproductive season and its span and temperature and latitude range (Fig. 7). These characters are less influenced by phylogeny, and consequently, more variable between closely related species. This is in agreement with the above observation that invasive fish species spawn before and come from higher and wider ranges of latitudes than native ones.

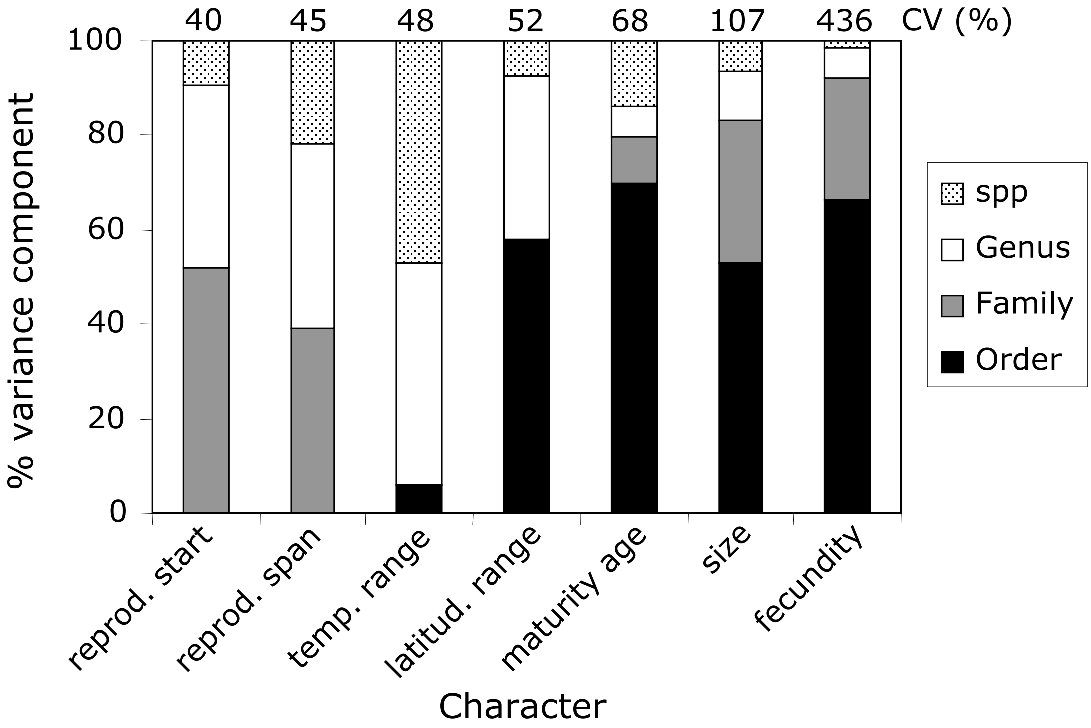

Figure 7. Variance components of different taxonomic levels (order to species) for selected quantitative traits of Iberian inland fish species. Figures at the top are the coefficients of variation (CV = 100 SD/mean).

Age at maturity, length and fecundity are more variable (coefficient of variation (CV), Fig. 7) and differences are mainly related to orders, whereas midpoint of reproductive season, reproductive season span, and temperature and latitude range are less variable (Fig. 7) and differences are mainly attributable to genus and species. CV was significantly related to the percentage of variation explained at the ordinal level (Spearman's r_S = 0.81, N = 7, P = 0.027), so characters more variable have more phylogenetic inertia (Blomberg & Garland 2002).

DISCUSSION

Differences between native and invasive fish species: the importance of phylogeny

The number of species by taxonomic order and family significantly differed between native and invasive species. In contrast to native fish, invasive species belong to only five taxonomic orders (Cypriniformes, Salmoniformes, Perciformes, Cyprinodontiformes and Siluriformes) but to a wide spectrum of families not native to the Iberian Peninsula (Centrarchidae, Cichlidae, Esocidae, Fundilidae, Ictaluridae, Percidae, Poeciliidae and Siluridae). We also found that salmoniform fish species are significantly overrepresented among invasives in relation to their world richness. The taxonomic distribution of invasive fish species is thus not random, as has been previously found for plants (Pyšek 1998, Richardson & Rejmánek 2004) and birds (Lockwood 1999, Blackburn & Duncan 2001). As for plants and birds, this seems clearly related to the purposeful introduction of species of human interest. In the Iberian Peninsula, invasive fish species were mainly introduced as game fish or for use in aquaculture or in aquarium. As also pointed out by Lockwood *et al.* (2001), there is a tendency for invaders to come from taxa that are not represented in the native fauna. Invasive species may be more successful if they are ecologically distinct from members of the community that they are invading (Lockwood *et al.* 1993, Moyle & Light 1996b; Williamson 1996) because they may be able to exploit a resource untapped by native species, avoiding competition with native species or may be free of predators or parasites (Lockwood *et al.* 2001), although biotic resistance has been suggested to be less important than abiotic factors in determining the success of invasive fish in California streams and estuaries (Moyle & Light 1996a).

If fish introduced to the Iberian Peninsula has a taxonomic bias, this may affect the comparison of life-history traits between native and invasive species. Without correcting for phylogeny, there were not significant mean differences in any trait between native and invasive species but after controlling for phylogeny, midpoint of reproductive season, mean latitude and latitude range became significantly different between native and invasive species. In general, trait differences vanish after accounting for phylogenetic effects, indicating that the differences between groups were caused at least in part by phylogeny (Jordano 1995). But in some cases, nonsignificant differences may turn significant (Villar *et al.* 1998, Duncan *et al.* 2001) as was also observed in our study, indicating that phylogenetic correction is thus necessary to partial out strong taxonomic effects and allow more powerful comparisons between native and invasive species within orders or families. Because many fish life-history traits are highly dependent on phylogeny (Vila-Gispert *et al.* 2002), phylogenetic methods may detect subtle differences within families. Since most Iberian fish species are cyprinids (51%) or salmonids (10%) this seem particularly worthy.

Predicting the traits of invasive fish species: variable human interests

Taxonomy and human use are the main factors differentiating native and invasive fish species in the Iberian Peninsula. It is more difficult to make generalizations about which life-history traits are characteristic of invasive fish species. We found that several life-history traits were not significantly different between native and invasive fish species on average but showed differences in variability (Table 3). Reproductive season span of invasive species is more variable, from species with protracted spawning seasons (e.g. *G. holbrooki*) to those with short spawning seasons (e.g. *R. rutilus*). In contrast, native fish fauna is more variable in latitude range as a result of a mixture of many Iberian endemisms (with small ranges) and some species with wide distributions. PCA also showed that invasive species are more variable in a suite of attributes such as fecundity, longevity, and temperature tolerance. The higher variability of life-history traits among invasive species should be expected from the contrasting human uses of these species that range from small species mainly selected for aquarium purposes such as *P. reticulata* or *G. holbrooki* to large species typically used in aquaculture such as salmoniforms. Human interests in fish introductions are diverse and thus obscure life-history trait characterization of invasive fish species.

After controlling for phylogeny, midpoint of reproductive season, mean latitude and latitude range significantly differed between native and invasive species. Invasive fish species came from higher and wider latitudes. Many researchers have previously noted the importance of latitudinal range to invasiveness (Scott & Panetta 1993, Ricciardi & Rasmussen 1998, Duncan *et al.* 1999, Goodwin *et al.* 1999, Duncan *et al.* 2001). Invasive species with a wide distribution are likely to succeed in a new environment because of their wide environmental tolerances (Goodwin *et al.* 1999, Marchetti *et al.* 2004). Wide geographical range could indicate flexible or generalist species that have a high chance of success because they are likely to encounter conditions suitable for establishment (Williamson 1996).

The difference in reproductive season was only significant after accounting for phylogeny and was depended on family. Introduced cyprinids spawned later than native cyprinids, whereas the opposite was the case for salmonids, of which, the two native species spawn in autumn, whereas the invasive spawn rather in winter or spring. The species-specific timing of salmonid recruitment and its relationship to the local hydrologic regime has been recently suggested as a key factor in explaining the invasive success of salmonids (Fausch *et al.* 2001).

In a previous study, we found that fecundity and age at maturity significantly distinguished native and invasive fish species from Catalan streams (Vila-Gispert *et al.* 2005), in contrast to the present results that did not find such differences even prior to phylogenetic correction. We believe that these contrasting results are caused by the wider geographical scale of the present study, which implies a larger set of different species.

In conclusion, the identification of life history and ecological traits differential of invasive species features will benefit from the incorporation of phylogenetic methods and an appraisal of variability in addition to central tendency of characters. Studies at different geographical scales may also yield different results and an understanding of the underlying mechanisms.

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Chapter II

First data on the use of a flooded saltmarsh habitat by an endangered cyprinodont

ABSTRACT

We report the first data on the use of occasionally-inundated habitats by the Spanish toothcarp (*Aphanius iberus*) in a Mediterranean coastal lagoon, which is the usual habitat of this endangered cyprinodontid fish. During a flooding period, we sampled the macroinvertebrate community and the fish population in three contrasting habitats: mats of green macroalgae, open water, and a habitat dominated by glasswort (*Salicornia patula*) that is occasionally inundated. The three habitats displayed strong variation in density and species composition of invertebrates in the water column and the benthos. In general, algal mats had higher invertebrate biomasses but glasswort had higher diversity of organisms, in part of terrestrial origin. The density of toothcarp was very low in the open water. The habitat that is occasionally inundated (glasswort) significantly had the highest density of mature toothcarp, whilst immature fish were similarly abundant in the glasswort and algal mat habitats. Condition (weight-length relationship) and total food biomass in the gut contents of immature toothcarp was significantly higher in algal mats than in glasswort, whereas there were no such differences for mature fish. Therefore, glasswort was positively selected by mature fish but seemed a disadvantageous habitat for immature toothcarp individuals.

Keywords

Coastal lagoon, flooding, cyprinodontid fish, *Aphanius iberus*, Spanish toothcarp.

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* Alcaraz C., Pou-Rovira Q. & García-Berthou E. (submitted).

INTRODUCTION

Marshes around the Mediterranean Sea typically consist of coastal lagoons without tidal influence supporting submerged meadows of eelgrasses (mainly *Ruppia* and *Zostera*). Such lagoons are surrounded by seasonal wetlands (De Szalay & Resh 2000) with salt marshes dominated by glasswort (*Salicornia* and *Sarcoconia*) that are dry in summer and only occasionally flooded by sea water intrusion or precipitation. The role of inundated habitats for fish has been well investigated in North American marshes. One of the most abundant fish, the cyprinodontiform *Fundulus heteroclitus*, has been shown to play an important role in energy transfers within U.S. salt marshes (Meredith & Lotrich 1979). Mummichogs (*F. heteroclitus*) follow the rising tide onto the salt marsh to feed in these highly productive habitats (Kneib 1981, Kneib & Wagner 1994) and displayed higher growth rates when experimentally allowed to access the intertidal marsh surface, due to increased food availability (Weisberg & Lotrich 1982). However, the use of flooded habitats by European fish species is largely unknown, particularly in Mediterranean marshes, which have negligible tidal influence.

The Spanish toothcarp (*Aphanius iberus*) is a cyprinodontid fish endemic to the Mediterranean coast of Spain and one of the few Iberian fish species protected by law and considered in danger of extinction by the Spanish National Catalogue of Endangered Species and the Convention on the Conservation of European Wildlife and Natural Habitats (Bern Convention) (Doadrio 2002). The toothcarp originally inhabited a range of lowland waters but its current distribution is reduced to brackish waters, due to the impact of invasive species such as the mosquitofish (*Gambusia holbrooki)*, habitat destruction and water pollution (García-Berthou & Moreno-Amich 1991, Rincón *et al.* 2002). Moreno-Amich *et al.* (1999) pointed out the importance of submerged vegetation, mainly *Ruppia* spp., as a habitat of the toothcarp but its behaviour during flooding and the importance of infrequently inundated habitats, such as glasswort, is unknown. Although several studies on the life-history and conservation of the toothcarp are available (García-Berthou & Moreno-Amich 1992, Planelles-Gomis 1999, Rincón *et al.* 2002), no study has previously shown whether the Spanish toothcarp uses the salt marsh during flooding. In contrast to both North American and European Atlantic wetlands, the flooding events in Mediterranean marshes are not linked to tidal influence and thus unpredictable but may last for long periods (weeks to months).

The objectives of our paper are to report the first data on: (i) the use of an occasionally flooded habitat (glasswort) by the Spanish toothcarp; and (ii) the population density and structure of the toothcarp and the macroinvertebrate community in three habitats (algal mats, open water and glasswort). In a companion paper (Alcaraz & García-Berthou 2006), we analyse in detail the diet and prey electivity for the same fish.

METHODS

Study area

The study was undertaken in Fra Ramon, a hyperhaline coastal lagoon located behind the dune line of the Baix Empordà salt marshes (NE Spain) (Fig. 1). It is situated at 42º 1’ 49’’ N, 3º 11’ 29’’ E and 1.75 m below the sea level. The salinity ranges between 28 ppt (during rainfall periods) to 49 ppt (in summer) and the temperature from 5 ºC to 30 ºC. It is eutrophic according to the concentrations of total nitrogen and chlorophyll but hypereutrophic according to the concentration of total phosphorous (A. Badosa, unpublished data). There is no tidal influence or freshwater inflow to the lagoon and water entry occurs mainly through rainfall and marine intrusions. The water area of the lagoon ranges from ca. 2200 m^2 during the dry season (summer) to 13000 m^2 in heavy rainfall periods (when we sampled the lagoon) (A. Badosa, unpublished data).

The permanently inundated zone of the lagoon supports dense submerged meadows of ditch-grass (*Ruppia cirrhosa*) and floating mats of green algae (*Enteromorpha* sp. and *Chaetomorpha* sp.). Algal mats are more abundant in summer (when high blooms occur) and rare in winter. The salt marsh is dominated by the *Puccinellio-Arthrocnemetum fruticosi* plant association, including *Arthrocnemun fruticosum* and other glasswort species. The lagoon shore that is occasionally inundated during flooding periods displays the *Suaedo-Salicornietum patulae* plant association, dominated by glasswort (*Salicornia patula*) and others succulent plants (e.g. *Suaeda maritima* and *Suaeda splendens*). This habitat is usually flooded only about two months per year (Fig. 1). *Aphanius iberus* is the most abundant fish in the lagoon, although other species of marine origin such as eel (*Anguilla anguilla*) and mullets (Mugilidae) are occasionally observed (Alcaraz *et al.*, pers. obs.). The Spanish toothcarp population was monitored bimonthly during a year. Our study was undertaken during one of the occasional periods of inundation of the glasswort belt (Fig. 1).

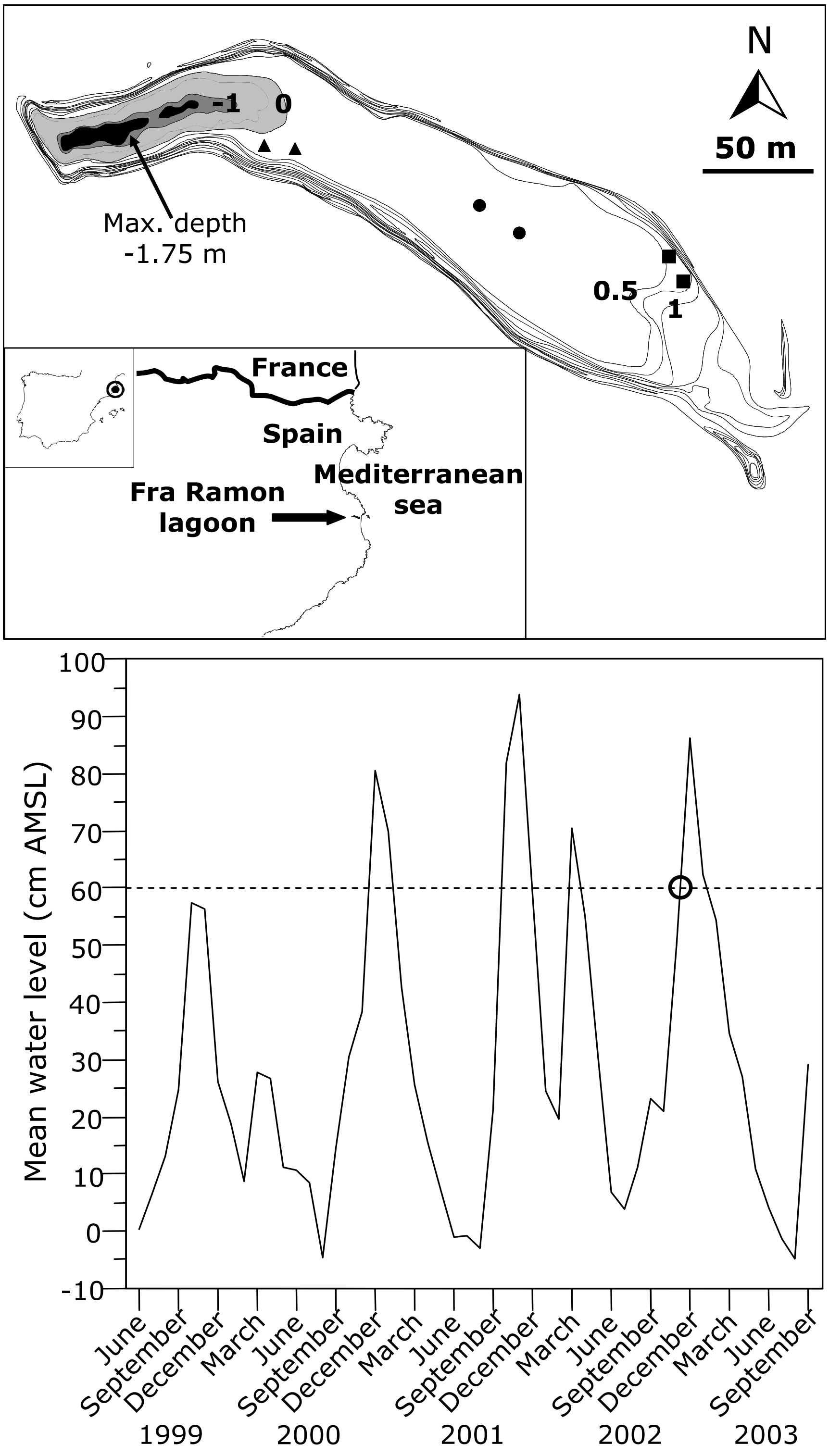

Figure 1. Top, location of the study area and map of the Fra Ramon lagoon with the main sampling points per habitat (■ = Glasswort, ● = Open water, and ▲ = Algal mats); depths are meters above mean sea level. Bottom, mean water level (in cm above mean sea level) in the lagoon from 1999 to 2003; dashed line shows the water level above which glasswort is flooded and available for fish and the circle marks the sampling date.

Field and laboratory methods

The lagoon was sampled on the mornings of 21-22 of November 2002, during a flooding period due to heavy rainfall. Three different habitats were sampled: "glasswort" (corresponding to the *Suaedo-Salicornietum patulae* plant association that is occasionally inundated), "algal mats" (dominated by *Enteromorpha* sp. and *Chaetomorpha* sp. and mostly present close to the littoral); and "open water", unvegetated habitat that dominates most of the lagoon. In each of the habitats we sampled organisms in the water column and benthos (both two replicates per habitat) and fish (9-20 replicates per habitat, see below) (Fig. 1). At the sampling sites for invertebrates, these three habitats were 14, 37 and 33 cm deep respectively. Zooplankton and macroinvertebrates in the water column were sampled with a 50 µm mesh dip net in a 1 m long transect (volume sampled = 0.038 m^3). All the samples were immediately preserved in 10% formaldehyde. Benthic organisms were sampled with a 15 × 15 cm benthic grab and immediately frozen. In the laboratory, the benthos samples were unfrozen and sifted through a 0.1 mm sieve. All the organisms were then fixed in 10% formaldehyde.

Organisms were usually identified to the genus or species level, except Nematoda and Ostracoda. All organisms were counted and a minimum of 20-30 individuals (if present) of each taxon were measured under a dissecting microscope with an ocular micrometer. The measurements were converted to dry mass according to published length-dry mass relationships (mainly Dumont *et al.* 1975, Smock 1980).

The fish sampling technique consisted of quickly throwing a cylinder (90 cm diameter) and then removing all the fish contained within using a dip net. The mean water depth within the throw trap was measured at each sampling point and the captures were transformed to density (fish l^{-1}). Although throw traps may produce estimates that are about 37% lower than actual population densities, they provide relatively accurate estimates of fish density and size across a range of environmental conditions (Jordan *et al.* 1997). Our objective was not to have absolute estimates but to compare abundances and population structure among habitats, which were also evident by visual observations. Fish were sampled throughout the lagoon with 38 throws of the cylinder, distributed among three habitats (9 in glasswort, 9 in open water and 20 in algal mats due to its dense cover of the lagoon). In the laboratory, all individuals were measured (standard and total lengths to the nearest 0.5 mm) and weighed (total and eviscerated weight to the nearest 0.1 mg). Sex was determined externally due to the sexual dimorphism of the species. The total number and biomass of prey in the gut contents was measured for a minimum of 25 fish in each habitat. The gut contents of toothcarps were examined under a dissecting microscope, sorted usually to the species or genus level, counted, and a minimum of 20-30 individuals (if present) of each taxon were measured with an ocular micrometer. As with water and benthos organisms, the measurements were converted to dry mass using published length-dry mass relationships. The volume of uncountable food categories (detritus, plant debris, plant leaves, invertebrate eggs and digested material) were estimated to the nearest 0.00025 mm^3 with a Neubauer counting chamber (haemacytometer) as in García-Berthou (1999) and transformed to biomass using a conversion of 0.27 mg of dry weight / mm^3 (Dumont *et al.* 1975, Lindegaard 1992).

Data analysis

Differences in abundance among habitats were analyzed with analysis of variance (ANOVA), followed by Games-Howell post-hoc tests (hereafter, GH tests). GH tests are post-hoc multiple comparison tests that are among the most robust to unequal variances and most powerful (Day and Quinn 1989). The proportions of sex categories were compared among habitats with a *G*-test of independence. Analysis of covariance (ANCOVA) was also used to compare the condition (weight-length relationship) and total food biomass in the gut contents among sex categories and habitats, using fish length as the covariate. We started with the most complex model, introducing all possible interactions (including interactions of covariates × factors, following García-Berthou & Moreno-Amich (1993)). Then, we simplified the general linear model by removing non-significant interactions ($p >> 0.10$) to increase statistical power, that otherwise would be seriously compromised. When the covariate was non-significant ($p > 0.10$) it was also deleted from the model (so an ANOVA was used). All the quantitative variables were log-transformed for the analyses, because homoscedasticity and linearity were clearly improved. Open water was omitted for some of the fish analyses because only three fishes were captured in that habitat. All data analyses were performed with SPSS 12.

RESULTS

Habitat variation in the invertebrate community

The three habitats significantly differed in the total density (ANOVA, $F_{2,\,3} = 117.6$, $P = 0.001$) and total dry biomass ($F_{2,\,3} = 10.41$, $P = 0.045$) of water column invertebrates (Fig. 2).

In both cases, algal mat habitat showed the highest values but while glasswort and algal mats presented a similar total density/total dry biomass ratio, open water had high total density but the lowest dry biomass because it only consisted of small organisms (mainly copepod nauplii and rotifers).

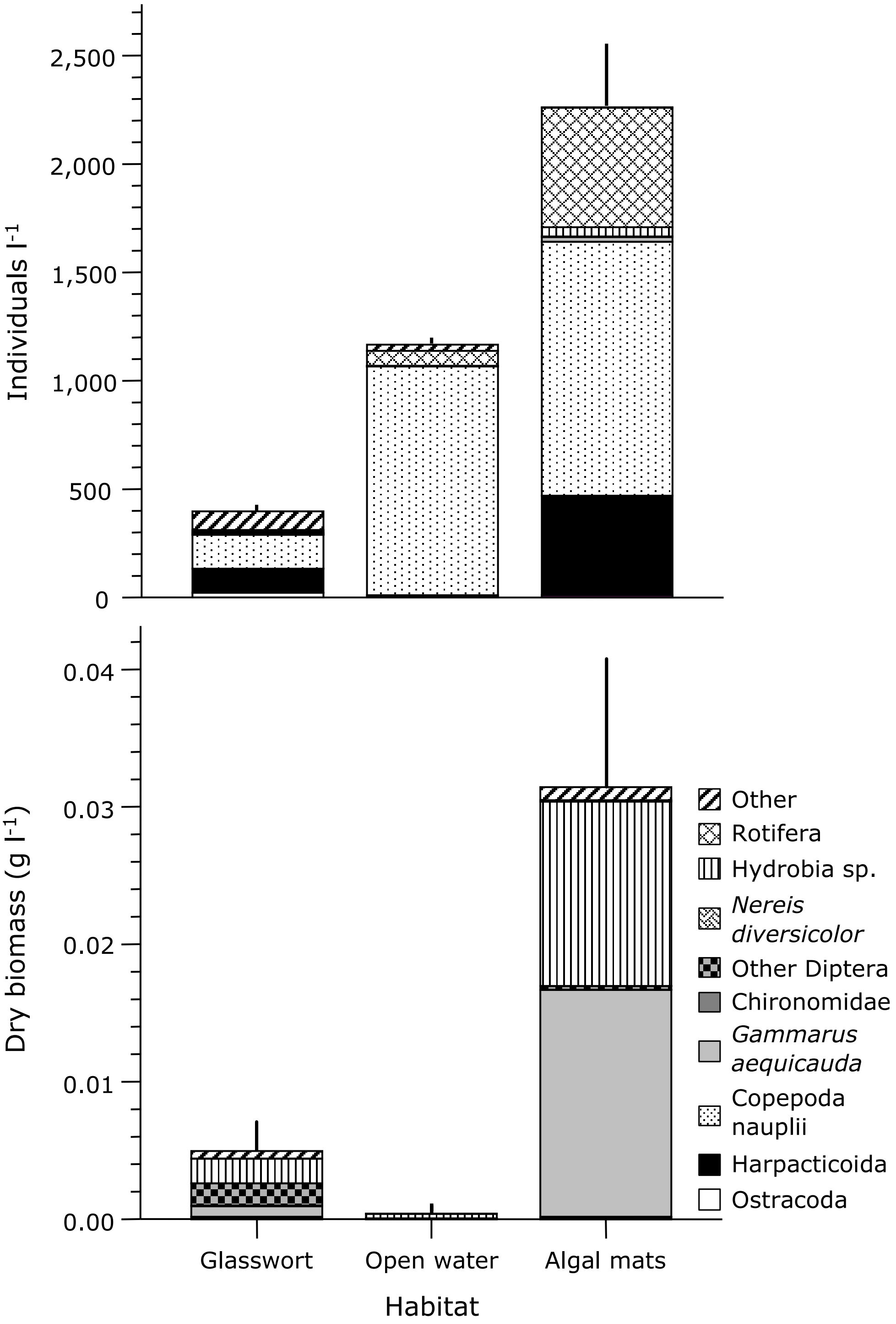

Figure 2. Density (top) and dry biomass (bottom) of the different taxa in the water column for the three habitats. Error bars show the standard error of the mean of the total density (top) and total dry biomass (bottom) by habitat (2 replicates per habitat).

The mean individual weight was lowest in open water but not significantly different between glasswort and algal mats (Fig. 3). In glasswort and algal mat habitats, the water column was also dominated in number by small organisms, mainly copepod nauplii, harpacticoids, and in algal mats by rotifers (Fig. 2). By biomass the results were quite different and while algal mats were dominated by amphipods (*Gammarus aequicauda*) and gastropods (*Hydrobia* sp.), glasswort was dominated by gastropods (*Hydrobia* sp.) and dipterans (mostly Stratyomidae) (Fig. 2).

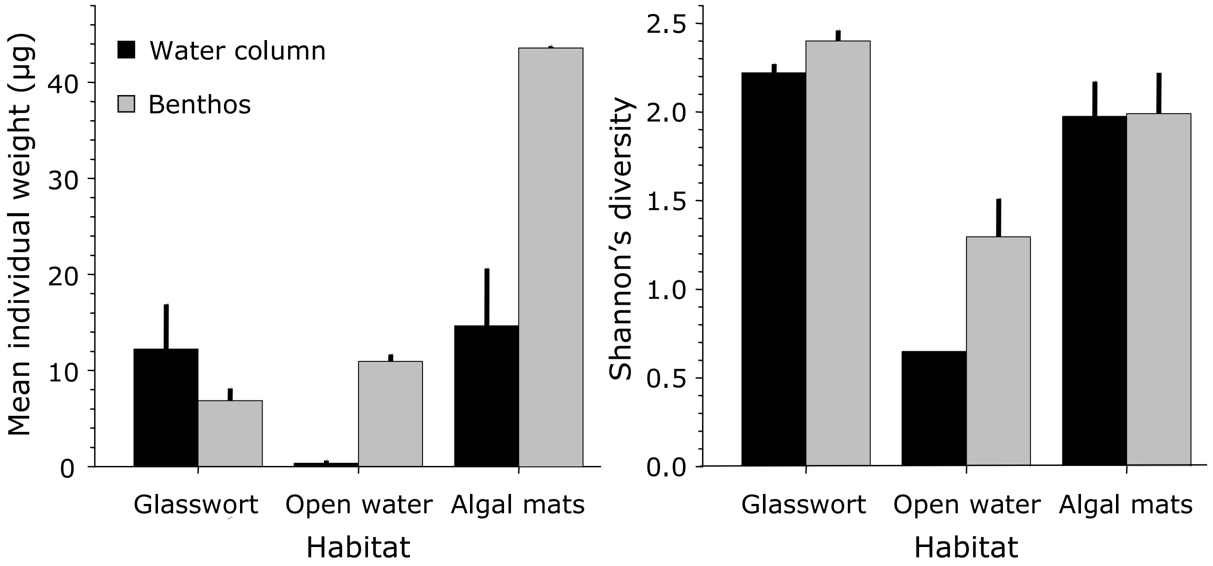

Figure 3. Mean individual weight in water column and benthos. Errors bars show the standard error of the mean (left). Shannon's diversity index (log 2 base) in the water column and benthos by habitat. Error bar is the standard error of the mean (right). *n* = 2 replicates per habitat.

Habitat variation was also significant for total dry biomass of the zoobenthos ($F_{2, 3}$ = 10.51, P = 0.044) but not for total density ($F_{2, 3}$ = 1.32, P = 0.39). By number, the three habitats presented high abundance of harpacticoids (mainly *Mesochra lilljeborgi* in glasswort and *Canuella perplexa* in open water and algal mats), but open water and algal mats were clearly dominated by ostracods and in glasswort chironomids and copepod nauplii were also common. By biomass, chironomid larvae in the three habitats and ostracods in open water and algal mats were more important (Fig. 4). Glasswort and open water showed a similar total density/total dry biomass ratio, but algal mats showed a much higher total biomass because of the dominance of chironomids, much larger than microcrustaceans.

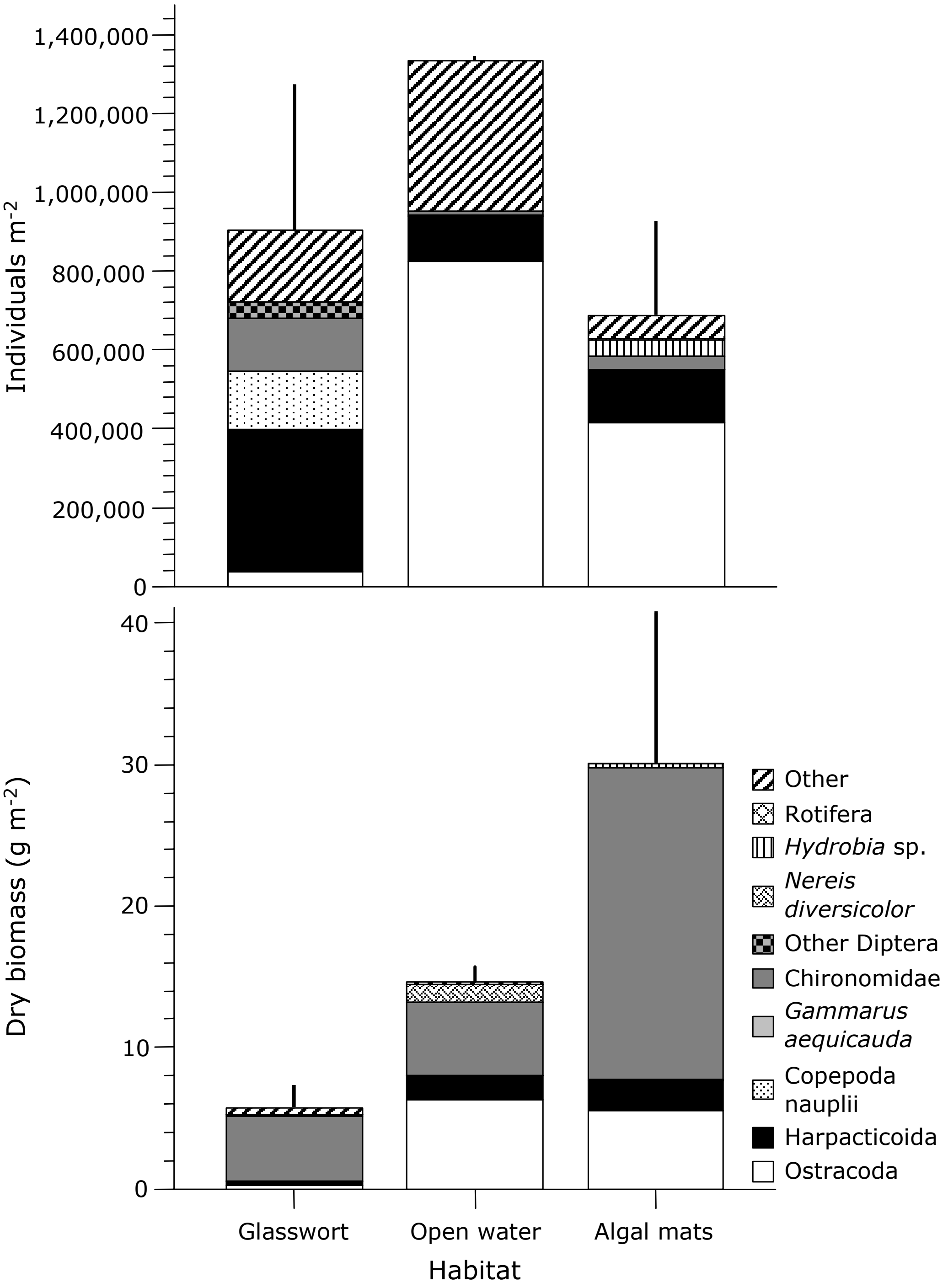

Figure 4. Density (top) and dry biomass (bottom) of the different taxa in the benthos for the three habitats. Error bars show the standard error of the mean of the total density (top) and total dry biomass (bottom) by habitat (2 replicates per habitat).

Overall mean size for all taxa (Fig. 3) presented significant differences among habitats for the water column ($F_{2,3} = 6.88$, $P = 0.076$) but not for the benthos ($F_{2,3} = 2.06$, $P = 0.27$). However, this difference was mainly due to different species composition since only four of the water column taxa showed significant differences when analyzed separately: the polychaete *Nereis diversicolor* ($F_{1, 1}$ = 49.5, P = 0.090), the snail *Hydrobia* sp. ($F_{2, 2}$ = 21.9, P = 0.044), chironomid larvae ($F_{2, 1}$ = 4166.2, P = 0.011), and copepod nauplii ($F_{2, 3}$ = 12.29, P = 0.036) (Fig. 5). Only two of the benthic taxa showed significant differences: chironomid larvae ($F_{2, 3}$ = 15.85, P = 0.025) and the harpacticoid *Tisbe longicornis* ($F_{2, 2}$ = 15.69, P = 0.060) (Fig. 5). In general, mean size was greater in the algal mat habitat and lowest in glasswort, except for *Nereis diversicolor* that were largest in open water.

The diversity of the invertebrate community (Fig. 3) was significantly different among habitats (Fig. 4) in the water column ($F_{2, 3}$ = 52.01, P = 0.005) and the benthos ($F_{2, 3}$ = 8.95, P = 0.054). Overall diversity was highest in glasswort (in part because of the presence of many organisms of terrestrial origin, such as isopods, acari, and dipterans adults) and much lower in the open water habitat, for both water column and benthos (Fig. 3).

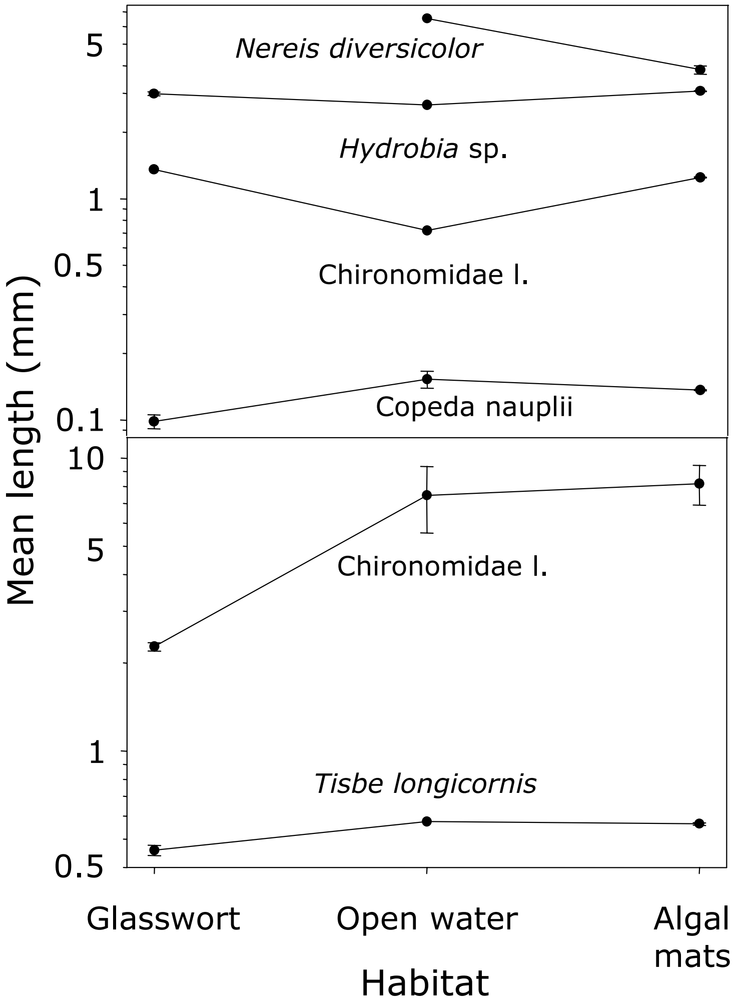

Figure 5. Habitat variation in length (mean ± standard error) of different taxa in the water column (top) and the benthos (bottom). Only taxa with significant difference variation are shown.

Habitat variation in fish features

The density of toothcarp significantly differed among habitats ($F_{2, 35} = 14.87$, $P < 0.001$) and was much higher in glasswort and lowest in the two other habitats (Games-Howell post hoc tests, $P < 0.05$) (Fig. 6). However, the higher densities in glasswort habitats were significant for mature fish ($F_{1, 27} = 22.82$, $P < 0.001$) but not for immature individuals ($F_{1, 27} = 0.93$, $P = 0.344$). Identical conclusions were obtained when density was referred to surface area instead of volume.

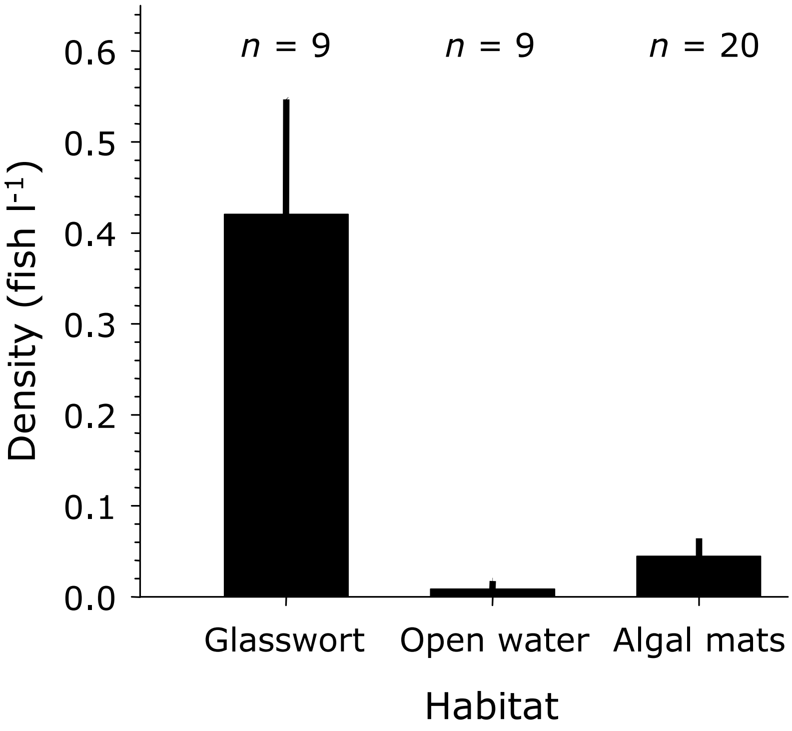

Figure 6. Density of *Aphanius iberus* in the three habitats. Mean, standard error (error bar) and number of samples (*n*) are shown.

Standard length of toothcarp also differed among habitats ($F_{2, 300} = 25.41$, $P < 0.001$) and was higher in glasswort than in algal mats (Games-Howell test, $P < 0.001$) (Fig. 7). This difference was due to a higher proportion (52%) of immatures in algal mats ($G = 54.57$, $df = 4$, $P < 0.001$) (Fig. 8), compared to only 10% in glasswort. For matures alone, although females were larger than males ($F_{1, 241} = 30.61$, $P < 0.001$) due to sexual dimorphism, there was no significant effect of habitat on standard length ($F_{2, 243} = 0.25$, $P = 0.78$) or sexual proportion ($G = 0.82$, $df = 2$, $P = 0.66$) (Fig. 8).

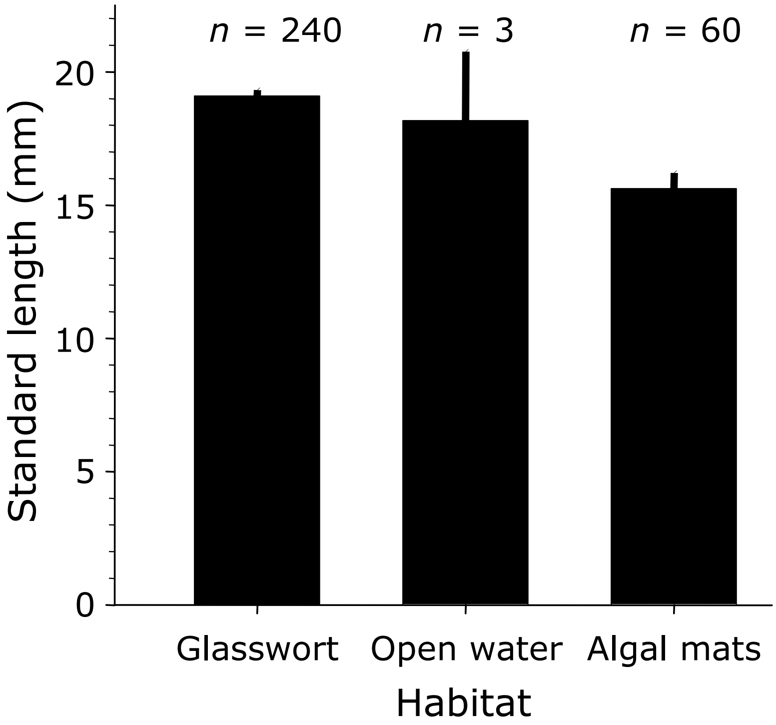

Figure 7. Standard length of *Aphanius iberus* in the three habitats. Mean, standard error (error bar) and number of fish (*n*) are shown.

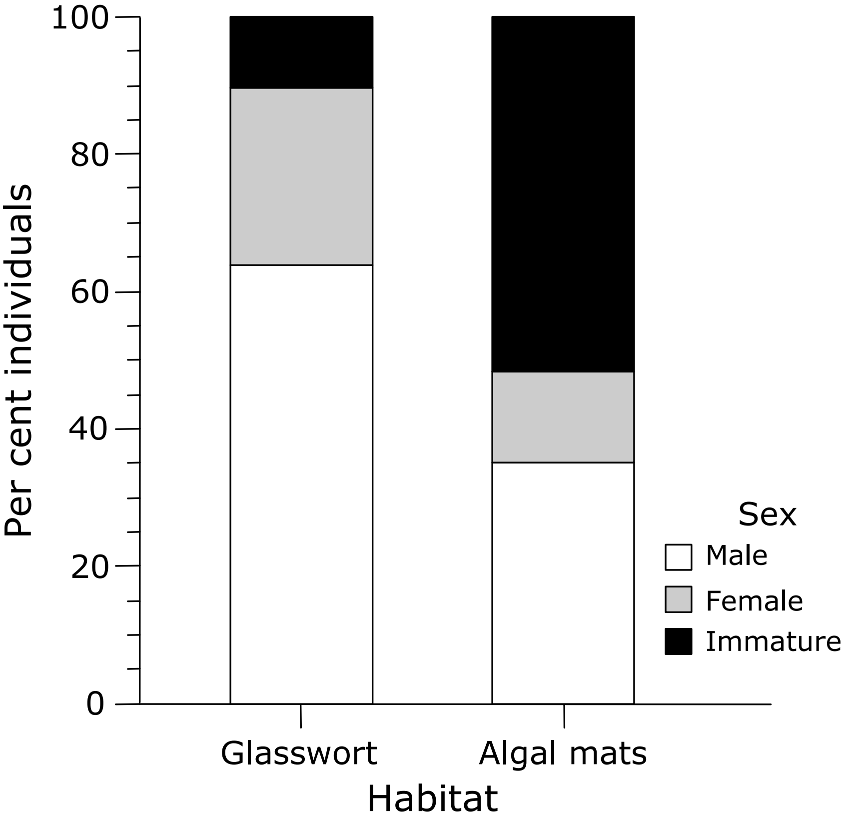

Figure 8. Frequency of sex categories by habitat. Open water habitat is omitted because only three individuals were captured.

The weight-length relationship differed significantly among sex groups (male, female and immature) (ANCOVA, $F_{2,\ 292} = 9.30$, $P < 0.001$) and there was also some indication of sex × habitat interaction ($F_{3,\ 292} = 2.44$, $P = 0.065$) (Fig. 9). Mature females were significantly heavier than mature males for a given length ($F_{1,\ 238} = 8.95$, $P = 0.003$) but condition did not depend on habitat ($F_{1,\ 238} = 0.014$, $P = 0.91$).

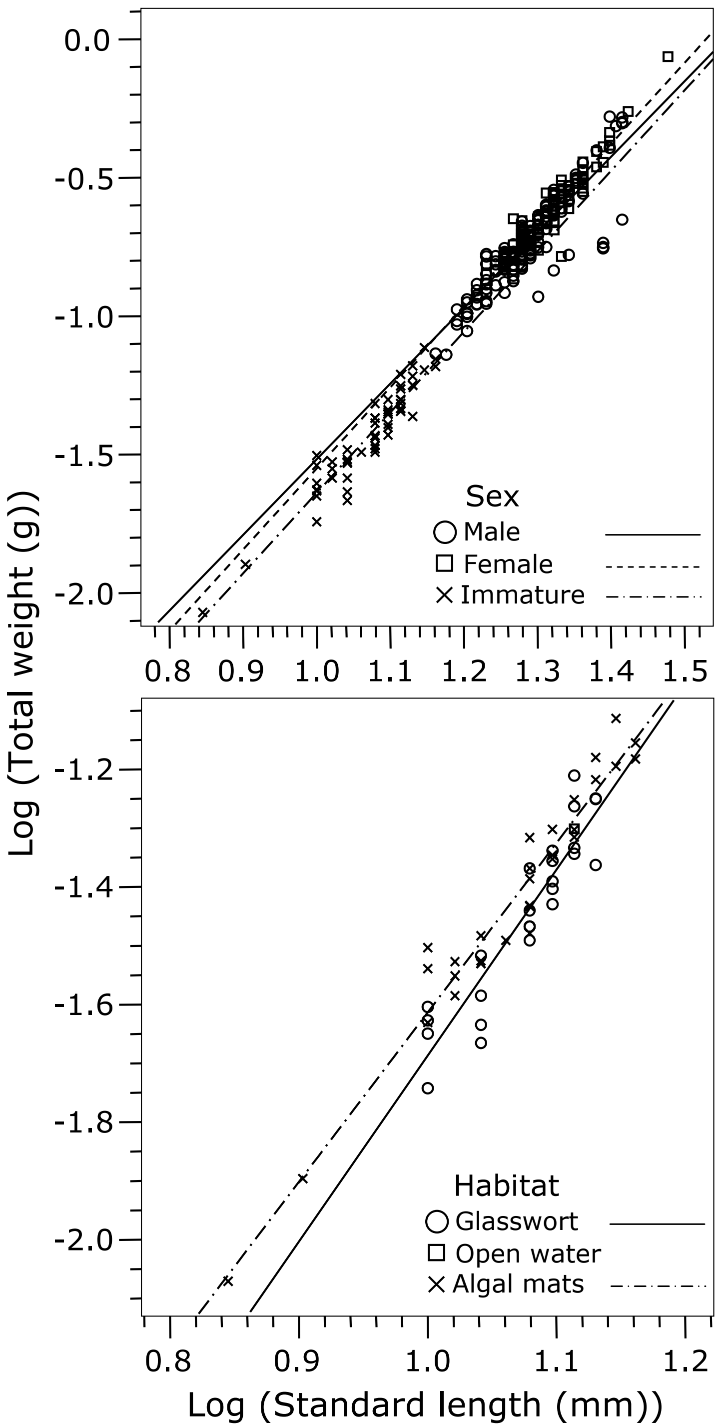

Figure 9. Weight-length relationship of *Aphanius iberus* for different sex categories (top) and by habitat for immatures (bottom). All regression had $r^2 >> 0.80$.

In contrast, immatures were significantly in better condition (controlling for length with ANCOVA) in algal mats than in glasswort habitat ($F_{1, 53}$ = 14.68, P < 0.001) (Fig. 9). The adjusted mean of total weight in algal mat habitat (-1.412) indicated than toothcarps were 3.7% heavier than in glasswort (adjusted mean, -1.465). These condition differences were the same when eviscerated weight was used instead of total weight for mature (sex, $F_{1, 238}$ = 8.2, P = 0.005; habitat, $F_{1, 238}$ = 0.30, P = 0.59) and for immature fish (habitat, $F_{1, 53}$ = 13.7, P = 0.001).

For total food biomass in the gut contents, there were significant effects of fish size (ANCOVA, $F_{1, 52}$ = 15.05, P < 0.001) and habitat ($F_{1, 52}$ = 13.45, P = 0.001) but not of sex ($F_{2, 52}$ = 1.68, P = 0.20). However, there was significant sex × habitat interaction ($F_{2, 52}$ = 5.52, P = 0.007), because the habitat effects were only significant for immatures ($F_{1, 11}$ = 31.38, P < 0.001), with higher food biomass in algal mats than in glasswort, after accounting for fish size (Fig. 10).

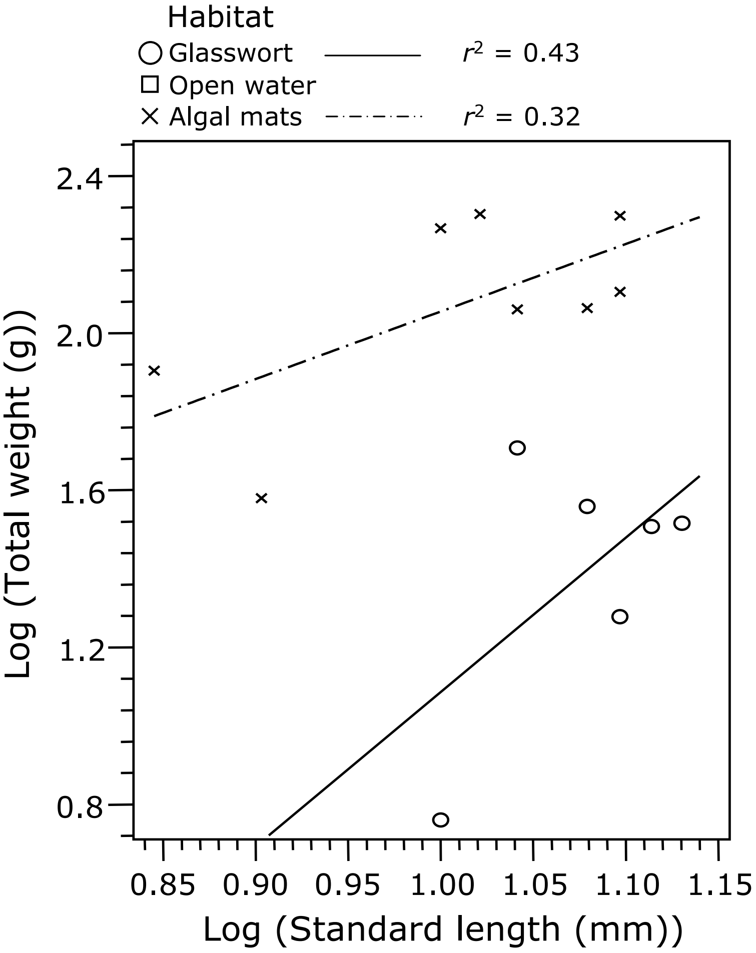

Figure 10. Total food biomass in the gut of the immature toothcarp by habitat and fish length.

DISCUSSION

Habitat variation in the invertebrate community

We found that the highest biomass and density of water column invertebrates occurred in algal mats. The algal mat community was dominated in number by zooplankton (rotifers, harpacticoids and copepods nauplii). In contrast, the abundance of benthic organisms was lower in algal mat habitat than in the two other habitats. Although dense macroalgal mats increase habitat complexity and oxygen availability in the water column, periods of high oxygen consumption due to macroalgal respiration and decomposition processes create anoxic conditions (Hansen & Kristensen 1997, Franz & Friedman 2002). These anoxic conditions modify the macrofaunal composition by mortality or by forcing emigration towards algae (Raffaelli *et al.* 1991, Norkko *et al.* 2000) or the open water, where predation risk may increase (Raffaelli *et al.* 1998).

The benthic fauna of algal mats was dominated by chironomid larvae (by dry biomass), ostracods and harpacticoids (mainly by number). Franz & Friedman (2002) have shown that a decrease of oxygen level due to presence of dense algal mats may produce a small increase in phytal species of harpacticoids. Although previous studies have shown that invertebrate density depends on the presence and structure of vegetation, glasswort presented the lowest values among habitats for density of water column invertebrates and for benthos biomass. We found that water column and benthos communities in glasswort were dominated in number by harpacticoids and copepods nauplii, while dry mass was dominated by dipterans, presenting the most important abundances of dipterans among the three habitats. Early colonization stages in marshes are characterized by lower macrofauna densities and by greater proportion of organisms with aerial (insects) or planktonic dispersion (Talley & Levin 1999, De Szalay & Resh 2000).

Habitat effects on the Spanish toothcarp

Vegetation density strongly mediates fish habitat selection by decreasing predation risk and increasing food supplies and stable substrates (Hindell *et al.* 2001). In the lagoon, the two vegetated habitats showed the highest fish densities, in contrast to open water where only three fishes were captured. This pattern of distribution agrees with patterns previously shown for many others fishes (Hindell *et al.* 2001, Jordan 2002, Lewin *et al.* 2004).

The glasswort habitat had significantly higher densities of mature fish than algal mats but immature density was not significantly different in both habitats. Kneib & Wagner (1994) also showed that larger individuals were the first to reach marshes recently inundated, whereas the proportion of smaller fish was higher in permanent habitats. Interestingly, immatures in glasswort significantly were in poorer condition than in algal mats, despite both habitats were only a few hundred meters away and fully connected. We are not aware of studies showing such a small-scale spatial difference in condition, that suggests a restricted home range by this small-sized fish. The home range of another cyprinodontiform fish (*F. heteroclitus*) has been shown to be smaller (36 m) than usual for freshwater fish (Lotrich 1975). Immature fish from algal mats significantly had more food biomass than immatures from glasswort, so the poorer condition was due to lower ingestion rate of immatures in the latter habitat. This lower ingestion rate and poorer condition in glasswort suggests intraspecific competition. Rincón *et al.* (2002) showed in a mesocosm experiment that young stages of *Aphanius iberus* captured significantly less prey in the presence of adult conspecifics. Weisberg & Lotrich (1982) showed that crowding in newly colonized habitats may counteract the availability of unexploited resources.

The management of *Aphanius iberus* has mainly focused on the preservation of the few localities where it is still present. We have shown that different size groups of the toothcarp select different habitats and this affects ingestion rate and condition. Further data are needed to document the temporal dynamics and overall implications of these patterns for the persistence of this endangered cyprinodont. The functional role of flooded habitats in Mediterranean salt marshes is still poorly known but may have enormous implications for their management and the conservation of endangered species.

ACKNOWLEDGEMENTS

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Chapter III

Food of an endangered cyprinodont (*Aphanius iberus*): ontogenetic diet shift and prey electivity

ABSTRACT

We studied the ontogenetic diet shift and prey electivity of an endangered cyprinodontid fish endemic to the Iberian Peninsula, the Spanish toothcarp (*Aphanius iberus*). The toothcarp's diet was omnivorous, dominated by harpacticoid copepods (*Mesochra lilljeborgi* and *Tisbe longicornis*), copepod nauplii and detritus. Diet composition varied greatly among habitats, depending on prey availability. In a rarely inundated habitat (glasswort), there was more consumption of the isopod *Protracheoniscus occidentalis* and the harpacticoid copepod *Mesochra lilljeborgi*, while in algal mats another harpacticoid (*Tisbe longicornis*), chironomid dipterans and invertebrate eggs were more important in diet. Although a benthic feeding habitat has previously been suggested, in our study the diet was based rather on water column organisms for both glasswort and algal mat habitats. There was also an ontogenetic diet shift, with an increase of mean prey length with fish length, clearly linked to a microhabitat change. Smaller fish showed positive electivity and greater reliance on planktonic prey (e.g., copepod nauplii, the harpacticoid copepods *Mesochra lilljeborgi* and *Tisbe longicornis*, the rotifer *Brachionus plicatilis*, and ostracods), while larger fish elected and preyed on more benthic organisms (e.g., *Canuella perplexa*, *Mesochra rapiens*, and ephydrid dipterans).

Keywords

Cyprinodontidae, salt marsh, coastal lagoon, microhabitat, Iberian Peninsula.

* Alcaraz C. & García-Berthou E. 2007. *Environmental Biology of Fishes*, **78**, 193–207.

INTRODUCTION

The Spanish toothcarp (*Aphanius iberus*) is a cyprinodontid fish endemic to the Mediterranean coast of Spain and is considered in danger of extinction by the National Catalogue of Endangered Species and the Convention on the Conservation of European Wildlife and Natural Habitats (Bern Convention) (Doadrio 2001). It originally inhabited a wide range of lowland waters but nowadays its distribution is reduced to brackish and hypersaline waters in salt marshes and coastal lagoons. This is due to the impact of invasive species such as the poeciliid fish *Gambusia holbrooki*, habitat destruction by wetland desiccation, and water pollution (García-Berthou & Moreno-Amich 1992, Doadrio 2001, Rincón *et al.* 2002). The Atlantic populations of the Spanish toothcarp have recently been distinguished as a new species (*A. baeticus*) because of differences in morphometrics and genetics (Doadrio *et al.* 2002).

Although the Spanish toothcarp (*Aphanius iberus*) is one of the few protected fish species in Spain, 12 of the known 28 Mediterranean populations have disappeared and one is extinct in the wild with only a few individuals still preserved in captivity (Doadrio 2001). It is a small fish (total length usually < 5 cm), with external sexual dimorphism, short longevity (aged up to 2+) and strongly euryhaline and eurythermal (Fernández-Delgado *et al.* 1988, García-Berthou & Moreno-Amich 1992, Vargas & De Sostoa 1997). It reproduces from April to October, laying up to 900 eggs in successive spawns, and reaches sexual maturity in a few months (at a total length of less than 20 mm). The only published studies on *A. iberus* feeding ecology are Vargas & De Sostoa (1999) and experimental work by Rincón *et al.* (2002) and Caiola and De Sostoa (2005). Vargas & De Sostoa (1999) showed that the Spanish toothcarp in the Ebro river delta is omnivorous, with a diet composed of both animal prey (mainly benthic crustaceans such as harpacticoid copepods and amphipods) and plant debris and detritus. This population also showed a seasonal change in diet related to the hydrological cycle of the lagoon (Vargas & De Sostoa 1999). Rincón *et al.* (2002) and Caiola and De Sostoa (2005) studied the interactions of toothcarp and mosquitofish and found that small toothcarp captured less prey in the presence of adult conspecifics and adult mosquitofish. However, the role of resource availability in toothcarp feeding is largely unknown and there is no study on its prey electivity. In general, resource availability and electivity is often not considered in fish feeding studies, although without its measurement little can be said about niche overlap and niche breadth (Hurlbert 1978). The study of electivity is important in order to understand the response of the invertebrate community and the patterns of intraspecific and interspecific competition of predators (Gerking 1994: 249). Knowledge of the specific prey types consumed at different sites also allows a more mechanistic understanding of habitat selection (Nemerson & Able 2004).

In a previous study (Alcaraz *et al.*, submitted manuscript), we described the effect of habitat variation on the distribution and composition of the invertebrate community and the population structure of the Spanish toothcarp. The objectives of the present paper are (1) to analyze the effect of habitat variation on the Spanish toothcarp diet and, in particular, to assess the role of a rarely inundated habitat (glasswort); and (2) to study the toothcarp's prey electivity and its relationship with ontogeny and habitat variation. This is the second diet study of a natural population of this endangered toothcarp and the first measuring resource availability and electivity. Such data are essential to enhance its conservation programs. By simultaneously measuring diet and resource availability in the water column and the benthos, we also aim to assess its main feeding microhabitat.

METHODS

Study area

The study was undertaken in Fra Ramon, a coastal lagoon located behind the dune line of the Baix Empordà salt marshes (NE Spain) (Fig. 1). It is situated at 42º 1' 49'' N, 3º 11' 29'' E and 1.75 m below the sea level. The salinity ranges from 28 ppt (i.e. brackish) during rainfall precipitation to 49 ppt in summer (i.e. hypersaline) and the temperature ranges from 5 ºC to 30 ºC. It is eutrophic according to the concentrations of total nitrogen and chlorophyll but hypereutrophic according to total phosphorous (A. Badosa, unpublished data). There is no freshwater inflow to the lagoon and, as is usual in Mediterranean wetlands, no regular tidal influence, so water entries occur mainly through heavy rainfall and rough sea periods. The water area of the lagoon ranges from ca. 2,200 m^2 during the dry season (summer) to 13,000 m^2 in heavy rainfall periods (when the lagoon was sampled) and water level oscillates from -5 cm to 1 m above mean sea level (A. Badosa, unpublished data) (Fig. 1).

The permanently inundated zone of the lagoon presents dense submerged meadows of ditch-grass (*Ruppia cirrhosa*) and floating mats of green algae (*Enteromorpha* sp. and *Chaetomorpha* sp.). Algal mats are more abundant in summer (when high blooms occur) and rare in winter. The salt marsh is dominated by the *Puccinellio-Arthrocnemetum fruticosi* plant association. However, in the zone closest to the lagoon that is occasionally inundated there is the *Suaedo-Salicornietum patulae* plant association, dominated by glasswort (*Salicornia patula*) and other succulent plants (e.g. *Suaeda maritima* and *Suaeda splendens*). This habitat is only occasionally flooded, usually for about two months per year (Fig. 1). Only *Aphanius iberus* presents stable fish populations in the lagoon, although other species of marine origin, such as eel (*Anguilla anguilla*) and mullets (Mugilidae), are occasionally observed (Alcaraz *et al.*, pers. obs.). The Spanish toothcarp population was monitored once every two months or one year to estimate its abundance, population structure, and habitat use. Our study was undertaken during one of the rare periods of flooding to examine whether the toothcarp used infrequently inundated habitats and assess the differences among habitats.

Field and laboratory methods

The lagoon was sampled on the mornings of 21-22 November 2002, during a period of flooding caused by heavy rainfall. We sampled three different habitats: “glasswort”, corresponding to the *Suaedo-Salicornietum patulae* plant association that is rarely inundated; “algal mats”, dominated by *Enteromorpha* sp. and *Chaetomorpha* sp. and mostly present close to the littoral; and “open water”, unvegetated habitat that dominates most of the lagoon. At the sampling sites, these three habitats were 14, 37 and 33 cm deep respectively. In each of the habitats we sampled organisms in the water column, the benthos (in both cases making two replicates per habitat), and fish (Fig. 1). Zooplankton and macroinvertebrates in the water column were sampled with a 1 m long transect of 50 μm mesh dip net (volume sampled = 0.038 m^3). All the samples were immediately preserved in 10% formalin. All the material collected, including algal mats when present, was included in the samples and invertebrates were sorted later in the laboratory under a dissecting microscope. Benthic organisms were sampled with a 15 × 15 cm benthic grab and frozen in-situ with a portable freezer. In the laboratory, the benthos samples were unfrozen and sifted with a 0.1 mm sieve. All the organisms were then fixed in 10% formalin.

Organisms were usually identified to the genus or species level, except in the cases of Nematoda and Ostracoda. All organisms were counted and a minimum of 20-30 individuals (if available) of each taxon were measured under a dissecting microscope with an ocular micrometer. The measurements were converted to dry mass according to published length-dry mass relationships (mainly Dumont *et al.* 1975, Smock 1980).

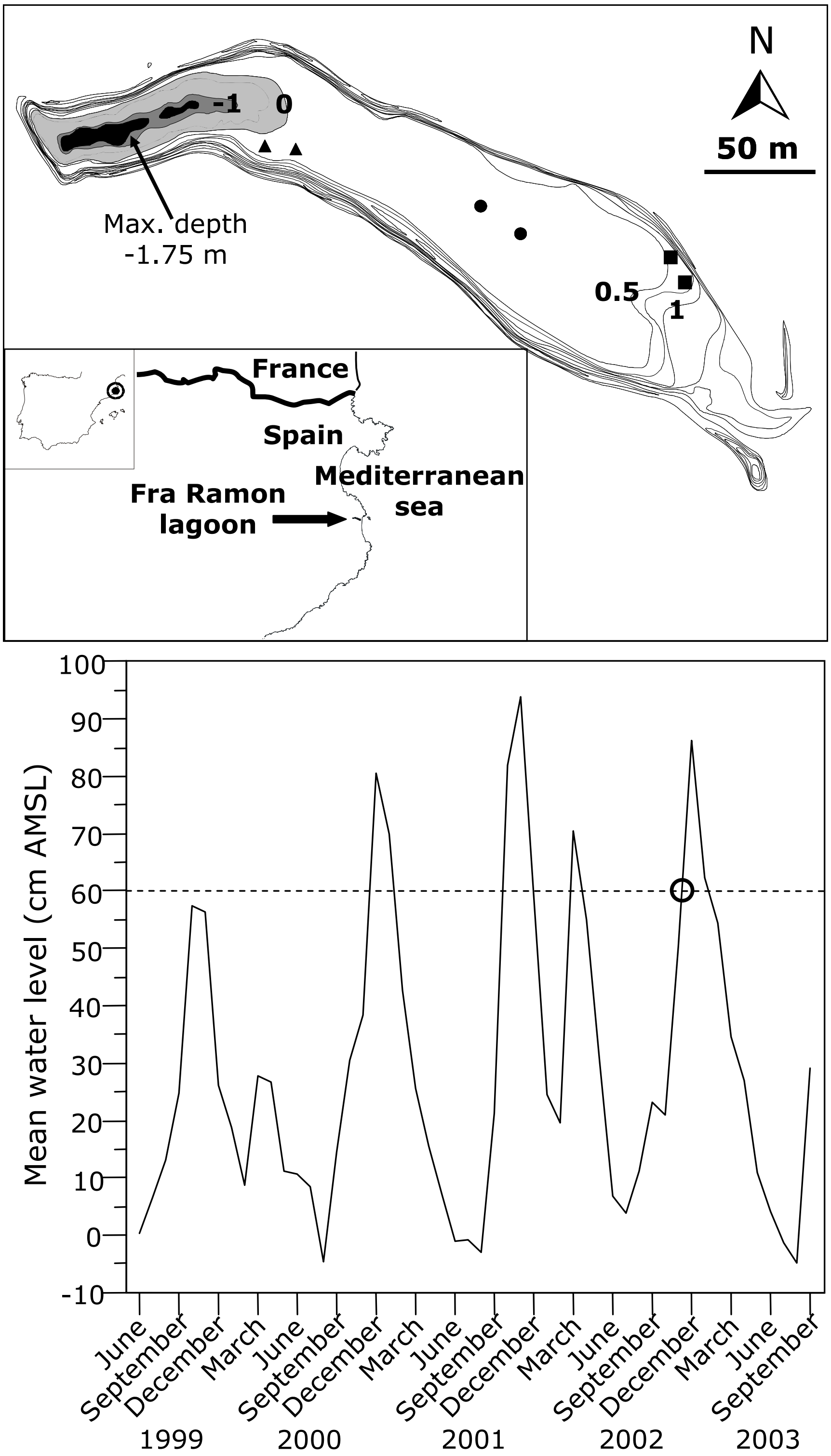

Figure 1. Top, location of the study area and map of the Fra Ramon lagoon with the main sampling points per habitat (■ = Glasswort, ● = Open water, and ▲ = Algal mats); depths are meters above mean sea level. Bottom, mean water level (in cm above mean sea level) in the lagoon from 1999 to 2003; dashed line shows the water level above which glasswort is flooded and available for fish and the circle marks the sampling date.

The fish sampling technique consisted of quickly throwing a 1 m high cylinder of 90 cm in diameter and then removing all the fish with a dip net. The mean depth of each sampling site was measured and the fish captured were expressed as densities (fish l^{-1}). Although this sampling technique may underestimate total density, it allows abundance comparison among habitats. Fish were sampled with 38 throws of the cylinder, systematically distributed troughout the lagoon, and habitat was then characterized. The number of sampled points thus resulted in 9 in glasswort, 9 in open water and 20 in algal mats, due to the dense cover of the latter habitat in the lagoon. Fish were frozen in-situ with a portable freezer to avoid digestion of the stomach content. In the laboratory, all individuals were measured (standard and total lengths to the nearest 0.5 mm), eviscerated, sexed (generally from external sexual dimorphism), and weighed (total and eviscerated weight to the nearest 0.1 mg). The entire gut was preserved in 10% formalin until analysis. The entire gut contents of the toothcarp were examined under a dissecting microscope; in the largest samples a subsample of 31 (glasswort) and 26 (algal mats) fish were examined. All the prey present in the gut were sorted, usually to the species or genus level, counted, and a minimum of 20-30 individuals (if available) of each prey taxon were measured with an ocular micrometer. As for the water and benthos samples, the measurements were converted to dry mass according to published length-dry mass relationships. The volume of uncountable categories (detritus, plant debris, plant leaves, invertebrate eggs and digested material) in the gut contents was estimated to the nearest 0.00025 mm^3 with a Neubauer counting chamber (haemacytometer) as in García-Berthou (1999) and the volume later transformed to biomass using a conversion of 0.27 mg of dry weight / mm^3 (Dumont *et al.* 1975, Lindegaard 1992).

Data analysis

Differences in density among habitats and mean length were analyzed with analysis of variance (ANOVA), followed by Games-Howell post-hoc tests (hereafter, GH tests). GH tests are among the most powerful and most robust to unequal variances of post-hoc multiple comparison methods (Day & Quinn 1989). The frequencies of sex categories among habitats were compared with a *G*-test of independence.

The analysis of diet data follows our previous work (García-Berthou 1999, García-Berthou & Moreno-Amich 2000, García-Berthou 2001). Percent number (% number), percent biomass (% biomass), and frequency of occurrence were used to estimate the dietary importance of each food category. Percent number is the number of individuals of a prey type divided by the total number of individuals and expressed as a percentage, after pooling the gut contents of all fish. Percent biomass is the equivalent measure for biomass data. Frequency of occurrence is the percentage of guts in which a food category was present. To describe prey importance and feeding strategy, Costello's (1990) graphical method was used, i.e. a plot of % number or % biomass versus frequency of occurrence. The most important prey are closer to the top right corner. The other diagonal corresponds to feeding strategy: prey with low occurrence but important by number or biomass correspond to some sort of specialization and are closer to the top left corner. Diet diversity (for each fish) was measured with the complement of Simpson's index (*D*), calculated as

$$D = 1 - \sum_i \frac{n_i(n_i - 1)}{N(N - 1)}$$

where n_i is the number of individuals of prey type i, and *N* is the total number of prey (Hurlbert 1971).

Analysis of covariance (ANCOVA) was also used to compare variables (e.g. fish weight or prey length) among sex groups and habitats, using fish length as the covariate. We started with the most complex model, introducing all possible interactions (including interactions of covariates × factors, following García-Berthou & Moreno-Amich (1993)). We then simplified the general linear model by removing nonsignificant interactions ($p >> 0.10$) to increase the statistical power of the remaining sources of variation, which would otherwise be seriously compromised. When the covariate was not significant ($p > 0.10$) it was also deleted from the model (so an ANOVA was used). Quantitative variables were log-transformed for the analyses because homoscedasticity and linearity were clearly improved. Open water was omitted for most analyses because only three fish were captured in that habitat.

All factors were considered fixed effect factors. We used partial η^2 (partial eta squared) as a measure of effect size (i.e. importance of factors). Similarly to r^2, partial η^2 is the proportion of variation explained for a certain effect (effect SS / effect SS + error SS). Partial η^2 has an advantage over eta squared (effect SS / total SS) in that it does not depend on the number of sources variation in the ANOVA design used, because it does not use the total sum-of-squares (SS) as the denominator (Tabachnick & Fidell 2001: 191).

Following Lechowicz (1982), comparison between diet composition and resource availability was made using Vanderploeg & Scavia's (1979) relativized electivity index (E^*):

$$E_i^* = \frac{[W_i - (1/n)]}{[W_i + (1/n)]}, \quad \text{where} \quad W_i = \frac{r_i / p_i}{\sum r_i / p_i},$$

r_i is the relative (proportional) abundance of prey *i* in the diet, p_i is the relative abundance of prey *i* in the environment and *n* is the number of prey types included in the analysis. This index ranges from +1 (positive selection) to -1 (negative selection) and values near zero indicate neutral selectivity. Although other electivity indices such as Ivlev's or Strauss's are more widely used, these have several undesirable properties and Vanderploeg and Scavia's is the single best electivity index (Lechowicz 1982). The relative abundances of prey in the environment (p_i) were computed as the average of the proportions in the zooplankton and benthos samples; however, similar results were obtained when the electivity was analyzed separately for zooplankton and benthos data. The E^* index was arcsine transformed (arcsine $\sqrt{[(E_i^* + 1) / 2]}$) for statistical analysis, because homoscedasticity and normality were clearly improved. To test whether electivity significantly deviated from 0, a one-sample Student's *t*-test was used. Electivity was further analyzed with ANCOVA (see above), using fish length as the covariate. All statistical analyses were performed with SPSS 12 for Windows.

RESULTS

Habitat use and resource availability

As in previous studies, the toothcarp population size structure was bimodal (Fig. 2) and composed of two groups: immature young-of-the-year (0+) born in summer and mature fish (0+, born mostly in spring, and 1+). Toothcarp density differed significantly in the three habitats ($F_{2, 35} = 14.87$, $P < 0.001$). Only three fish were captured in open water, and density was also higher in glasswort than in algal mats (GH test, $P < 0.05$). However, the significantly higher density in glasswort was only significant for mature fish ($F_{1, 27} = 22.82$, $P < 0.001$) and not for immature fish ($F_{1, 27} = 0.93$, $P = 0.34$), showing size-specific differences in habitat use (Fig. 2).

Mean standard length (SL) of the toothcarp was 18.4 mm (SE = 3.9, range 7 – 30). Fish were larger in glasswort (mean =19.1 mm, range 10-30) than in algal mats (15.6 mm, range 7-25) ($F_{1, 13} = 9.02$, $P = 0.10$) (Fig. 2). This difference was due to a higher proportion of immature fish in algal mats ($G = 54.6$, df = 4, $P < 0.001$), since 52% of the individuals in algal mats were immature compared with only 10% in glasswort (Fig. 2). Taking mature fish alone, although females (mean = 21.2 mm, range 16.5 – 30) were larger than males (19.4 mm, range 14.5 – 26) ($F_{1, 17} = 9.71$, $P = 0.006$) due to sexual dimorphism, habitat produced no significant effect on standard length ($F_{1, 17} = 0.50$, $P = 0.49$) or on sex group composition ($G =$

0.82, df = 2, $P = 0.66$) (Fig. 2). Sex ratio significantly differed from 1 : 1 ($G = 45.7$, df = 1, $P < 0.001$), males (71.5%) being more abundant than females (28.5%).

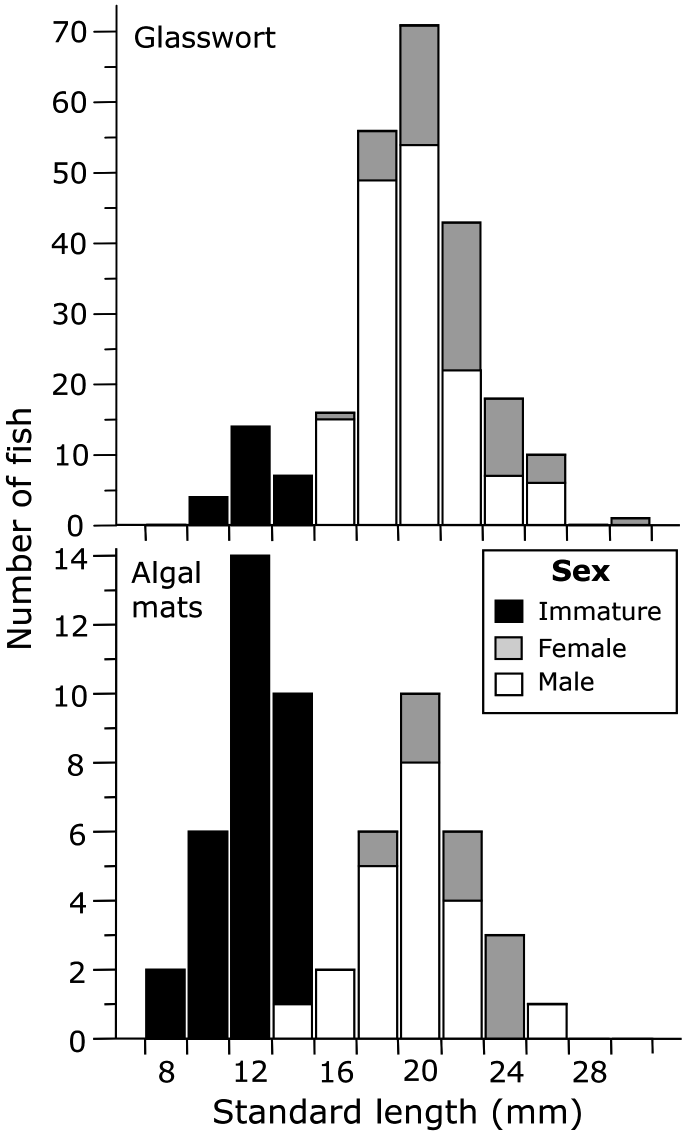

Figure 2. Length-frequency distribution of the toothcarp in Fra Ramon lagoon by habitat (top, glasswort; bottom, algal mats) and sex category (immature, female and male). The open water habitat is omitted because only three fish were captured.

The spatial heterogeneity of benthos and plankton is analyzed in detail elsewhere (Alcaraz *et al.*, submitted manuscript) and briefly summarized herein. The density of organisms (ANOVA, $F_{2,3} = 117.6$, $P = 0.001$) and total dry biomass ($F_{2,3} = 10.41$, $P = 0.045$)

of water column invertebrates showed significant differences among habitats. In both cases, the algal mat habitat presented higher densities of organisms. While density of invertebrates in glasswort was lower than in open water, dry biomass was higher because open water contained only smaller organisms (mainly copepod nauplii and rotifers). In glasswort and algal mat habitats, the water column was dominated in number by small organisms, mainly copepod nauplii, harpacticoids and, in algal mats, rotifers. By biomass the result differed and while algal mats were dominated by amphipods (Gammarus aequicauda) and gastropods (Hydrobia sp.), glasswort was dominated by gastropods (*Hydrobia* sp.) and dipterans (mostly Stratiomyidae).

Total dry biomass of the zoobenthos also differed significantly among habitats ($F_{2,\,3}$ = 10.51, P = 0.044), being lowest in algal mats, but not the total density of organisms ($F_{2,\,3}$ = 1.32, P = 0.39). The three habitats presented a high abundance of harpacticoids (mainly *Mesochra lilljeborgi* in glasswort and *Canuella perplexa* in open water and algal mats) but open water and algal mats were clearly dominated by ostracods. By biomass, the most important taxa were chironomid larvae (in all three habitats) and ostracods in open water and algal mats.

Ontogenetic and habitat variation in diet

The toothcarp diet was mostly based on the harpacticoids copepods *Mesochra lilljeborgi* and *Tisbe longicornis*, copepod nauplii and detritus (Table 1 and Fig. 3). The relationship between number, biomass, and occurrence (Fig. 3) pointed to some feeding variation among individuals. First, some prey were important by biomass but with low occurrence, particularly the semiaquatic isopod (*Protracheoniscus occidentalis*) but also the amphipod *Gammarus aequicauda*, rhagionid dipterans and hymenopterans. These were the largest prey, dominating in only a few large fish (see below). Chironomid larvae were more important by biomass and invertebrate eggs by number and both were eaten more in algal mats than in glasswort (Fig. 4).

The relative importance of prey eaten was highly dependent on habitat and toothcarp size (Fig. 4). The harpacticoid copepod *Mesochra lilljeborgi* was important for small (juvenile or immature) fish in glasswort, but consumed less in algal mats, where it was replaced by chironomid larvae and invertebrate eggs. The isopod *Protracheoniscus occidentalis* was very dominant in larger toothcarp in glasswort, but replaced by chironomids and other dipterans (mainly rhagionids) in algal mats (Fig. 4). Detritus was important for toothcarp present in both glasswort and algal mats; its importance significantly decreased with size in glasswort but increased in algal mats.

Table 1. Diet of the Spanish toothcarp in Fra Ramon lagoon: % number, % biomass, and frequency of occurrence of the main food components.

Food category	**% number**	**% biomass**	**Frequency of occurrence**
Detritus	–	24.69	67.74
Plant debris	–	0.07	14.52
Plant leaves	–	0.10	3.23
Brachionus plicatilis	0.21	0.02	12.90
Testudinella clypeata	0.01	0.00	1.61
Nereis diversicolor	0.20	1.46	24.19
Unid. Acari	0.01	0.15	1.61
Unid. Ostracoda	0.37	0.05	32.26
Mesochra lilljeborgi	61.24	5.11	93.55
Mesochra rapiens	0.04	0.01	4.84
Tisbe longicornis	15.20	4.63	82.26
Canuella perplexa	0.27	0.47	8.06
Copepoda nauplii	5.84	0.05	91.94
Gammarus aequicauda	0.02	3.59	3.23
Protracheoniscus occidentalis	0.18	39.13	11.29
Unid. Crustacea	0.01	0.05	1.61
Chironomidae (l.)	0.48	14.34	41.94
Stratiomyidae (l.)	0.05	0.35	3.23
Stratiomyidae (ex.)	0.01	0.00	1.61
Rhagionidae (l.)	0.02	2.11	3.23
Ephydridae (l.)	0.03	0.18	4.84
Ephydridae (ex.)	0.01	0.00	1.61
Unid. Hymenoptera (a.)	0.02	1.14	3.23
Invertebrate eggs	15.68	2.32	46.77
Terrestrial snails	0.12	0.00	16.13

Number of guts analyzed = 62; total number of prey in the gut contents = 10,839; total biomass = 42.454 mg., l. = larvae, ex. = exuviae, a. = adult, unid. = unidentified.

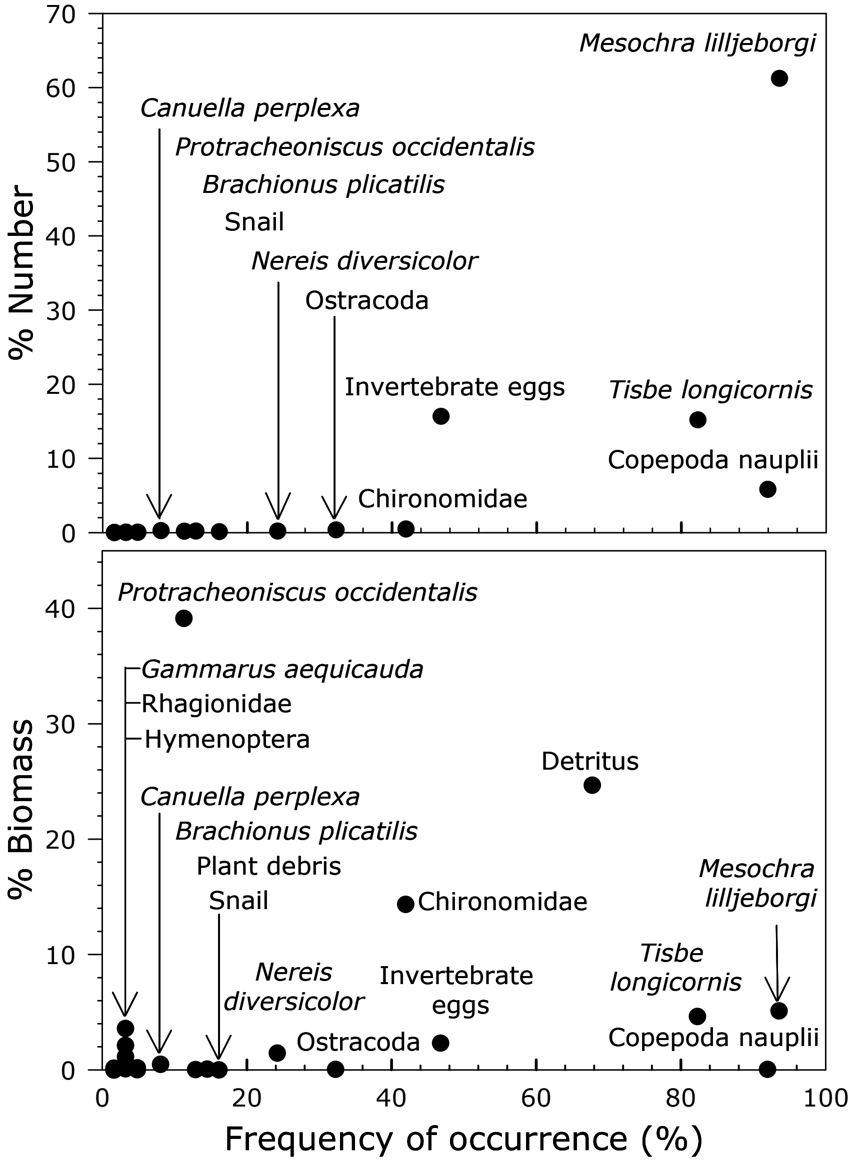

Figure 3. Relationship among % number, % biomass, and frequency of occurrence of food categories in the Spanish toothcarp diet. Plots based on Costello's (1990) method (see Methods). Food categories described are the items most important in diet.

Despite the ontogenetic variation in diet, the overall diversity of the toothcarp's prey did not significantly depend on length (ANCOVA, $F_{1,55} = 0.47$, $P = 0.50$) but it was significantly higher in algal mats ($D = 0.40 \pm SD = 0.18$) (ANOVA, $F_{1,56} = 4.96$, $P = 0.030$) than in glasswort ($D = 0.31 \pm SD = 0.16$). Females ($D = 0.40 \pm SD = 0.16$) presented a marginally higher diversity than males ($D = 0.32 \pm SD = 0.17$) ($F_{1,42} = 3.92$, $P = 0.054$). In contrast, the number of different prey items in gut contents did not show any significant relationship with standard length (ANCOVA, $F_{1,55} = 1.94$, $P = 0.17$) or depend on habitat (ANOVA, $F_{1,56} = 0.98$, $P = 0.33$).

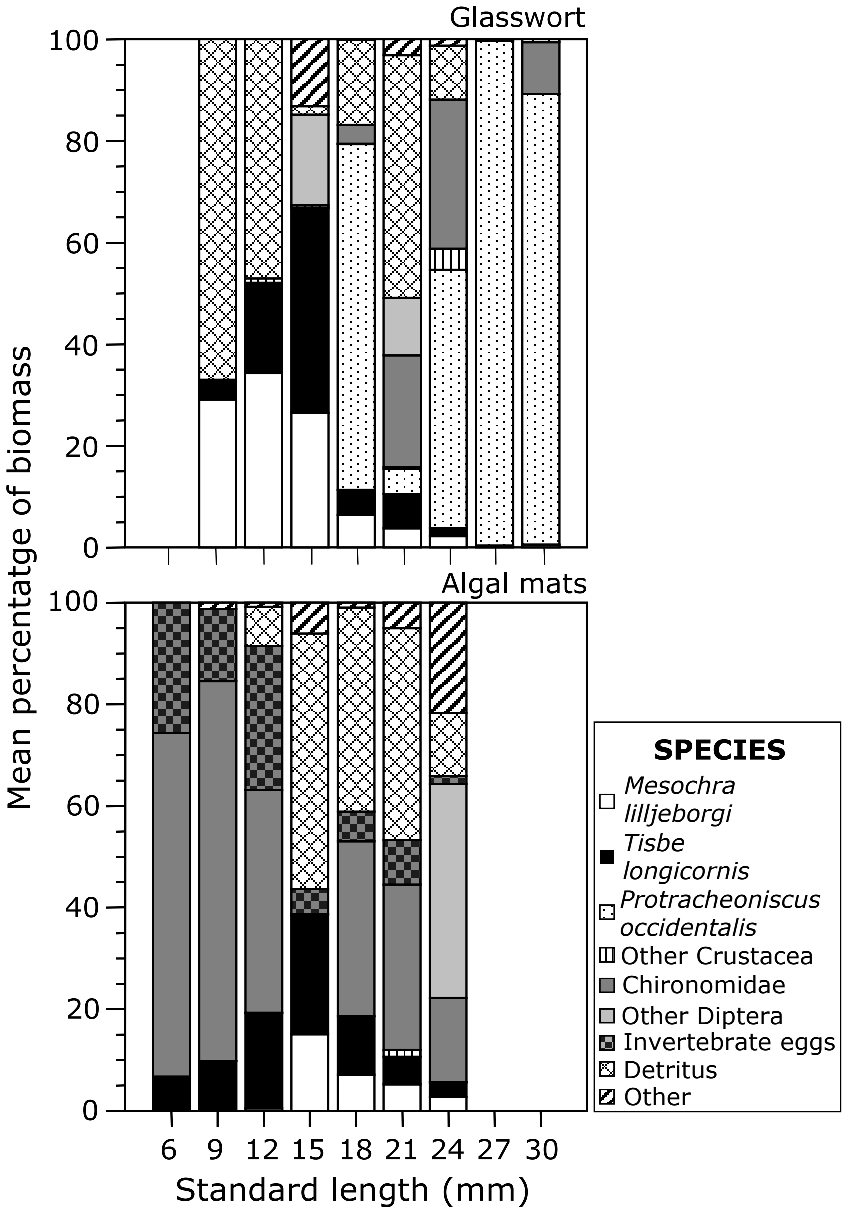

Figure 4. Ontogenetic and habitat variation in the diet of the toothcarp. Data are the percentage of gut content biomass, averaged for 3 mm-length classes of toothcarp.

The mean length of prey captured increased significantly with the standard length of the toothcarp (ANCOVA, $F_{1,\ 55} = 10.9$, $P = 0.002$), but only in glasswort and not in algal mat habitat (habitat × SL interaction, $F_{1,\ 55} = 4.53$, $P = 0.038$) (Fig. 5). Total biomass of gut contents also depended significantly on fish size (ANCOVA, $F_{1,\ 55} = 87.38$, $P < 0.001$), habitat ($F_{1,\ 55} = 11.13$, $P = 0.002$), and their interaction ($F_{1,\ 55} = 9.01$, $P = 0.004$). Food biomass in the gut was higher in algal mats than in glasswort, after accounting for fish size, but only for small, immature fish (Fig. 6).

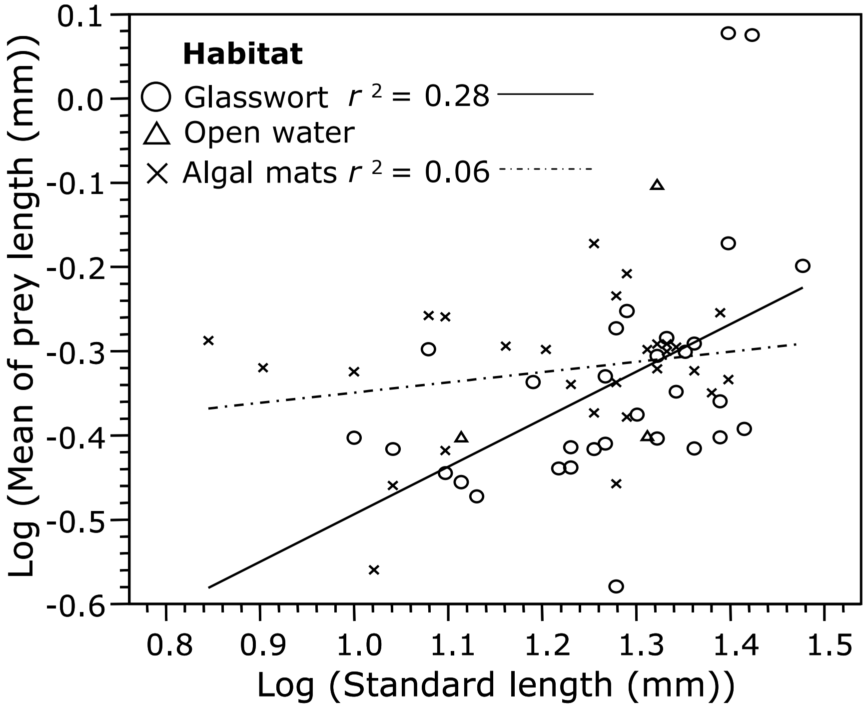

Figure 5. Relationship of mean prey length in the gut contents with standard length of toothcarp by habitat. The linear regression functions are also shown.

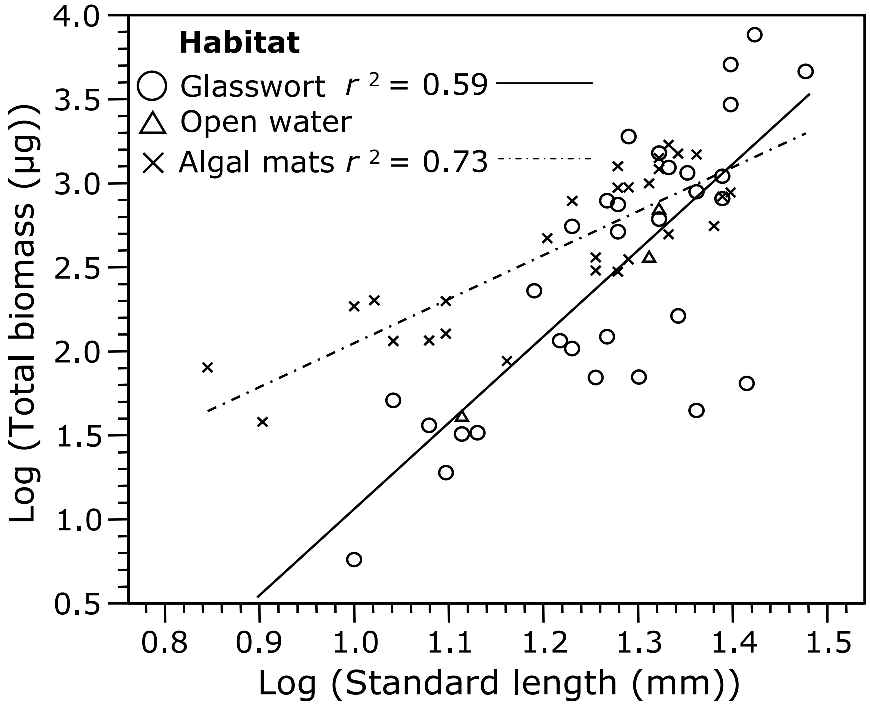

Figure 6. Relationship of total food biomass in the gut contents with standard length of toothcarp by habitat. The linear regression functions are also shown.

Habitat (partial $\eta^2 = 0.088$) was more important than fish size (partial $\eta^2 = 0.013$) in explaining variation of prey diversity and composition in the toothcarp's diet; whereas for mean prey length and total food biomass, standard length (partial $\eta^2 = 0.17$ and 0.61 respectively) was more important than habitat (partial $\eta^2 = 0.084$ and 0.17 respectively).

Toothcarp electivity

Mature toothcarp showed significant positive electivity (*t*–tests, $P < 0.05$) for the harpa cticoid copepod *Mesochra lilljeborgi*, rhagionid dipterans and the polychaete *Nereis diversicolor*, and significant negative electivity for copepods nauplii, the rotifer *Brachionus plicatilis*, ostracods, the harpacticoids *Mesochra rapiens* and *Canuella perplexa*, chironomids, and stratiomyid dipterans (Fig. 7). For the remaining categories (e.g., *Tisbe longicornis*, snail, ephydrid dipterans, hymenopterans, *Protracheoniscus occidentalis*, and *Gammarus aequicauda*) electivity did not significantly deviate from zero ($P > 0.10$) (Fig. 7). Immature toothcarp selected positively for *Tisbe longicornis*, while copepod nauplii and ostracods were negatively selected (Fig. 7). Electivity could not be statistically tested for some prey that only appeared in a single fish, but it was clearly positive for water mites (Acari) and terrestrial snails due to lower relative abundance in the water, while it was negative for the rotifer *Testudinella clypeata* and exuviae of ephydrid and stratiomyid dipterans, which were widely available (Fig. 7).

Furthermore, electivity of prey depended significantly on standard length for *Tisbe longicornis* ($F_{1,46} = 18.4$, $P < 0.001$), chironomids ($F_{1,21} = 29.9$, $P < 0.001$), copepod nauplii ($F_{1,51} = 10.6$, $P = 0.002$), ostracods ($F_{1,15} = 4.49$, $P = 0.051$) and snails ($F_{1,7} = 5.32$, $P = 0.054$) (see e.g. Fig. 8). The remaining categories were not significantly related to standard length ($P >> 0.10$). In general, electivity for many small prey was positive in small fish and negative in large fish.

Electivity also showed differences among habitats. Prey such as copepod nauplii ($F_{1,51} = 4.78$, $P = 0.033$), *Tisbe longicornis* ($F_{1,46} = 4.65$, $P = 0.036$), terrestrial snails ($F_{1,7} = 6.47$, $P = 0.038$), and *Nereis diversicolor* ($F_{1,11} = 31.2$, $P < 0.001$) showed higher electivity in glasswort than in algal mats, while *Mesochra lilljeborgi* ($F_{1,52} = 25.6$, $P < 0.001$) and chironomids ($F_{1,21} = 9.84$, $P = 0.005$) were more selected in algal mats. Furthermore, prey such as *Testudinella clypeata* and water mites only appeared in toothcarp from algal mats, while *Mesochra rapiens*, larvae and exuviae of ephydrids and stratiomyids, *Protracheoniscus occidentalis*, and *Gammarus aequicauda* were only present in fish from glasswort.

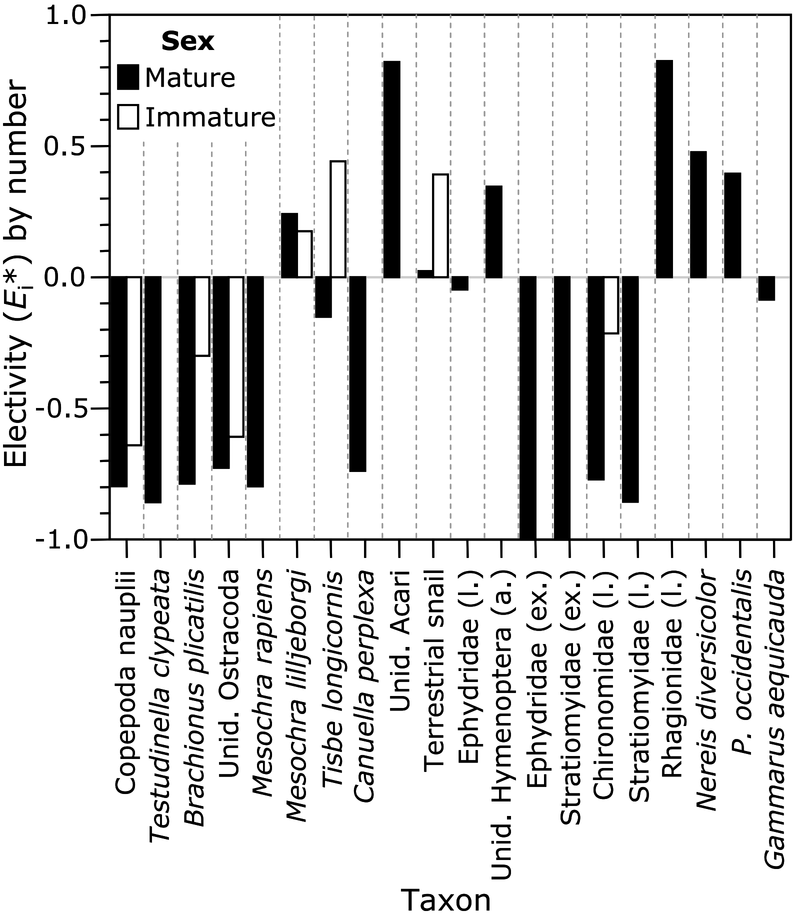

Figure 7. Electivity (Vanderploeg and Scavia's selectivity coefficient) in number by habitat for immature and mature Spanish toothcarp. The different prey items are ordered by species mean length in the lagoon. l. = larvae, ex. = exuviae, a. = adult, unid. = unidentified.

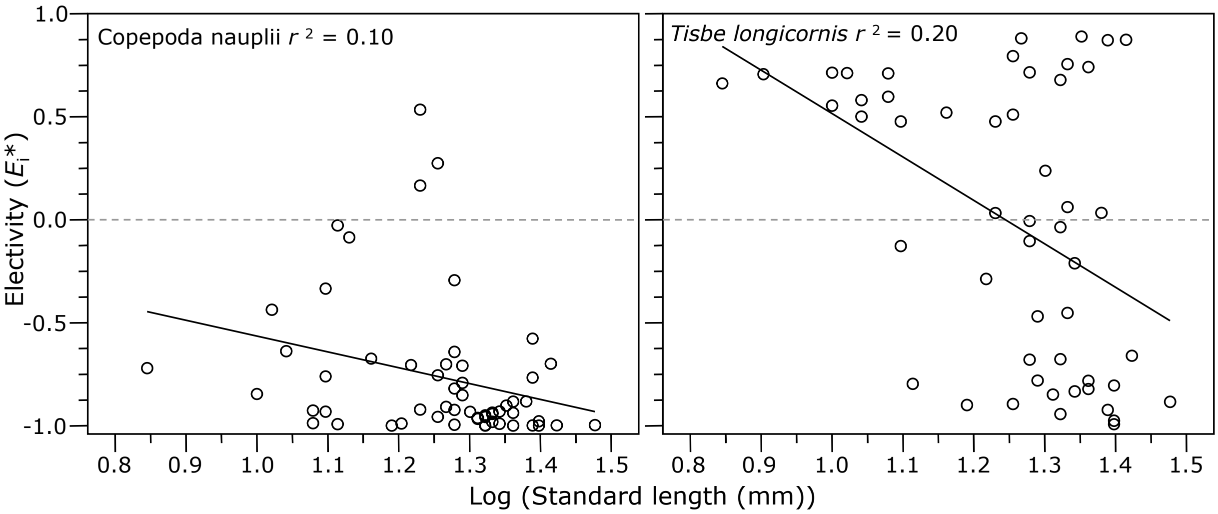

Figure 8. Relationship of electivity with standard length of toothcarp for copepod nauplii (left) and the harpacticoid copepod *Tisbe longicornis* (right).

Microhabitat use and toothcarp feeding

Although some taxa were very abundant in both the water column and the benthos (e.g. *Mesochra lilljeborgi*, *Tisbe longicornis* or copepod nauplii), abundances in both microhabitats were not significantly related (Spearman's $r_s = 0.33$, $N = 20$, $P = 0.16$, by % number and $r_s = 0.26$, $N = 20$, $P = 0.26$ by % biomass) because some taxa were abundant in the water column but not in the benthos (e.g. *Testudinella clypeata*, *Gammarus aequicauda* or stratiomyids dipterans) and vice versa (e.g. *Mesochra rapiens*, snails or ephydrid dipterans) (Fig. 9). This relationship (see caption to Fig. 9) allows us to distinguish objectively between planktonic prey (e.g. rotifers such as *Brachionus plicatilis* and *Testudinella clypeata*, *Tisbe longicornis*) and benthic prey (e.g. *Mesochra rapines*, ephydrid dipterans) and thus examine whether the toothcarp feeds more from the water column or from the benthos.

In glasswort, the importance of prey in diet was positively correlated with percent abundance in the water column ($r_s = 0.54$, $N = 18$, $P = 0.021$) but not with abundance in the benthos ($r_s = 0.33$, $N = 18$, $P = 0.18$), because some typically planktonic taxa (e.g. copepod nauplii) are very important in the toothcarp diet. In algal mats, percent number of prey categories was correlated with percent number in both the water column ($r_s = 0.64$, $N = 13$, $P = 0.020$) and the benthos ($r_s = 0.68$, $N = 13$, $P = 0.011$). By percent biomass, importance in diet was correlated only with percent biomass in the water column for both glasswort ($r_s = 0.52$, $N = 18$, $P = 0.028$) and algal mats ($r_s = 0.49$, $N = 13$, $P = 0.090$).

The ontogenetic diet shift previously described (Fig. 4) was clearly related to variation in microhabitat use. Small fish showed a preference for feeding on plankton or general food prey (organisms present in both the water column and the benthos), whereas larger fish fed on benthic prey, such as ephydrid and stratiomyid dipterans or the harpacticoid copepod *Canuella perplexa* or *Mesochra rapines* (Fig. 9). This ontogenetic shift in microhabitat and diet was clearer in algal mats than in glasswort (Fig. 4), as expected, given the shallowness of the latter habitat.

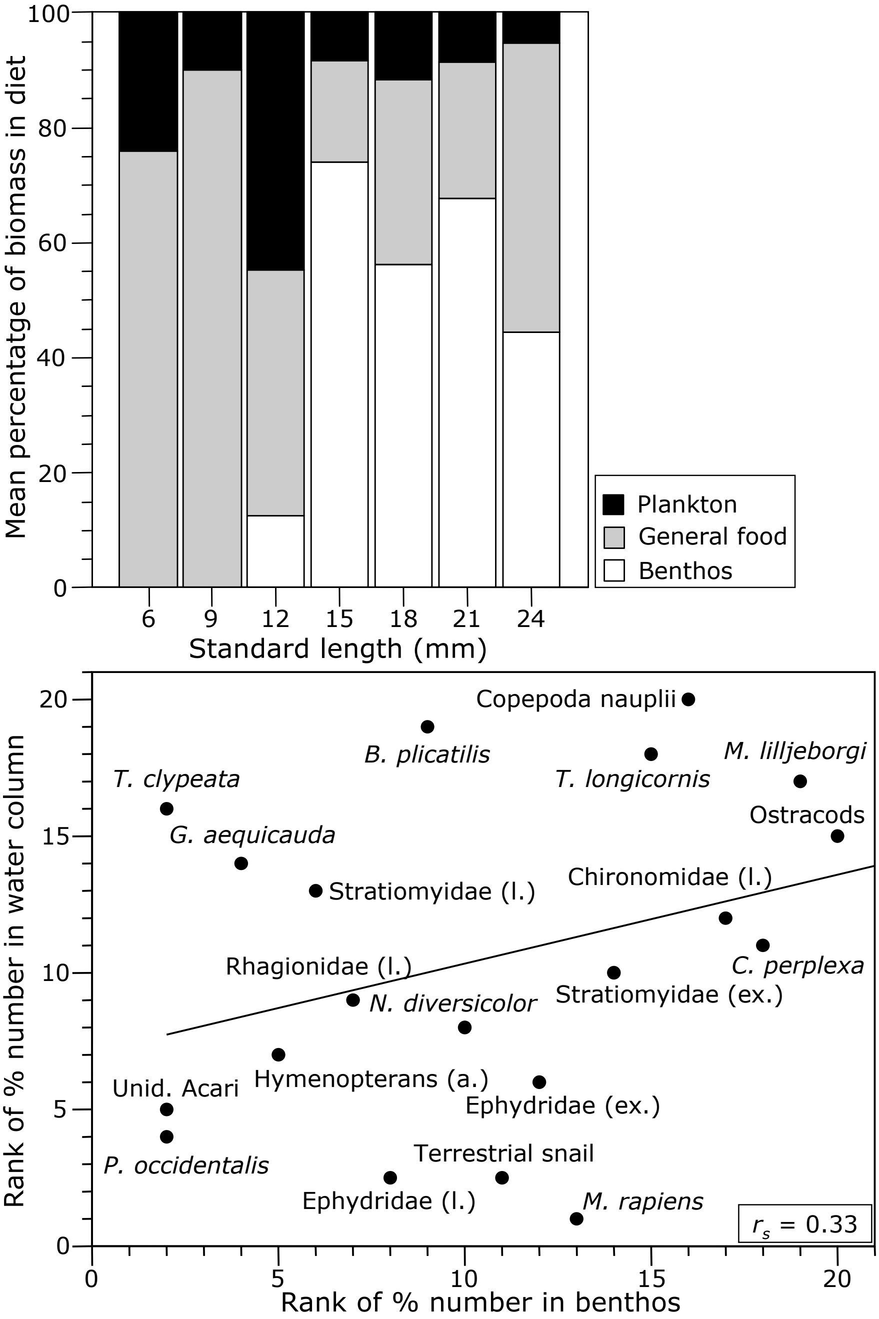

Figure 9. Discrimination of planktonic and benthic prey (bottom) and relative importance of both with ontogeny in the toothcarp's diet (top). The figure at the bottom shows the relationship of the abundance (% number) of invertebrates in the water column and the benthos (rank transformed, in ascending order, because of strong asymmetry and nonlinearity). Species on top were the most abundant in the water column, while species on the right were the most abundant in the benthos. A larger distance from the linear regression function indicates taxa disproportionately more abundant in the water column (positive residuals) or the benthos (negative residuals). The figure at the top shows the relative importance of plankton and benthos in the toothcarp's diet in the algal mat habitat. Data are biomass proportion of planktonic (prey with standardized residual ≥ 1 in the bottom figure), benthic (residual ≤ − 1), and general food (not discriminating, present in both the water column and the benthos) in gut contents, averaged for 3 mm-length classes of toothcarp.

DISCUSSION

Toothcarp's diet

The most common prey, by number and occurrence, in the toothcarp's diet were microcrustaceans, particularly harpacticoid copepods (*Mesochra lilljeborgi* and *Tisbe longicornis*). There was an ontogenetic diet shift, as well as among-habitat variation in diet, as a result of different prey availability (e.g. the presence of the isopod *Protracheoniscus occidentalis* on glasswort, or the higher abundance of chironomids on algal mats habitat). The only field study on the diet of *Aphanius iberus* is the study carried out in the Ebro delta (Vargas 1993, Vargas & De Sostoa 1999), which did not measure prey availability. The main difference between our study (hereafter Fra Ramon) and the Ebro delta population was in the occurrence and relative importance of different prey. Harpacticoid copepods were the most important prey by number in both populations, with a similar percentage in number but a higher frequency of occurrence in Fra Ramon (> 90%) than in the Ebro (36 % overall, and less important in autumn and winter), while amphipods were more abundant in the Ebro (15% in number and 36% occurrence on average, more in autumn and winter). Ostracods and chironomids presented similar occurrence in both populations but were more important by number in the Ebro population, while polychaete were more important in Fra Ramon. These differences were probably due to differences in resource availability. Percentage of guts with detritus or plant material was very similar in both populations (69% in the Ebro and 68% in Fra Ramon). By contrast, the number of empty guts in the Ebro population was very high (50%) and the mean number of prey per gut very low (7.8), whereas in Fra Ramon no gut was absolutely empty and the mean number of prey per gut was 174.8. These differences may be due to much larger prey sizes or methodological differences in the Ebro study.

The Spanish toothcarp's diet, combining prey of both animal (mainly crustaceans) and plant or detritic origin, is similar to that of other *Aphanius* species (Al-Daham *et al.* 1978, Haas 1982, Al-Akel *et al.* 1987) and other cyprinodontiform fish such as *Fundulus luciae* (Kneib 1978). However, *A. dispar*, *A. mento*, and *A. sophiae* presented a more herbivorous diet (Al-Daham *et al.* 1978, Haas 1982, Al-Akel *et al.* 1987), although under experimental conditions *A. mento* ate plants but *A. dispar* and *A. sophiae* preferred animal food (Al-Daham *et al.* 1978, Haas 1982). By contrast, another Spanish endemic and threatened cyprinodontiform (*Valencia hispanica*) showed a diet based only on invertebrate prey, mainly amphipods (Caiola *et al.* 2001). Two introduced species competing with the Spanish endemic cyprinodontiforms present a similar diet, with *Fundulus heteroclitus* also preferring harpacticoid copepods (Kneib 1986) and *Gambusia holbrooki* being zooplanktivorous and consuming cladocerans, ostracods and copepods (Crivelli & Boy 1987, García-Berthou 1999).

Habitat variation in diet

The toothcarp diet presented significant differences among habitats. Habitat was more important than fish size (power analysis, partial eta squared) in explaining the variation of diversity and prey composition on toothcarp guts. There was higher diet diversity in algal mats and immature fish presented more biomass in gut contents here than in glasswort. In glasswort, larger toothcarp captured larger prey and there was a closer relationship between mean prey length and fish length. Furthermore, glasswort toothcarp fed on larger prey than those in algal mats. Similarly, *F. heteroclitus* showed positive electivity for larger prey in the presence of less dense vegetation (Vince *et al.* 1976).

Immature fish were relatively more abundant in algal mats habitats, where they had more gut biomass, and they were also in better condition (Alcaraz *et al.*, submitted manuscript). Rincón *et al.* (2002) showed in a mesocosm experiment that young stages of *A. iberus* captured significantly less prey in the presence of adult conspecifics. Our results point to interactive segregation in habitat use among size classes of toothcarp.

Food electivity and ontogenetic diet shift

Toothcarp presented significant positive electivity for a reduced number of prey. Electivity depended on prey availability and fish size. Overall, smaller fish positively selected small prey such as harpacticoid copepods (*Mesochra lilljeborgi* and *Tisbe longicornis*), while larger fish showed a greater preference for diverse larger prey (e.g. rhagionids dipterans, *Nereis diversicolor* and *Protracheoniscus occidentalis*). The positive electivity for terrestrial or semi aquatic prey, such as the terrestrial snail, the isopod *Protracheoniscus occidentalis* and hymenopterans, suggests the importance of these large, visible prey as a food resource for the toothcarp.

In Fra Ramon, the toothcarp diet was diverse but displayed an ontogenetic shift from harpacticoid copepods to larger prey (isopods and dipterans larvae). Standard length was a more important factor than habitat (power analysis) for explaining differences among mean prey length and total gut biomass. Mean prey length increased with fish length in glasswort, where harpacticoid copepods and detritus were very important for small fish, but were replaced by chironomids and mainly isopods as the fish grew in length. In algal mats, smaller fish consumed fewer harpacticoids, preferring chironomids and invertebrate eggs, and the importance of detritus and other dipterans increased with fish length. A similar ontogenetic diet shift was shown by Vargas (1993): in autumn, the smallest fish showed a preference for chironomids, increasing the abundance in their diet of other prey such as harpacticoids copepods as they grew in size (Vargas 1993).

In Fra Ramon the ontogenetic diet shift in glasswort toothcarp involved other prey items, very different from those of algal mats or the Ebro population, demonstrating the importance of habitat variation and prey availability in dietary studies. In glasswort, harpacticoids copepods were more abundant than they were in algal mats (Alcaraz *et al.*, submitted manuscript) being consumed more by small fish, and progressively replaced by larger prey such as dipterans or the isopod *Protracheoniscus occidentalis*. This pattern agrees with the Ebro study, where in spring harpacticoid copepods were more abundant than chironomids and more important in diet (Vargas 1993).

However, the overall diversity and number of prey categories in gut contents did not change significantly with fish length. This pattern was surely due to the fact that with growth, the Spanish toothcarp did not completely change its trophic niche and feeding habitat, but only added some larger prey to its diet (new species or larger sizes of the same species) these being more important in biomass.

Although several authors suggest a benthic feeding habitat (Vargas & De Sostoa 1999, Doadrio 2001, Rincón *et al.* 2002), the toothcarp diet was more closely correlated with organisms in the water column in both the glasswort and algal mats habitats. This pattern might be due to the fact that *A. iberus* is the only fish with a stable population in the Fra Ramon lagoon. In the presence of planktivorous aggressive species, such as *G. holbrooki*, the toothcarp restricts its microhabitat to a lower position in the water column (Rincón *et al.* 2002), producing niche segregation and compression in synoptic populations (Vargas 1993). There are no field studies showing the generality of these niche dynamics between the toothcarp and invasive species.

In Fra Ramon, where *A. iberus* is the only fish species with a stable population, we have shown that the ontogenetic diet shift is linked to a microhabitat change. Smaller fish showed positive electivity and the importance of more planktonic prey (e.g., copepods nauplii, the harpacticoid copepods *Mesochra lilljeborgi* and *Tisbe longicornis*, the rotifer *Brachionus plicatilis*, and ostracods), while larger fish presented more benthic prey (e.g., *Canuella perplexa*, *Mesochra rapiens*, and ephydrids dipterans) and showed more electivity for benthic prey such as rhagionids dipterans or the polychaete *Nereis diversicolor*. Among habitats, this microhabitat change in diet was more apparent in algal mats than glasswort. This was as expected, due to the shallowness of the latter habitat. Our findings demonstrate the usefulness of measuring resource availability and electivity in dietary studies.

ACKNOWLEDGEMENTS

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Chapter IV

Life history variation of invasive mosquitofish (*Gambusia holbrooki*) along a salinity gradient

ABSTRACT

The mosquitofish (*Gambusia holbrooki*) is among the most invasive fish worldwide, with well documented ecological impacts on ecosystem functioning and on several taxa such as amphibians and small native fish. Although it has been suggested that salinity limits the invasive success of mosquitofish, there are few studies on how salinity affects its ecology. We examined differences in fish assemblages and the life history traits of mosquitofish at 24 sampling points from fresh (ca. 0.2‰ of salinity) to polysaline waters (ca. 23‰). Salinity significantly affected the density and life history traits of both male and female mosquitofish. They did not vary in size with salinity but their density showed a unimodal response with salinity, stronger for females and thus affecting the sex ratio. On the other hand, the endemic Spanish toothcarp (*Aphanius iberus*) was only present in polysaline and eusaline waters, where mosquitofish were generally absent. Both mosquitofish sexes increased reproductive investment in oligosaline waters, showing higher gonadal weight, at the cost of somatic condition in females. That increase in reproductive investment also implied an increase in total weight as opposed to somatic weight, demonstrating that the latter is a better indicator of condition. Females from higher salinity waters presented more embryos in advanced developmental stages, indicating earlier reproduction than in fresh water. Furthermore, after correction for brood and fish size, females from saltier environments presented heavier embryos (independently of maturation state). Although *G. holbrooki* has previously been considered a lecithotrophic species, we observed a weight gain of oocytes during development, thus demonstrating that some mosquitofish populations can be matrotrophic (i.e., embryos receive post-fertilization nourishment from the mother). In summary, polysaline waters mainly affected mosquitofish females which, despite presenting earlier maturation and higher reproductive investment, reduced their condition and density, hence supporting the hypothesis that salinity limits mosquitofish invasive success.

Keywords

Invasive species, water conductivity, life history traits, *Gambusia holbrooki*, Iberian Peninsula.

* Alcaraz C. & García-Berthou E. (submitted).

INTRODUCTION

Biotic homogenization through the introduction of invasive species and the extinction of native species is now recognized as one of the main threats to biodiversity and ecosystem function, particularly in freshwater ecosystems (Mack *et al.* 2000, Rahel 2002). Invasions by non-native species are of widespread concern because of their detrimental effects on ecosystem processes and their economic consequences (Mack *et al.* 2000), and human activities have recently accelerated the intentional and accidental spread of species across their natural dispersal barriers (Alcaraz *et al.* 2005, García-Berthou *et al.* 2005). The mosquitofish (in fact two very closely related species: *Gambusia holbrooki* and *G. affinis*) is only native to the United States and Mexico but has been introduced into more than 50 countries (García-Berthou *et al.* 2005) in order to control mosquito populations and hence malaria (Krumholz 1948). The mosquitofish is probably the most widely distributed freshwater fish in area occupied (Krumholz 1948) and considered to be one of the world's 100 worst invasive alien species by the GISP (Global Invasive Species Programme, http://www.issg.org/database/).

The strong ecological impact of introduced mosquitofish is quite well known. Mosquitofish profoundly alter ecosystem functioning through trophic cascades (Hurlbert *et al.* 1972). Several studies have demonstrated their negative effects on the decline and extinction of native amphibians (e.g, Kats & Ferrer 2003) as well as native fishes, especially fish of similar size such as the American cyprinodontiforms *Heterandria formosa* and *Poeciliopsis occidentalis* (e.g, Meffe *et al.* 1983, Belk & Lydeard 1994) or the endemic Spanish cyprinodontiforms *Aphanius iberus* and *Valencia hispanica* (Rincón *et al.* 2002, Caiola & de Sostoa 2005), both considered to be species in danger of extinction by the National Catalogue of Endangered Species (Doadrio 2002) and listed in the Annex II (Strictly protected fauna species) of the Convention on the Conservation of European Wildlife and Natural Habitats ("Bern Convention"). The Spanish toothcarp (*Aphanius iberus*) originally inhabited a wide range of lowland waters but nowadays its distribution is reduced to polysaline, eusaline and hypersaline waters. Several factors have been proposed to explain this decline (García-Berthou & Moreno-Amich 1992, Elvira 1995) but invasive *Gambusia holbrooki* plays a dominant role, replacing native cyprinodontiforms in fresh and oligosaline waters. Some works have reported lower mosquitofish density with salinity and it is often suggested that salinity limits its invasive success (Elvira 1995, Nordlie & Mirandi 1996). However, there are few studies on the effects of salinity on mosquitofish biology, particularly metabolism

(Nordlie & Mirandi 1996). Mosquitofish are able to cohabit in highly saline waters with other species that are displaced in fresh water (Al-Daham *et al.* 1977), but the ecological mechanism by which salinity limits the invasive success of mosquitofish is largely unknown. Although the life history plasticity of mosquitofish has been proposed as an important factor to understand its invasive success (Haynes & Cashner 1995), the only studies of the effects of salinity on mosquitofish life history traits are Stearns & Sage (1980) and Brown-Peterson & Peterson (1990), who studied short salinity gradients in North America (two populations from fresh (0‰) to mesosaline (10‰) and oligosaline (2.5‰) waters, respectively). The objective of our paper is to assess the variation of life history traits of invasive mosquitofish along a long salinity gradient (from 0‰ to 23‰) in northeastern Spain, in order to understand why salinity may limit its invasive success.

METHODS

Study area and sampling methods

We studied the fish assemblage and the life history traits of mosquitofish at 24 sampling sites ranging from fresh water (438 µS/cm) to eusaline water (55600 µS/cm) along three river basins in northeastern Spain (Figure 1, Table 1). Mosquitofish were not captured at only 5 of the 24 sites (mostly the sites with the highest salinity). The main physical and chemical features of the sampling sites are summarized in Table 1. The three river basins sampled were: the Ter River, with 208 km of length, 3010.5 km^2 of basin area, and 17.15 m^3/s of average flow, from fresh water sites at 35 km from the coast (close to Banyoles Lake) to mixosaline and eusaline water sites at the Baix Empordà wetlands, close to the river mouth; the Muga River with a length of 58 km, 854 km^2 of area, and 3.34 m^3/s of average flow; and the Tort River, a small stream in the Alt Empordà wetlands (Fig. 1).

Because the main objective was to assess the effect of salinity on life history traits, all samples were taken during May 2003 (Table 1), when the reproductive period of the mosquitofish that had survived the winter started but new born were still not present (Fernández-Delgado & Rossomanno 1997). All the fish were captured during daylight hours using dip nets (60 cm diameter and a stretched mesh size of 1 mm) and preserved in situ in 10% formalin. The sampling time was measured in order to estimate fish abundance (catch per unit effort, CPUE, as the number of fish per minute). Temperature, electrical conductivity (EC_{25}), pH and dissolved oxygen were measured in situ. An estimate of the water salinity concentration was obtained from conductivity with the conversion equations of APHA (2001). The distance to the coast was measured from maps as a perpendicular line from sampling sites to the sea.

Table 1. Features of the 24 sites sampled in May 2003. All values refer to the sampling date. See Figure 1 for location of the sampling sites. CPUE are number of fish captured per minute.

Site	Sampling date	Latitude	Longitude	Mosquitofish CPUE	Total fish CPUE	pH	Water conductivity (µS/cm)	Salinity (‰)	Dissolved O_2 (mg/l)	Temperature (°C)	Depth (m)
E1	5-May	42° 17' 57"	3° 6' 15"	0.35	0.39	6.32	443	0.25	8.2	17.1	0.34
E2	5-May	42° 17' 23"	3° 6' 48"	0.16	0.22	6.72	438	0.24	9.0	18.5	0.23
E3	5-May	42° 16' 54"	3° 7' 6"	3.05	4.10	7.02	1127	0.62	5.5	20.4	0.35
E4	5-May	42° 16' 49"	3° 7' 4"	0.77	0.80	7.02	740	0.40	5.8	19.9	1.00
E5	7-May	42° 16' 34"	3° 7' 28"	0.92	1.20	8.10	698	0.36	5.3	22.2	1.00
E6	5-May	42° 16' 25"	3° 7' 58"	0	25.80	6.58	24200	17.45	6.8	17.5	0.10
E7	5-May	42° 16' 4"	3° 7' 37"	0	2.62	7.90	27400	17.54	10.5	23.1	>2.00
E8	5-May	42° 15' 34"	3° 8' 50"	0	0.57	7.87	55600	38.68	11.3	22.9	1.00
E9	7-May	42° 14' 20"	3° 7' 30"	2.60	14.10	8.10	6350	3.57	21.4	23.5	0.50
E10	7-May	42° 14' 53"	3° 6' 23"	0.38	2.56	8.98	1546	0.78	20.8	24.6	0.50
E11	7-May	42° 15' 9"	3° 5' 57"	0.52	1.40	8.51	693	0.35	12.3	23.2	0.75
E12	7-May	42° 15' 22"	3° 4' 17"	0	0	7.68	718	0.38	5.0	21.3	0.35
E13	7-May	42° 15' 30"	3° 4' 9"	0.02	1.60	7.97	718	0.38	8.3	21.5	0.45
E14	7-May	42° 14' 3"	3° 4' 15"	0.53	0.75	8.09	888	0.61	10.7	17.8	0.20
B1	14-May	42° 7' 10"	2° 44' 55"	2.30	2.30	7.64	1332	0.74	9.5	20.3	0.50
B2	14-May	42° 6' 59"	2° 45' 38"	12.00	12.00	7.28	1330	0.72	6.3	21.4	4.50
B3	14-May	42° 6' 57"	2° 46' 38"	8.25	8.25	8.01	1326	0.74	9.7	20	0.20
B4	21-May	42° 7' 6"	2° 46' 40"	1.23	1.23	7.80	1406	0.78	8.1	20.3	0.30
T1	12-May	41° 59' 36"	2° 49' 24"	1.03	1.17	8.30	576	0.34	8.6	15.8	0.40
T2	12-May	42° 2' 49"	3° 11' 12"	23.00	23.00	8.51	1094	0.56	13.0	23.5	0.30
T3	12-May	42° 2' 55"	3° 11' 18"	2.84	2.84	7.10	1599	0.89	4.5	20.3	0.50
T4	12-May	42° 2' 46"	3° 11' 49"	15.20	15.20	7.70	35100	23.25	5.3	22.7	0.45
T5	12-May	42° 2' 55"	3° 11' 48"	8.67	8.67	7.94	10890	6.40	5.3	23.3	0.75
T6	12-May	42° 1' 50"	3° 10' 55"	0	2.21	8.84	32400	20.90	15.5	23.5	0.38

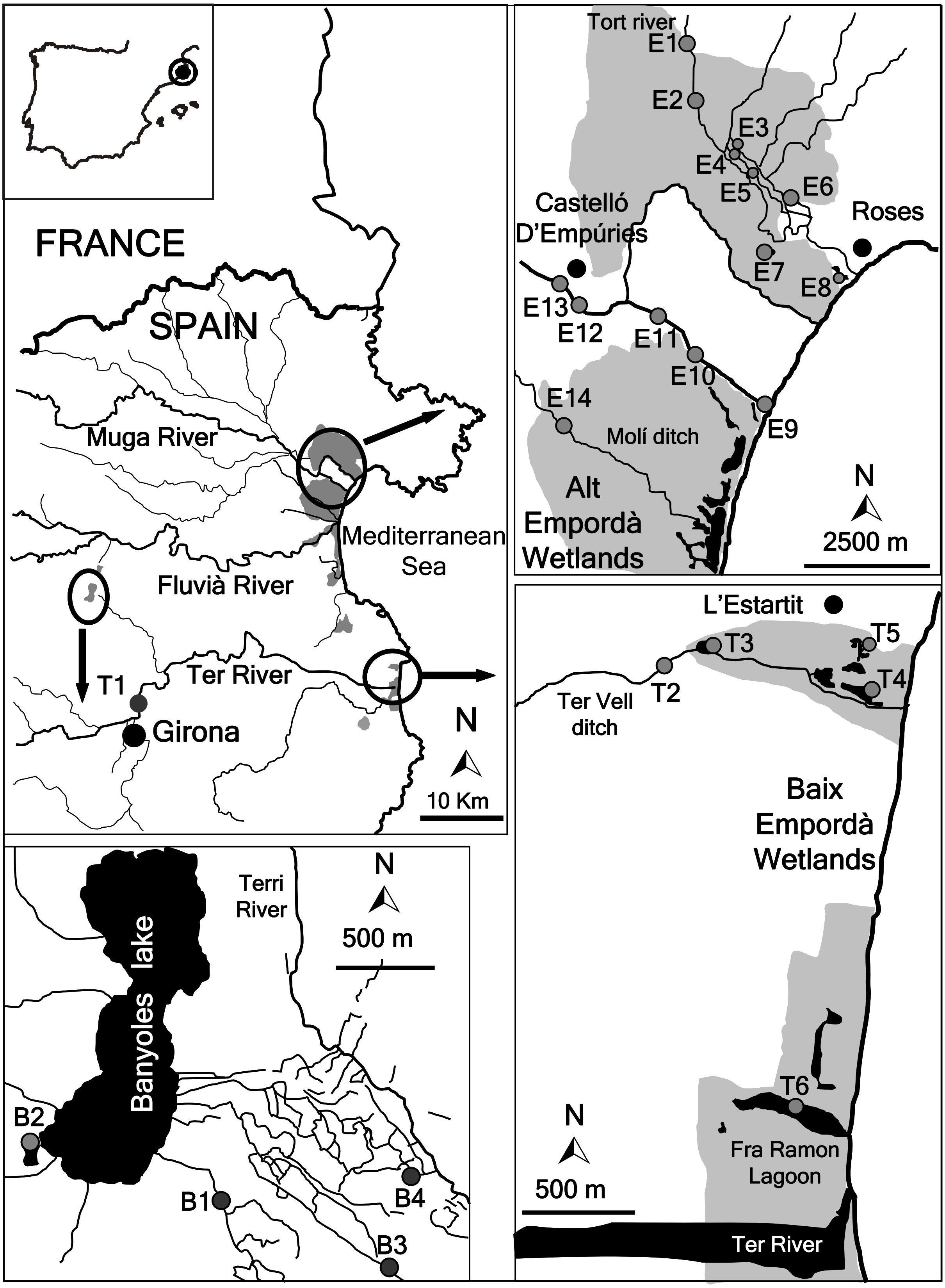

Figure 1. Location of the 24 sampling sites in the three study areas: Alt Empordà wetlands (sites starting with E), Banyoles Lake drainage (B sites) and Ter River basin that includes Baix Empordà Wetlands (T sites).

Laboratory procedures

In the laboratory, all the fish were classified to species level, weighed (total weight to the nearest 0.1 mg) and measured (total and standard length (SL) to the nearest 0.01 mm with a digital caliper). All mosquitofish were sexed according to anal fin morphology (Turner 1941); when this was not possible (usually fish with SL ≤ 11 mm), sex was determined by the direct observation of the gonads, and when it was not possible to discern the sex, the fish were classified as immature. For each sample, about 30 mosquitofish (15 males and 15 females, when available) were eviscerated, and their eviscerated weight and gonad weight were measured to the nearest 0.1 mg. The gonadosomatic index (GSI) was calculated as the percentage of gonadal weight of total weight.

For females, ovaries were removed and processed under a dissecting microscope. Mosquitofish are live-bearers that develop one clutch of eggs at a time; the offspring must be expelled before another clutch develops, i.e. there is no superfetation (Reznick 1981). Consequently, the ovary of mature females typically shows two kinds of oocytes: a group in development and another group of immature oocytes that may develop when the first ones have been released. Oocytes were classified in six categories according to Reznick (1981): I, no development (yolked but non-fertilized, development is not apparent); II, un-eyed (development is already apparent); III, early-eyed; IV, mid-eyed; V, late-eyed; VI, very late-eyed. Stages II to VI were considered fertilized eggs (Fernández-Delgado & Rossomanno 1997). All the oocytes present in the ovaries were classified, counted and weighed (to the nearest 0.1 mg) by stage group, after elimination of intra-ovarian tissue. Malformed embryos were detected in a very low percentage of females (< 1%) and they represented a low percentage (< 1%) of the total number of embryos. Following Fernández-Delgado & Rossomanno (1997), each group of oocytes (non-fertilized and fertilized) was used to calculate two fecundity measures: potential fecundity (including all the oocytes) and real fecundity (including only fertilized oocytes).

Data analysis

The effect of conductivity on fish composition and abundance was assessed with canonical correspondence analysis (CCA), a direct gradient ordination technique. In CCA, the ordination results are constrained to be a linear relationship of environmental variables (in our case, water conductivity), providing an ordination using the two matrices (fish composition and environmental variables) simultaneously in a single analysis (McCune 1997). CCA was obtained with CANOCO 4.5 (ter Braak & Šmilauer 2002), downweighting rare species, and the significance of conductivity was tested with Monte Carlo permutation tests (499 permutations). To further describe the relationship of mosquitofish abundance and one of the species most affected by it (the endemic cyprinodont *Aphanius iberus*), we also fitted generalized additive models (GAMs) (Lepš & Šmilauer 2003), as available in the CANOCO program, to fit the response of CPUE of both species to water conductivity. GAMs are an extension to generalized linear models that, unlike more conventional regression methods, do not require the assumption of a particular shape for the species distribution along the environmental gradient (Lepš & Šmilauer 2003). The model complexity of GAMs was selected by the stepwise selection procedure using the Akaike information criterion (AIC), as available in CANOCO 4.5. AIC considers not only the goodness of fit but also parsimony, penalizing very complex models (Burnham & Anderson 1998).

Spearman's correlations coefficient (r_s) was used to test differences in mean length with water conductivity. Variation of life history traits among populations were analyzed with analysis of covariance (ANCOVA) using fish length as the covariate, except for the gonadosomatic index (GSI), for which no covariate was used (i.e. an ANOVA was used). For mean number and weight of oocytes per developmental stage, an ANCOVA with fish length and developmental stage was used. When the covariate was not significant ($P > 0.10$), it was deleted from the models (i.e. an ANOVA was used), to increase statistical power (García-Berthou & Moreno-Amich 1993). The significant variation among populations was decomposed in a linear relationship with water conductivity and a residual component with polynomial orthogonal contrasts (Sokal & Rohlf 1995) and the SPSS "metric" option (SPSS Inc. 2004). By default, polynomial contrasts assume equally-spaced levels, whereas with the metric option unequal spacing for the factor levels (real distances between log-transformed water conductivities) may be specified (SPSS Inc. 2004). Estimated marginal (or adjusted) means of a dependent variable are the means for each level of the factor, adjusted for covariates (see e.g, García-Berthou & Moreno-Amich 1993) and were used to describe the differences among populations.

Water conductivity and distance to the coast of the sampling sites were, as expected, significantly related (Spearman's $r_s = -0.61$, $n = 24$, $P = 0.002$). However, all the life history variables were more related to water conductivity than to distance to the coast, e.g, male (Spearman's $r_s = 0.58$ vs. $r_s = 0.24$) and female ($r_s = 0.51$ vs. $r_s = 0.14$) gonadal weight, respectively. Therefore, we only used water conductivity in further analysis to avoid collinearity effects.

The sex group composition was compared among populations with a *G*-test of independence (Sokal & Rohlf 1995), followed with Spearman's correlation coefficient to test the relationship with water conductivity. Quantitative variables were log-transformed for parametric analyses because homoscedasticity and linearity were clearly improved. All statistical analyses were performed with SPSS 13.0.

RESULTS

Variation of fish assemblages with water conductivity

A total of 1546 fish were captured, namely 921 mosquitofish and 625 of other species. When we assessed the relationship of the fish assemblages with water conductivity, the first two CCA axes, respectively, explained 13.4% (eigenvalue = 0.273) and 33.6% (eigenvalue = 0.689) of the variation in fish species composition (total inertia in species data = 2.05).

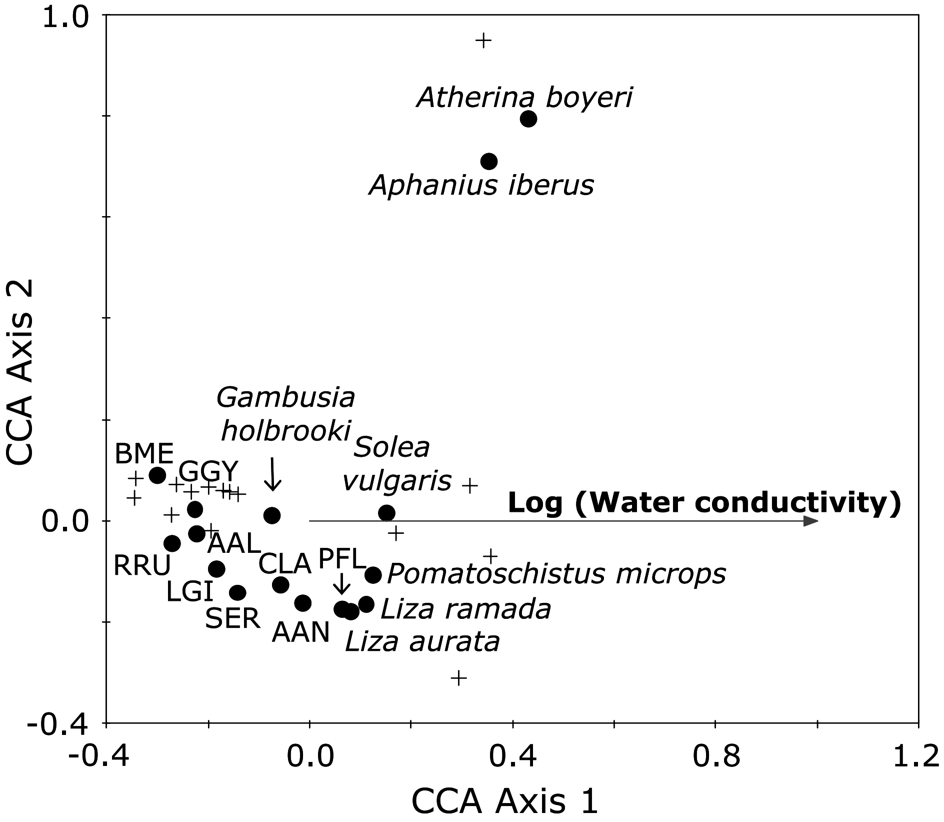

Figure 2. Canonical correspondence analysis triplot of fish composition (CPUE) in the 23 sampling sites and water conductivity. Crosses correspond to sampling sites and dots to fish species (BME = *Barbus meridionalis*, RRU = *Rutilus rutilus*, GGY = *Gasterosteus gymnurus*, AAL = *Alburnus alburnus*, LGI = *Lepomis gibbosus*, SER = *Scardinius erythrophthalmus*, CLA = *Chelon labrosus*, AAN = *Anguilla anguilla*, and PFL = *Platichthys flesus*).

The variation with water conductivity (Fig. 2) was displayed by the first CCA axis and showed a species-environment correlation of 0.593, distinguishing species that preferentially inhabited fresh water in the middle reaches (e.g, *Barbus meridionalis*) from species such as *Atherina boyeri* or *Solea vulgaris* (marine species inhabiting some estuaries and inland waters with high salinity levels) and *Aphanius iberus* (a highly euryhaline fish mostly present in hypersaline lagoons). Euryhaline species inhabiting waters with a wide range of salinities (e.g, mosquitofish and some diadramous species such as the eel (*Anguilla anguilla*) and the thicklip grey mullet (*Chelon labrosus*)) were located in the centre of the CCA.

The response curves (GAMs) of mosquitofish and toothcarp abundance to water conductivity (Fig. 3) confirm these patterns. AIC selected a unimodal response for mosquitofish (non-linear $F_{1,\,19} = 2.34$, $P = 0.045$), its abundance peaking at intermediate water conductivity (ca. 3500 µS/cm), while toothcarp showed a linear CPUE-conductivity relationship (linear $F_{1,\,20} = 9.63$, $P = 0.006$), being mostly present in polysaline and eusaline waters where the mosquitofish was generally not present (Fig. 3).

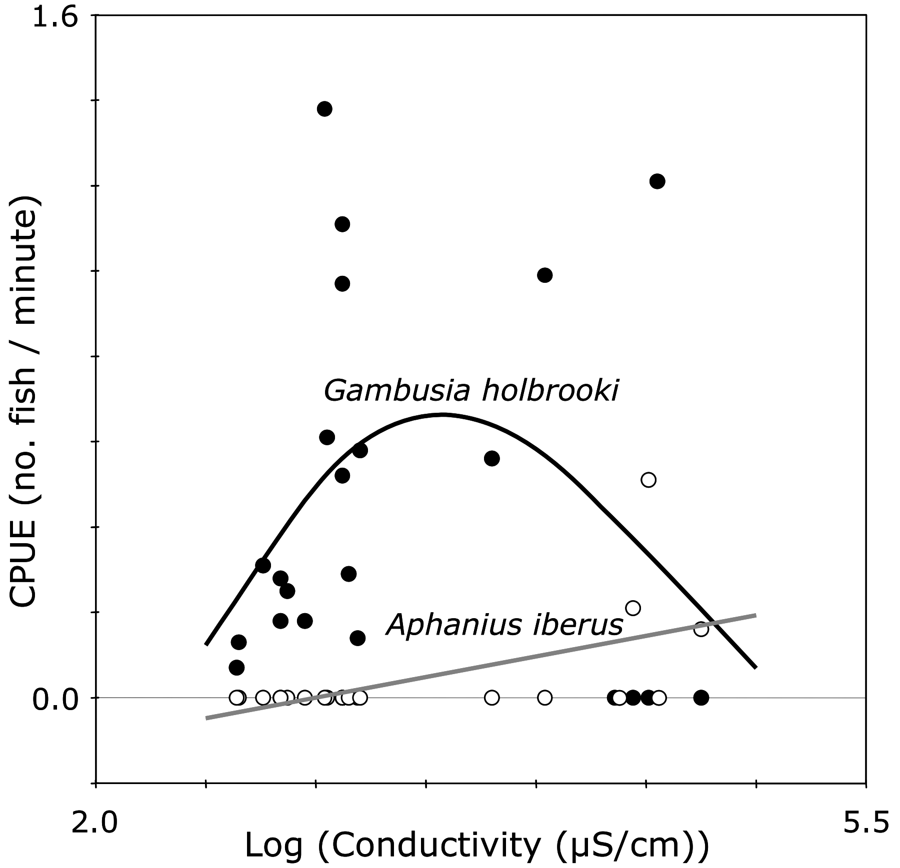

Figure 3. Response of *Gambusia holbrooki* (●, deviance = 3.14, model df = 19) and *Aphanius iberus* (○, deviance 0.20, model df = 20) abundance (CPUE) with water conductivity. Lines are the generalized additive models selected by the Akaike information criterion.

Mosquitofish population structure

The mosquitofish standard length was not correlated with water conductivity for females (r_s = 0.31, n = 15, P = 0.25), males (r_s = -0.30, n = 15, P = 0.27) or immatures (r_s = -0.10, n = 15, P = 0.78) (Fig. 4).

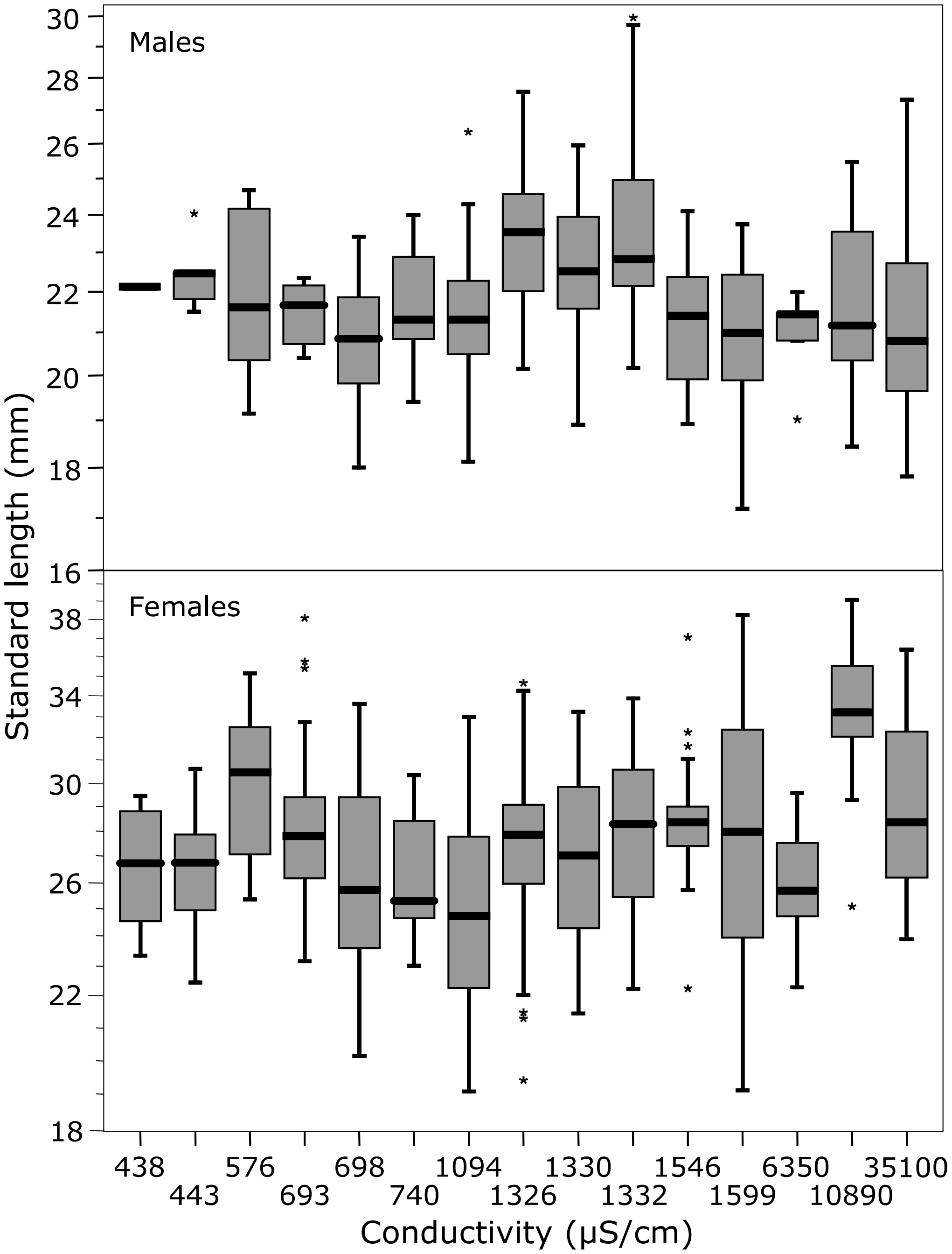

Figure 4. Box-plot of standard length with water conductivity for male (top) and female (bottom) mosquitofish. The box corresponds to the 25th and 75th percentiles, the dark line inside the box represents the median, error bars are the minimum and maximum except for outliers (asterisks, corresponding to values beyond 1.5 boxes from the box).

Mosquitofish abundance (CPUE) showed a parabolic relationship with water conductivity (see also Fig. 3), the quadratic component being significant ($P << 0.05$) for males (polynomial regression, adjusted $r^2 = 0.16$), females (adjusted $r^2 = 0.25$) and immatures (adjusted $r^2 = 0.21$). Density reduction in higher salinity was more marked for females than for males and thus the sex ratio varied significantly among populations ($G = 77.8$, df = 14, $P < 0.0001$) and the female proportion decreased with water conductivity (Spearman's $r_s = -0.58$, $n = 15$, $P = 0.022$) (Fig. 5). Although the proportion of immature fish differed among populations ($G = 76.7$, df = 14, $P < 0.0001$), these differences were not related to water conductivity ($r_s = -0.14$, $n = 15$, $P = 0.62$).

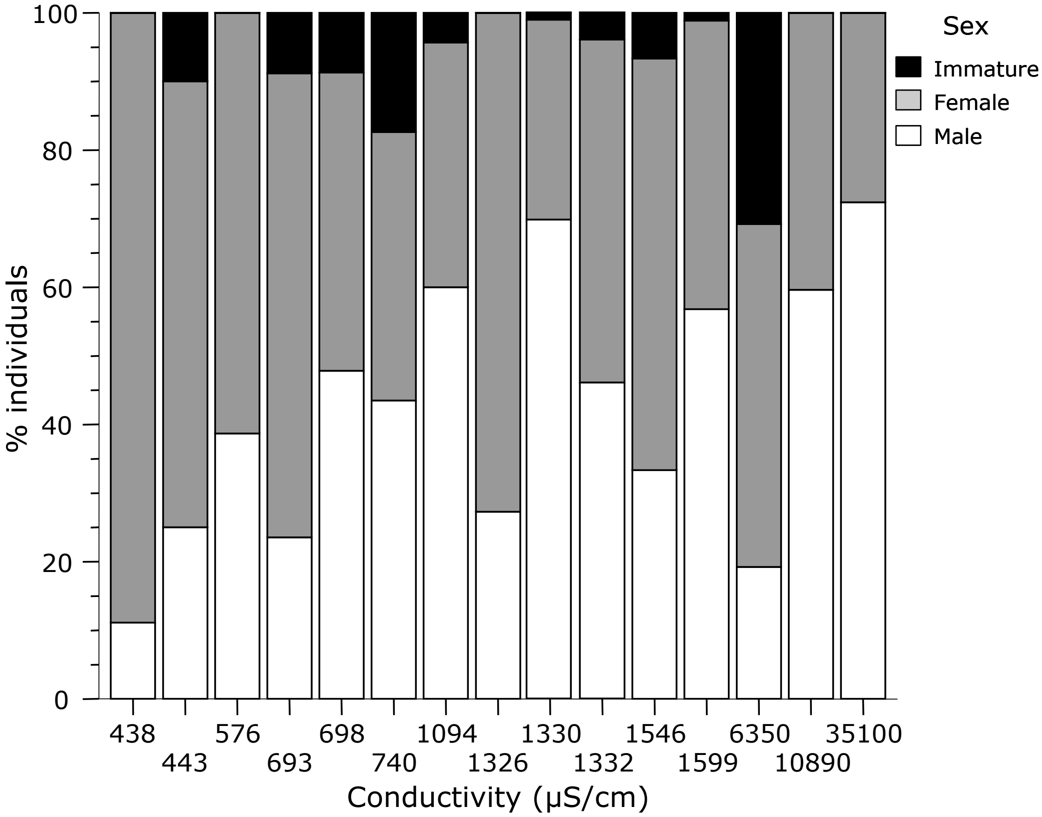

Figure 5. Variation of sex group composition of mosquitofish with water conductivity.

Mosquitofish life history traits along the salinity gradient

All reproductive variables depended significantly on standard length (Table 2). After accounting for that, all reproductive variables for both sexes significantly differed among populations and for most of them the linear component with water conductivity was highly significant (Table 2). Only for total and eviscerated weight of males and potential fecundity and mean embryo weight of females was the linear contrast not significant; however, for these cases the deviation contrast was significant and in several cases a curvilinear relationship was observed.

Table 2. ANCOVAs of the life history traits of mosquitofish with conductivity (factor) and standard length (covariate). The variation among populations was decomposed in a linear component and a residual term (deviation) with polynomial contrasts. All variables were $\log_{10}$ transformed except GSI. No covariate was used for the analysis of GSI (i.e. ANOVA was used).

	Explained variation (Adjusted R^2)	**Among populations**			**Log Conductivity (μS/cm)**		**Log Standard length (mm)**		
					Linear contrast	**Deviation**			
		F	**Df**	***P***	***P***	***P***	***F***	**df**	***P***
MALES									
Total weight	0.914	6.09	14, 380	<0.0001	0.237	<0.0001	3465.1	1, 380	<0.0001
Eviscerated weight	0.918	4.40	14, 158	<0.0001	0.548	<0.0001	1658.6	1, 158	<0.0001
Gonadal weight	0.647	12.06	14, 159	<0.0001	<0.0001	<0.0001	113.84	1, 159	<0.0001
GSI	0.472	12.00	14, 158	<0.0001	<0.0001	<0.0001			
FEMALES									
Total weight	0.958	7.34	14, 341	<0.0001	0.002	<0.0001	5958.2	1, 341	<0.0001
Eviscerated weight	0.969	9.11	14, 201	<0.0001	0.002	<0.0001	5243.9	1, 201	<0.0001
Gonadal weight	0.799	3.33	14, 201	<0.0001	0.001	0.001	553.21	1, 201	<0.0001
GSI	0.268	6.65	14, 202	<0.0001	<0.0001	<0.0001			
Potential fecundity	0.546	6.37	14, 201	<0.0001	0.159	<0.0001	103.05	1, 201	<0.0001
Real fecundity	0.619	3.41	14, 201	<0.0001	0.001	0.001	189.93	1, 201	<0.0001
Mean oocyte weight	0.603	4.11	14, 200	<0.0001	0.010	<0.0001	189.50	1, 200	<0.0001
Mean embryo weight	0.596	11.41	13, 99	<0.0001	0.377	<0.0001	28.84	1, 99	<0.0001

For males, gonadal weight and the gonadosomatic index showed a significant increasing relationship with water conductivity (Table 2) and the quadratic contrast was also significant ($P < 0.0001$ and $P = 0.011$, respectively); so, mosquitofish inhabiting fresh water showed lower gonadal weight (after accounting for fish length) and GSI values than mosquitofish populations of higher salinities (Fig. 6).

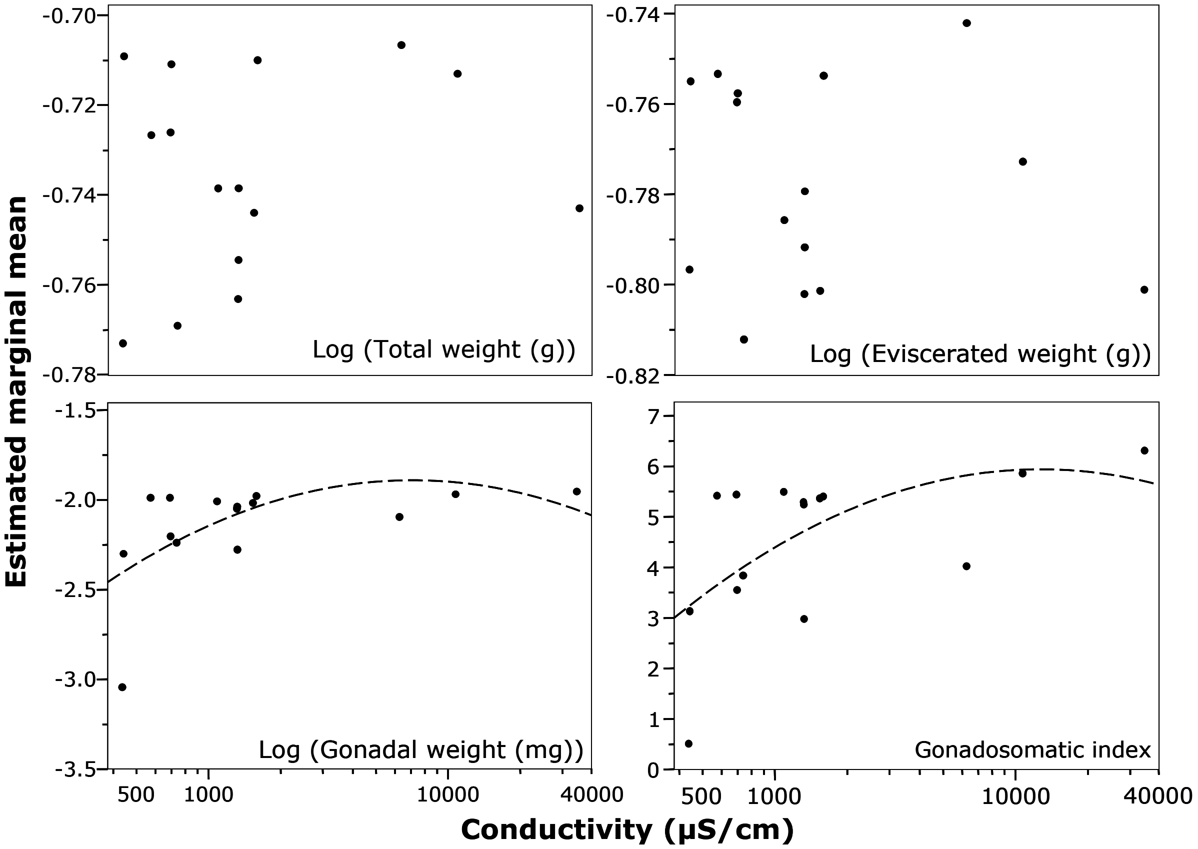

Figure 6. Relationship of conductivity with size-adjusted means (ANCOVAs of Table II) of life history traits for male mosquitofish. For gonadosomatic index the observed means without adjusting for length are given (i.e. ANOVA was used). Significant (*P* < 0.05) linear or quadratic components are also shown.

For females, water conductivity had a positive linear effect on total weight, gonadal weight and GSI, but a negative one on eviscerated weight (Table 2, Fig. 7). Potential fecundity (total number of oocytes) was not related to water conductivity, but real fecundity (number of embryos at stages II to VI) significantly decreased with lower water conductivity (Table 2, Fig. 7, quadratic contrast *P* = 0.016). On the other hand, mean oocyte weight was positively affected by water conductivity but mean embryo weight was not (Table 2, Fig. 7). Therefore, female mosquitofish from fresh waters significantly decreased reproductive investment, as indicated by lower gonadal weight (adjusted for length),GSI and total weight, but had better somatic condition (eviscerated weight adjusted for length) (Fig. 7). Potential fecundity did not vary with water conductivity but freshwater females significantly presented fewer embryos (real fecundity) and mean oocyte weight was lower than in higher salinity populations.

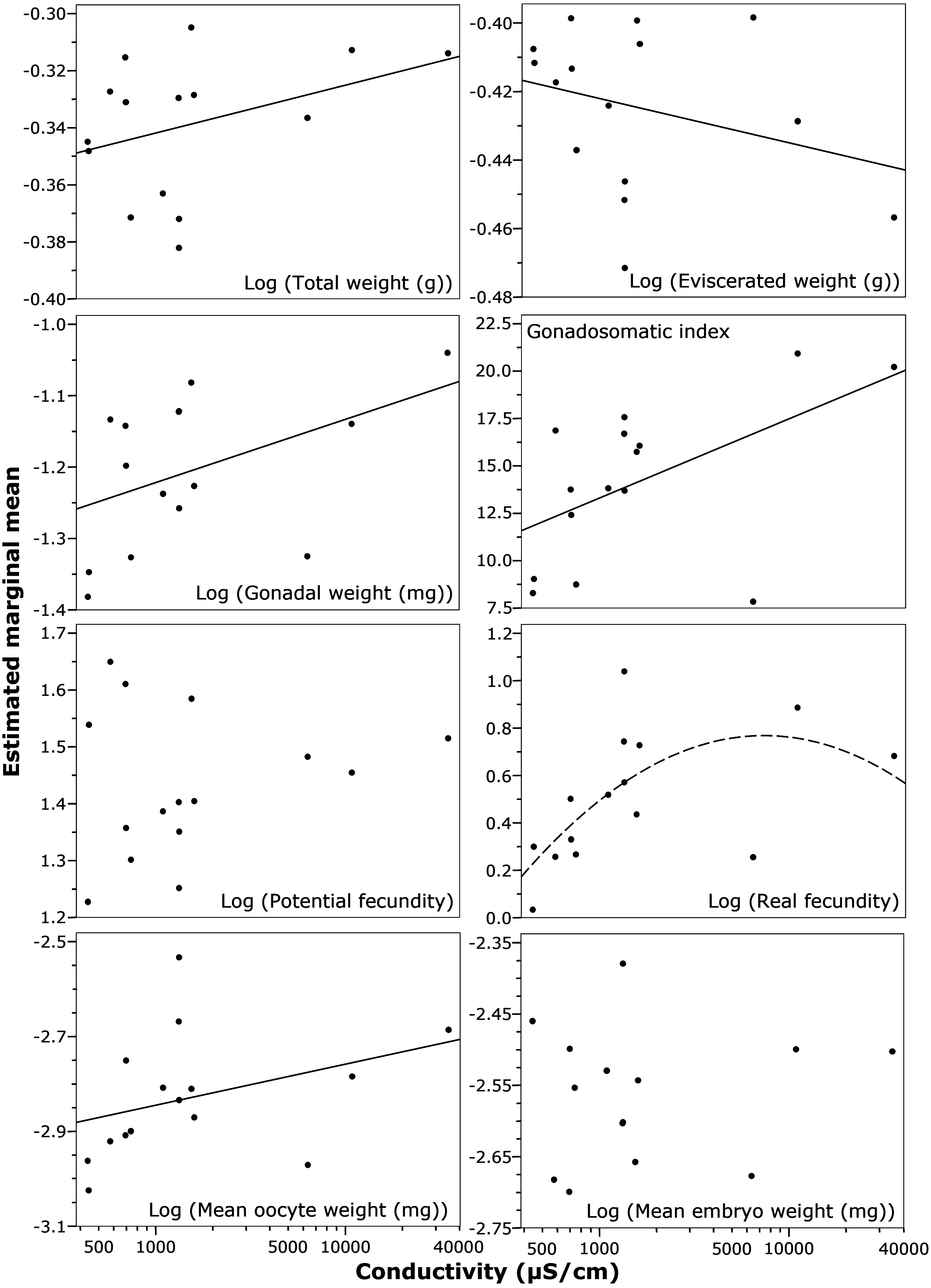

Figure 7. Relationship of conductivity with size-adjusted estimated means (ANCOVAs of Table II) of life history traits for female mosquitofish. For gonadosomatic index the observed means without adjusting for length are given. Significant (*P* < 0.05) linear or quadratic components are also shown.

Oocyte developmental traits

The detailed analysis of oocyte developmental traits (ANCOVA with standard length and developmental stage as covariates) showed that the mean oocyte weight averaged for each developmental stage (from I to VI) was not related to water conductivity (linear and quadratic contrasts, $P > 0.18$). The mean oocyte weight increased significantly during development ($F_{1, 337} = 202.1$, $P < 0.0001$) (Fig. 8) and with female standard length ($F_{1, 337} = 38.0$, $P < 0.0001$). However, standard length was only significant for stage I oocytes ($F_{1, 172} = 49.81$, $P < 0.0001$) because no relationship was observed for the embryos (i.e. stages II to VI) ($P > 0.15$). For embryos, the same weight gain during development was observed ($F_{1, 150} = 94.64$, $P < 0.0001$) (Fig. 8) independently of water conductivity ($P > 0.10$).

However, oocyte weight was significantly correlated with brood size for all developmental stages (ANCOVA, $P < 0.001$). After partialling out the effect of brood size, the same pattern was observed except that the weight of stage II oocytes (linear and quadratic contrasts: $P = 0.93$ and $P = 0.033$, respectively) and stage III and IV oocytes (linear and quadratic contrasts: $P < 0.05$ and $P > 0.68$, respectively) varied with water conductivity (Fig. 8), showing a weight gain independent of female size.

The number of oocytes per developmental stage (from I to V) significantly depended on female standard length (ANCOVA, $P < 0.0001$ for all the stages). The number of stage I oocytes significantly decreased in larger females, whereas the number of the other stages significantly increased with female standard length (Fig. 9). After accounting for female size, all stages (except stage III) showed a significant linear relationship with water conductivity (linear and quadratic contrasts: $P \ll 0.05$ and $P \gg 0.05$ respectively for all the stages). The number of oocytes of stages I and II were negatively related to water conductivity (linear and quadratic contrasts: $P = 0.001$ and $P = 0.016$, respectively), while stages IV and V presented a significant positive relationship with water conductivity (linear and quadratic contrasts: $P < 0.0001$ and $P = 0.001$, respectively); stage III oocytes did not depend on water conductivity (linear contrast, $P = 0.73$) (Fig. 9). Therefore, oocyte weight did not depend on standard length (except stage I), but females inhabiting saltier waters presented heavier embryos (stages II to IV, after correcting for brood size), less oocytes (after correcting for standard length) in initial developmental stages (I and II) and more embryos in the latest developmental stages (IV and V).

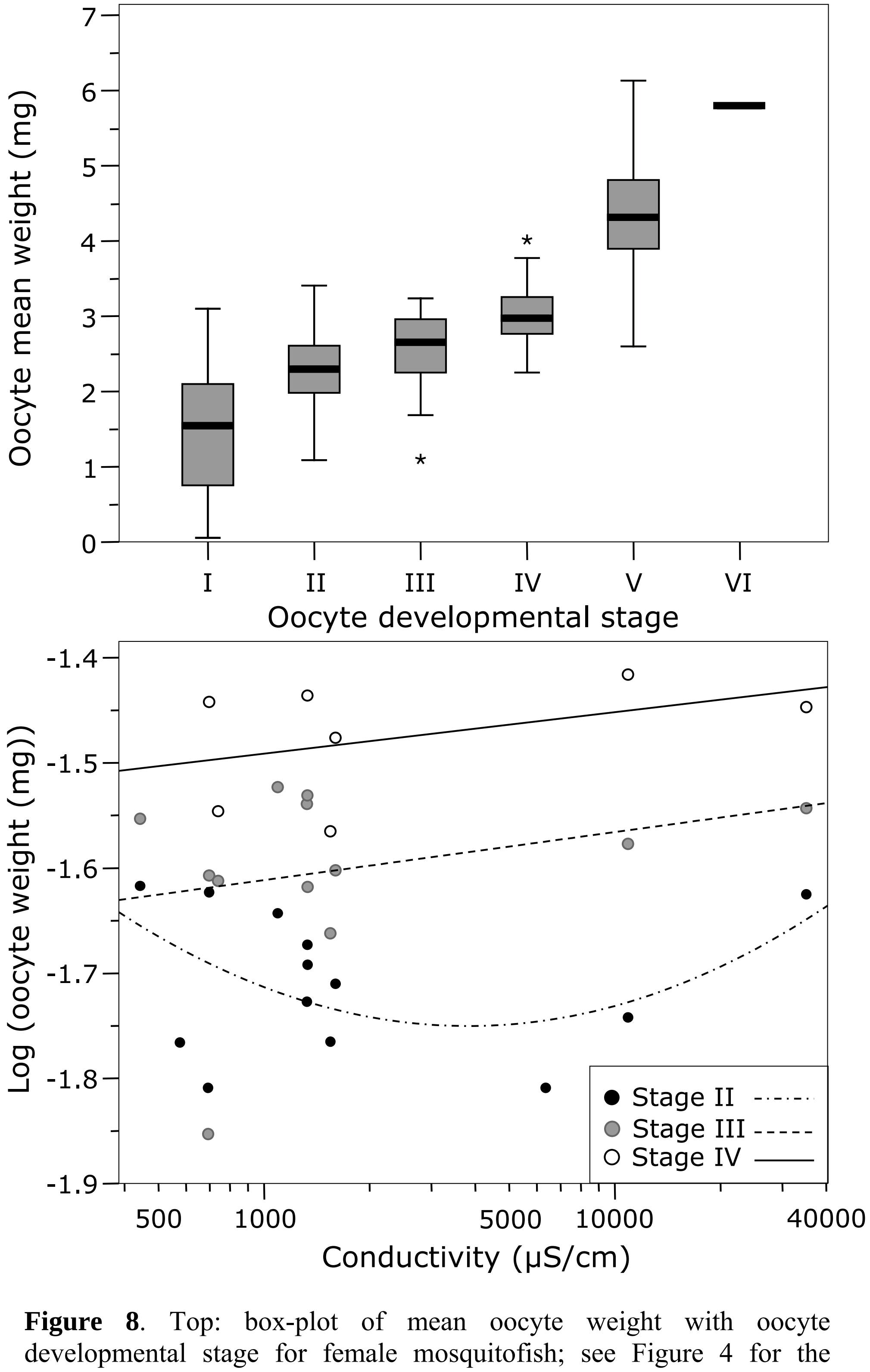

Figure 8. Top: box-plot of mean oocyte weight with oocyte developmental stage for female mosquitofish; see Figure 4 for the statistics given. Bottom: significant relationship of oocyte weight (size-adjusted means) per developmental stage with water conductivity.

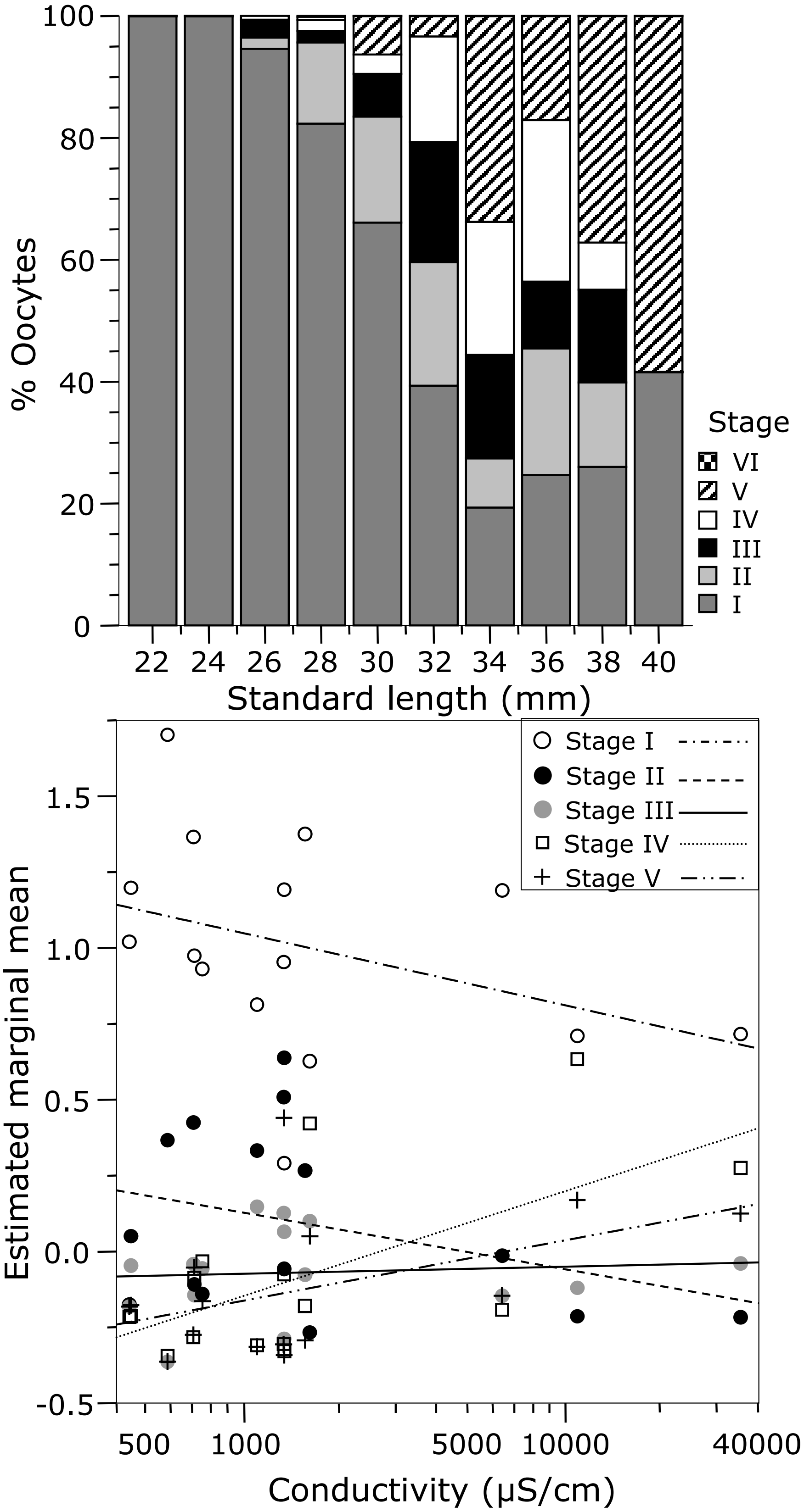

Figure 9. Top: variation of oocyte stage composition with standard length of female mosquitofish. Bottom: relationship of oocyte number (size-adjusted means) per developmental stage with water conductivity.

DISCUSSION

Salinity effects on mosquitofish life history traits

Water conductivity had highly significant effects on the life history traits of both male and female mosquitofish at the start of the reproductive season, with both sexes increasing reproductive investment in higher salinity, at the cost of somatic condition (eviscerated weight adjusted for standard length) in females. Mosquitofish inhabiting freshwater localities significantly showed less gonadal weight and GSI values than those from higher salinity waters, whereas female mosquitofish from higher salinities were in worse somatic condition. The increase of female reproductive investment also implied a gain in total weight, in contrast to somatic weight, indicating that the latter is a better indicator of condition. Females from higher salinity waters produced more embryos (real fecundity) and had larger oocytes than those in freshwater, but did not differ in total brood size (potential fecundity) and total embryo weight. These differences could be explained by the detailed analysis of oocyte stages. After accounting for fish length, females from saltier waters had significantly less oocytes in initial developmental stages (I and II) and more in advanced stages (IV and V), showing that the increase in reproductive investment is partly due to an earlier start of reproduction. However, in addition to earlier reproduction, females from saltier environments also presented heavier embryos (after correction for brood size and mosquitofish length).

Although it has often been thought that salinity may limit the invasive success of the mosquitofish, there are few studies on how salinity affects its ecology. Our results are similar to those reported by Stearns & Sage (1980) and Brown-Peterson & Peterson (1990), the only similar previous studies. Although they studied a shorter salinity range, from fresh (0‰) to mesosaline (10‰) and oligosaline waters (2.5‰), respectively, they also found that at the start of reproductive season, females from brackish waters increased reproductive effort and had larger GSI and brood sizes (similar to our real fecundity due to different oocyte classification), but produced smaller embryos. The contrasting results on embryo size might be due to a variety of reasons, including a different classification of developmental stages, a longer salinity range, or the existence of a matrotrophic strategy (see below). Brown-Peterson & Peterson (1990) also reported that females from fresh water were in better somatic condition and matured later, since ovarian development was significantly more advanced in oligosaline waters. Similar effects of salinity on life history traits have been observed in other fish species. For instance, both males and females of the poecilid *Poecilia latipinna* grew faster and matured earlier in relatively salty water (Trexler & Travis 1990), and environmental factors had a more pronounced influence on females than on males (Trexler *et al.* 1990), as we observed for mosquitofish. Frenkel & Goren (1997) showed that *Aphanius dispar* females from higher salinity had larger GSI and oocytes in more advanced developmental stages. Similarly, the fecundity and GSI values for both males and females of Caspian roach (*Rutilus rutilus caspicus*) (Naddafi *et al.* 2005) and the fecundity of tule perch (*Hysterocarpus traski*) (Baltz & Moyle 1982) were greater in saline habitats than in fresh water; while in environments with high salinity the black-chinned tilapia (*Sarotherodon melanotheron*) and the bonga shad (*Ethmalosa fimbriata*) reduced size-at-maturity and increased fecundity (Panfili *et al.* 2004a, 2004b). Therefore, the pattern of increased reproductive investment with salinity seems widespread among euryhaline fish.

Life history theory predicts that reduced adult survival will select for earlier maturation and increased reproductive effort, whereas fluctuating environments select for a configuration of traits marked by younger age- and smaller size-at-maturity, higher reproductive effort, larger brood sizes, and smaller offspring, relative to stable environments (Stearns 1992). Salinity is one of the most stressful factors in mosquitofish survival, affecting it at two levels: because of osmotic pressure capabilities and because saline habitats usually undergo much greater salinity fluctuations than freshwater habitats (Nordlie & Mirandi 1996). Both stress mechanisms may thus favor a similar response of life history traits. The above mentioned results, however, may also be due to indirect effects of salinity, because salinity is also related to distance to coast and, consequently, to temperature, pH and other water chemistry variables. For instance, Grether *et al.* (2001) and Reznick *et al.* (2001) have recently discussed that despite the well known effects of predation on the life history of guppies (*Poecilia reticulata*), others factors such as resource availability or forest cover co-vary with predation pressure. Thus, laboratory experiments are needed to further understand salinity effects on mosquitofish life history.

Mosquitofish matrotrophy

Gambusia holbrooki, as well as most species of the same genus, has generally been considered a lecithotrophic species, as suggested from both experimental (Reznick 1981, Reznick & Miles 1989) and field studies (Haynes & Cashner 1995, Fernández-Delgado & Rossomanno 1997). However, the weight gain of oocytes during the development that we observed, instead of the previously reported loss of ca. 30%, clearly demonstrates that matrotrophy was present in the Iberian mosquitofish populations. A decrease of embryo weight during the developmental process has been well described in *G. affinis* (Reznick 1981) and *G. holbrooki* (Fernández-Delgado & Rossomanno 1997). Scrimshaw (1945) indicated a post-fertilization maternal contribution in many poeciliids, including members of the genus *Gambusia.* More recently, Meffe & Snelson (1993) and Koya *et al.* (2000) experimentally demonstrated that some maternal nutrients were supplied to embryos during the gestation process of *G. holbrooki* and *G. affinis*, respectively. Marsh-Matthews *et al.* (2005) using radiolabeled leucine provided the first direct mother-to-embryo nutrient transference in *G. geiseri* and *G. affinis*, two species previously reported as lecithotrophic. Similarly, Trexler (1997) recently reported convincing evidence for post fertilization transfer in *Poecilia latipinna*, a species previously thought to be strictly lecithotrophic.

Our data thus support previous observations and recent laboratory experiments (Marsh-Matthews, pers. comm.) indicating that *G. holbrooki* can adopt a matrotrophic strategy. More data are needed to know how widespread this strategy is in *G. holbrooki* and whether it is related to invasive success. The evolution to matrotrophy by a lecithotrophic species can be treated as a life history adaptation but in spite of the importance of this transition, there is no well-developed theory of the environmental conditions that favor this shift (Trexler & De Angelis 2003).

Mosquitofish population structure

Because samples were taken at the start of the reproductive season, we only captured fish born at the end of the last reproductive season that had survived the winter, and the new born were still not present. Salinity effects are more important in female mosquitofish than in males (Trexler *et al.* 19990); in accordance with that, we found that the mosquitofish population size-structure did not vary with salinity but that sex ratio differed in saltier waters because of a stronger decrease of female density. Salinity affects mosquitofish metabolism (Nordlie & Mirandi 1996), which is positively correlated with body mass (Hölker 2003). Because female mosquitofish are much larger than males, higher energy expenditures and oxygen consumption to maintain osmotic regulation are expected. The somatic condition of females was also significantly reduced in saltier waters because of a stronger reproductive investment. Both factors suggest reduced female survival at higher salinities. Similarly, Timmerman & Chapman (2003) reported that *Poecilia latipinna* increased routine metabolic oxygen consumption during brood development and showed a significant increase of routine metabolic rate during late gestation, whereas the metabolic rate of males was lower. Moreover, they suggested that the higher oxygen demand of females caused an increase in the time spent in aquatic surface respiration, hence affecting maternal predation risk.

Mosquitofish and toothcarp interactions

Although the reproductive investment of mosquitofish increased with salinity, abundance showed a unimodal relationship, with a strong decline in highly polysaline waters, particularly of females. In contrast, the Spanish toothcarp were only present in high polysaline and eusaline waters where mosquitofish were not present. The toothcarp is endemic to the Mediterranean coast of Spain, considered in danger of extinction by the National Catalogue of Endangered Species and listed in the Annex II of the Bern Convention (Doadrio 2002). It originally inhabited a wide range of lowland waters but nowadays its distribution is reduced to polysaline, eusaline and hypersaline waters. Several factors have been proposed to explain this decline (García-Berthou & Moreno-Amich 1992, Elvira 1995) but the impact of invasive mosquitofish plays a dominant role. The change from fresh to oligosaline waters implied the increased abundance and reproductive investment of invasive mosquitofish; these patterns do not continue, however, with further salinity. The contrasting response of mosquitofish and toothcarp abundance with salinity, in addition to the lower somatic condition of mosquitofish with increasing salinity, support the hypothesis that polysaline waters limit the invasiveness of mosquitofish and constitute a refuge for native fauna such as Mediterranean cyprinodontiform fishes.

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Chapter V

Salinity mediates the competitive interactions between invasive mosquitofish (*Gambusia holbrooki*) and an endangered fish (*Aphanius fasciatus*)

ABSTRACT

Behavioral traits are increasingly studied in invasion biology to understand the mechanisms underlying the success of invasive species. Mosquitofish (*Gambusia holbrooki*) are among the most invasive fish worldwide, with well documented ecological impacts on several taxa such as amphibians and small native fish. It has been previously hypothesized, based on field observations, that salinity limits the invasive success of mosquitofish and provides a competitive refuge for Mediterranean cyprinodonts. We experimentally examined the agonistic behavior and food competition between mosquitofish and an endangered native cyprinodont (*Aphanius fasciatus*) and we tested the role of salinity (0, 15, 25‰) on these interactions. Intraspecific aggressive behavior for both species was not significantly affected by salinity. As salinity increased, mosquitofish decreased their aggressive behavior towards cyprinodonts and captured less prey. In contrast, the cyprinodonts did not change their behavior with different salinity treatments, with the possible exception of increased defensive acts in higher salinities, but captured more prey because the efficiency of mosquitofish was reduced with increasing salinity. Our study confirms previous field observations that salinity limits the invasive success of mosquitofish and provides one of the few experimental demonstrations that it may mediate behavioral and competitive interactions between fish species.

Keywords

Invasive species, interference competition, agonistic behaviour, cyprinodontiforms fish, coastal lagoon.

* Alcaraz C., Bisazza A. & García-Berthou E. (submitted).

INTRODUCTION

Biological invasions are a leading threat to global biodiversity, altering the structure and functioning of ecosystems, particularly freshwater ecosystems (Rahel 2002, MacDougall & Turkington 2005). While the huge ecological and economic consequences of biological invasions (Mack *et al.* 2000) have produced a considerable literature on the biological characterization of invaders (e.g. Ricciardi & Rasmussen 1998, Alcaraz *et al.* 2005), behavioral traits have received little attention (Holway & Suarez 1999, Rehage *et al.* 2005). Determining how behavioral mechanisms contribute to invasive success is also more relevant because successful control requires a detailed understanding of the mechanisms underlying invasion success (Holway & Suarez 1999). Two attributes commonly used to explain the displacement of native fauna and the success of invasive species are interspecific aggression and food competition (Vorburger & Ribi 1999, Rincón *et al.* 2002, Rehage *et al.* 2005).

The mosquitofish (in fact two closely related species, the eastern mosquitofish *Gambusia holbrooki* and the western mosquitofish *G. affinis*) is only native to the United States and Mexico but has been introduced into more than 50 countries (García-Berthou *et al.* 2005) in order to control mosquito populations and hence malaria (Krumholz 1948), being one of the most widespread vertebrates. In southern Europe the eastern mosquitofish was first introduced to the Iberian Peninsula in 1921, was transferred to Italy in 1922 and nowadays is very abundant in most Mediterranean countries (see e.g. Krumholz 1948). Several studies have demonstrated the role of mosquitofish in the decline and local extinction of native amphibians (Hamer *et al.* 2002, Kats & Ferrer 2003) and small fishes (Courtenay & Meffe 1989, Mills *et al.* 2004). Predation by mosquitofish on eggs and larvae, food competition and behavioral interference have been proposed as the causal mechanisms (Arthington & Lloyd 1989, Rincón *et al.* 2002, Mills *et al.* 2004).

The Mediterranean toothcarp (*Aphanius fasciatus*) is a small endemic Mediterranean fish, listed in Annex II (“Strictly protected fauna species”) of the Convention on the Conservation of European Wildlife and Natural Habitats (“Bern Convention”). As with other native Mediterranean cyprinodontiforms, its current distribution has mostly been reduced to polysaline, eusaline and hypersaline waters. Habitat alteration and the introduction of exotic species, mainly mosquitofish that have replaced native cyprinodontiforms in fresh and oligosaline waters, have been previously proposed as causes to explain this reduction (García-Berthou & Moreno-Amich 1992, Bianco 1995). There are no previous studies of the competitive interactions between *Aphanius fasciatus* and mosquitofish and the mechanisms that mediate these interactions. The competitive interactions between *Gambusia holbrooki* and the endemic Spanish cyprinodontiforms *Aphanius iberus* and *Valencia hispanica* have been investigated by Rincón *et al.* (2002) and Caiola & de Sostoa (2005), who experimentally demonstrated that the endemic cyprinodonts decrease feeding rates and receive more aggressions in the presence of mosquitofish. The occurrence and intensity of these potentially harmful effects, however, seemed to be modulated by water temperature, reproductive condition, relative size of the interacting fishes and species-specific habitat preferences (Rincón *et al.* 2002). Whether or not salinity mediates these interactions and the invasive success of mosquitofish is unknown. Although salinity is considered to limit the invasive impact of mosquitofish (Bianco 1995, Nordlie & Mirandi 1996) and to act as a refuge for native fauna from mosquitofish, its importance in behavioral interactions with native species has not been previously investigated. The objectives of this paper are: (i) to experimentally study aggressive behavior and food competition between mosquitofish and toothcarp (*A. fasciatus*); and (ii) to test the role of salinity, over the range of salinities encountered in the wild, in the mediation of the competitive interactions between mosquitofish and toothcarp. Although the interplay of salinity and competition has been well investigated in marsh plant assemblages (e.g. La Peyre *et al.* 2001, Crain *et al.* 2004), we are not aware of similar experimental work with fish.

METHODS

Fish capture and acclimation

Adult eastern mosquitofish (*G. holbrooki*) were collected in July 2005 with a hand net from a stream near Padova, Italy (Idrovia Padova-Venezia, Camin), whereas the toothcarp were obtained from the nearby Valle Averto (Venice lagoon system). All fishes were immediately brought to the laboratory, where they were acclimated before the experiments. Because the salinity in the place of capture was 24‰, the Mediterranean toothcarp were transferred to freshwater (0‰) in a drop-by-drop process with dechlorinated tap water during one week, and then were allowed to acclimate to laboratory conditions for at least two weeks before the start of the salinity adaptation schedule.

Until the experiments began, the mosquitofish and the toothcarp were maintained in groups of 20–25 individuals in several 150 l stock aquaria provided with natural gravel and an air filter. The aquaria were provided with artificial plants to avoid changing vegetation in the different salinity treatments, were illuminated with 15 W fluorescent lights and were maintained at a constant temperature (25 ± 1ºC) and photoperiod (08:00–21:00 hours).

The salinity adaptation protocol was done in the same tanks and consisted of the progressive renewal of the fresh water with hypersaline water (50‰, prepared by adding sea salt to dechlorinated tap water) through a drop-by-drop process until the experimental conditions (0, 15 and 25‰) were reached. Salinity was measured using an optical refractometer (with a precision of 1‰, compensated for temperatures of 10 to 30ºC), three times per day to regulate the water input to a maximum salinity increase of 2–3‰ per day. After 10 days all fishes were at the necessary experimental salinity and were maintained in these conditions for at least two weeks before starting the experiment. Mortality during acclimation was low (<5%). Fish were fed to satiation twice daily (in the morning and in the evening) with commercial food flakes, live *Artemia* nauplii and frozen dipteran larvae (*Chironomus salinarius*).

Experiments were done in three adjacent aquaria, one for each salinity treatment. The aquaria were of 96 l (60 × 40 cm and 40 cm high), filled with 32 cm of water and 2 cm of natural gravel, illuminated by two 15 W fluorescent lights and maintained at the same laboratory temperature (25 ± 1ºC). The apparatus was placed in a dark quiet room. A grey nylon net (2 × 2 mm) was attached against the glass wall inside the tank as a one-way screen to prevent the fish from seeing the observer and from distractions produced by the mirror effect.

All trials were videotaped for later observation and analysis. After testing, the standard length of the fish was measured with a digital caliper to the nearest 0.01 mm (Table 1). To ensure that fish were used only once during the experiments, they were placed into a post-experimental tank after the trials. No mortality was observed during the tests.

Only females were used in the trials. Both mosquitofish and toothcarp are dimorphic species, females being larger than males, and the sex ratio is usually skewed in favor of females (Snelson 1989, Leonardos & Sinis 1999). Although both mosquitofish sexes are highly aggressive towards other species (including fish much larger than themselves), females are particularly aggressive, attacking other fishes, shredding their fins, and sometimes killing them (Courtenay and Meffe 1989). The effect on the female toothcarp is more important in demographic terms as females are generally more abundant because of higher survival rates and greater longevity (Leonardos & Sinis 1998, 1999), like in many other cyprinodonts (Fernández-Delgado *et al.* 1988, Keivany & Soofiani 2004).

Table 1. Standard lengths (mm) of fish used in the experiments (mean ± standard deviation).

Experiment	Species	*N*	Salinity 0 ‰	 15 ‰	 25 ‰
Aggressive behaviour	*G. holbrooki*	20	32.0 ± 2.3	32.6 ± 3.1	31.1 ± 2.2
	A. fasciatus	20	37.6 ± 2.7	39.1 ± 3.0	36.6 ± 5.0
Food competition	*G. holbrooki*	24	35.0 ± 2.9	34.5 ± 2.9	33.5 ± 2.4
	A. fasciatus	24	37.7 ± 3.1	40.5 ± 4.1	38.5 ± 5.4

Experiment 1: Aggressive behaviour

In this experiment we studied the effects of salinity on agonistic interactions between mosquitofish and toothcarp. We used a two-way factorial design with three species treatments (1 mosquitofish and 1 toothcarp: 2 mosquitofish and 1 toothcarp: and 1 mosquitofish and 2 toothcarp) and three salinity treatments (0‰, 15‰ and 25‰), with 5 replicates per treatment combination. The species treatment used is a kind of response surface experimental design, which in contrast to additive or substitutive designs varies the densities of the two competing species independently, hence allowing the effects of fish density to be distinguished from the effects of species composition within competitive interactions (Inouye 2001). No food was provided 24 h before the experiment. The evening before the trial, fish were transferred to the experimental tanks and isolated by a transparent methacrylate wall. The following morning the wall was gently raised to the surface avoiding the fall of drops that might scare the fishes and then we waited for the fish to behave normally and for the first interaction between them. Thereafter, the following behavioral parameters were counted for each of the two species during 30 minutes: number of orientations (fish orienting itself and swimming towards another fish), nips, chases, and defensive acts (fish responding to an aggressive act started by other fish).

Experiment 2: Food competition

In this experiment we tested the effect of salinity on the proportion of prey captured by mosquitofish and toothcarp. The apparatus was similar to that used in a previous study of *Gambusia holbrooki* (Pilastro *et al.* 2003). In each aquarium, we placed five equally spaced plastic barriers (11 × 40 cm) to simulate plants in the natural environment. Each barrier was composed of a series of elongated bars, 1 cm wide and 0.5 cm apart, virtually dividing the aquarium in six identical sectors. Three mosquitofish and three toothcarp were introduced to the apparatus 20 min before starting the test (8 replicates per salinity). Previous trials showed that in a lower density, toothcarp did not behave normally and did not feed. After 20 min, 10 prey items (frozen *Chironomus salinarius*) placed at equal distances on a methacrylate sheet (70 × 2.5 cm) were released at the water surface. The sheet was turned upside down and the food items were scattered over the six sectors (one item at the two end sectors and two at the other). We recorded the time taken to eat each of the ten prey items and the species involved.

Statistical analyses

Differences among treatments in length of the fish used in the experiments, behavioral parameters and the proportion of prey items captured were analyzed with analysis of variance (ANOVA). In Experiment 2, the time needed for individual mosquitofish to capture the different prey items was analyzed with a repeated-measure ANOVA. Significant ANOVAs were followed by polynomial orthogonal contrasts (Sokal & Rohlf 1995) for the salinity factor and deviation contrasts (that compare each treatment with the overall mean) for the species treatment. All factors were considered as fixed effects. We used partial eta squared (η^2) as a measure of effect size. Like r^2, partial eta squared is the proportion of variation explained for a certain effect (effect SS / (effect SS + error SS)) and has the advantage over eta squared (effect SS / total SS) of not depending on the number of sources of variation used in the ANOVA design, because it does not use the total sum-of-squares (SS) as the denominator (Tabachnick & Fidell 2001: 191).

Lengths were log transformed, behavioral frequencies were square root transformed ($\sqrt{(X + 0.5)}$) and the proportion of prey items captured was arcsin transformed ($\arcsin \sqrt{X}$) (Sokal & Rohlf 1995) for statistical analyses, because homocedasticity and normality were clearly improved. All statistical analyses were performed with SPSS 13.

RESULTS

Experiment 1: Aggressive behaviour

The standard length of mosquitofish and toothcarp (Table 1) used in the aggressive behavior experiment did not show significant differences among treatment combinations (ANOVA, $P > 0.27$). The majority of aggressive acts were performed by mosquitofish on toothcarp (66.3%), whereas only about 21.1% of them were done by toothcarp on mosquitofish (Table 2). Mosquitofish performed significantly more orientations, chases and nips towards toothcarp in freshwater (0‰) and their aggressiveness decreased with increasing salinity (Table 2, Fig. 1). For both orientations and nips, the number of aggressive acts performed by mosquitofish on toothcarp significantly decreased (deviation contrasts $P < 0.05$) in the following species treatment sequence: 1 mosquitofish + 2 toothcarp > 2 mosquitofish + 1 toothcarp > 1 mosquitofish + 1 toothcarp (Fig. 2). Therefore, there was a density effect as well as a species composition effect. Although the same pattern was observed for the number of chases among species treatments, no significant differences were observed (Table 2, Fig. 2). For the number of orientations and nips performed, salinity (partial $\eta^2 = 0.416$ and 0.187 respectively) was a more important factor than treatment (partial $\eta^2 = 0.238$ and 0.155) or their interaction (partial $\eta^2 = 0.026$ and $\eta^2 = 0.031$).

In contrast, the toothcarp did not modify their aggressive behavior with salinity or species treatments, and only showed marginally significant effects of salinity on defensive acts (Table 2), in agreement with the less aggressive behavior of mosquitofish at higher salinity. Moreover, we observed that the number of aggressive acts (orientations, nips and chases) performed by mosquitofish towards toothcarp was higher than the number performed by toothcarp on mosquitofish in fresh water (0‰) and 15‰ salinity (*t*-student tests, $P < 0.001$ and $P < 0.005$ respectively), but no significant differences were observed at 25‰ salinity ($P > 0.28$). Therefore the mosquitofish reduced their aggressive behavior with salinity, whereas the toothcarp hardly modified it, both species having the same aggressiveness at the highest salinity treatment.

Salinity × species interactions were not significant for all the behavioral variables (Table 2), so the effects of salinity were similar for the three species treatments. Furthermore, salinity had no significant effect on intraspecific behavior relationships for both species (Table 2).

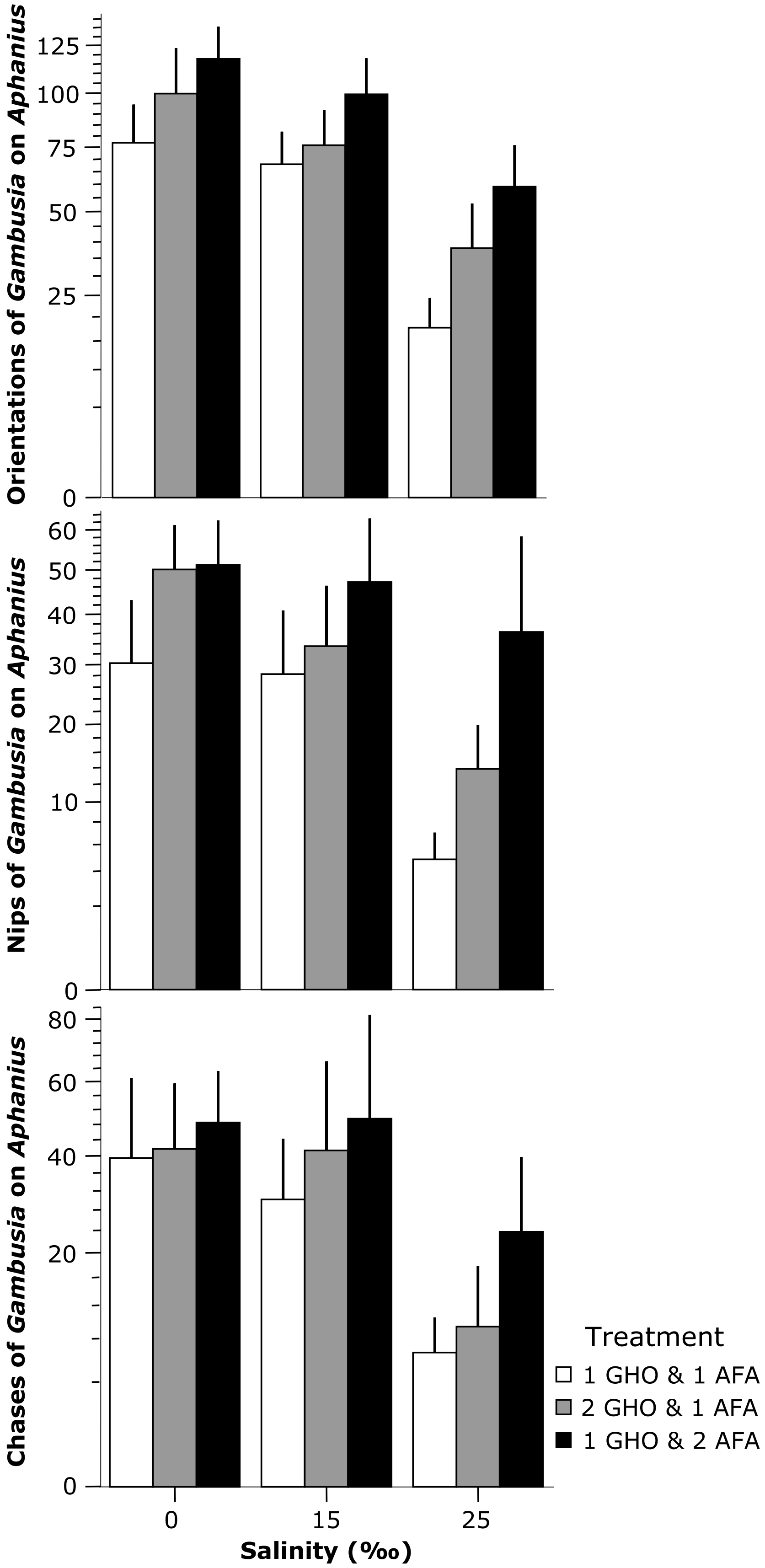

Figure 1. Number of aggressive acts (orientations, nips and chases) performed by mosquitofish towards toothcarp at the different species (GHO = *G. holbrooki*, AFA = *A. fasciatus*) and salinity treatments. Standard error (error bar) is shown.

Table 2. Two-way ANOVAs of the behavioural variables (Or, orientations; Ni, nips; Ch, chases; and Da, defensive acts) with the experimental factors (salinity and species treatment). Only the linear component of the polynomial contrasts for salinity is shown because all quadratic contrasts were not significant ($P >> 0.10$). Variables were square root transformed. The overall average (mean number of acts during the 30 min trial) for each behavioural variable is also given.

Behavioural variables		Mean number	Salinity			Linear contrast	Species Treatment		Salinity × Treatment	
			F	df	P	P	$F_{2, 36}$	P	$F_{4, 36}$	P
Gambusia on *Aphanius*	Or	72.4	12.8	2, 36	<0.0001	<0.0001	4.14	0.024	0.91	0.91
	Ni	32.7	5.61	2, 36	0.008	0.003	3.31	0.048	0.29	0.88
	Ch	32.3	3.31	2, 36	0.048	0.019	0.61	0.55	0.07	0.99
	Da	0.1	1.49	2, 36	0.24	0.093	1.05	0.36	1.31	0.29
Aphanius on *Gambusia*	Or	31.5	0.56	2, 36	0.58	0.42	1.41	0.26	0.64	0.64
	Ni	7.1	0.89	2, 36	0.42	0.60	0.31	0.74	0.31	0.87
	Ch	5.3	0.37	2, 36	0.69	0.87	1.57	0.22	0.54	0.70
	Da	9.3	2.92	2, 36	0.067	0.021	0.21	0.81	0.41	0.80
Gambusia on *Gambusia*	Or	24.1	0.10	2, 12	0.91	0.75				
	Ni	15.5	0.45	2, 12	0.65	0.83				
	Ch	14.1	0.65	2, 12	0.54	0.65				
	Da	0.2	1.20	2, 12	0.33	0.15				
Aphanius on *Aphanius*	Or	13.1	1.19	2, 12	0.83	0.55				
	Ni	7.5	0.49	2, 12	0.62	0.35				
	Ch	7.8	0.29	2, 12	0.75	0.71				
	Da	0.3	1.38	2, 12	0.29	0.13				

Experiment 2: Food competition

The proportion of prey items captured for both species varied with salinity. The mosquitofish captured significantly less prey items in higher salinity treatments ($F_{2, 21} = 5.74$, $P = 0.010$), showing a significant linear relationship (linear contrast, $P = 0.006$) (Fig. 2). In fresh water (0‰), the toothcarp did not capture any prey item because they were all captured by mosquitofish. With increasing salinity, the mosquitofish needed significantly more time to find and capture prey (repeated-measure ANOVA: time × salinity, Pillai's $F_{8,\,32} = 2.10$, $P = 0.066$; linear contrast of salinity, $P = 0.020$) (Fig. 2).

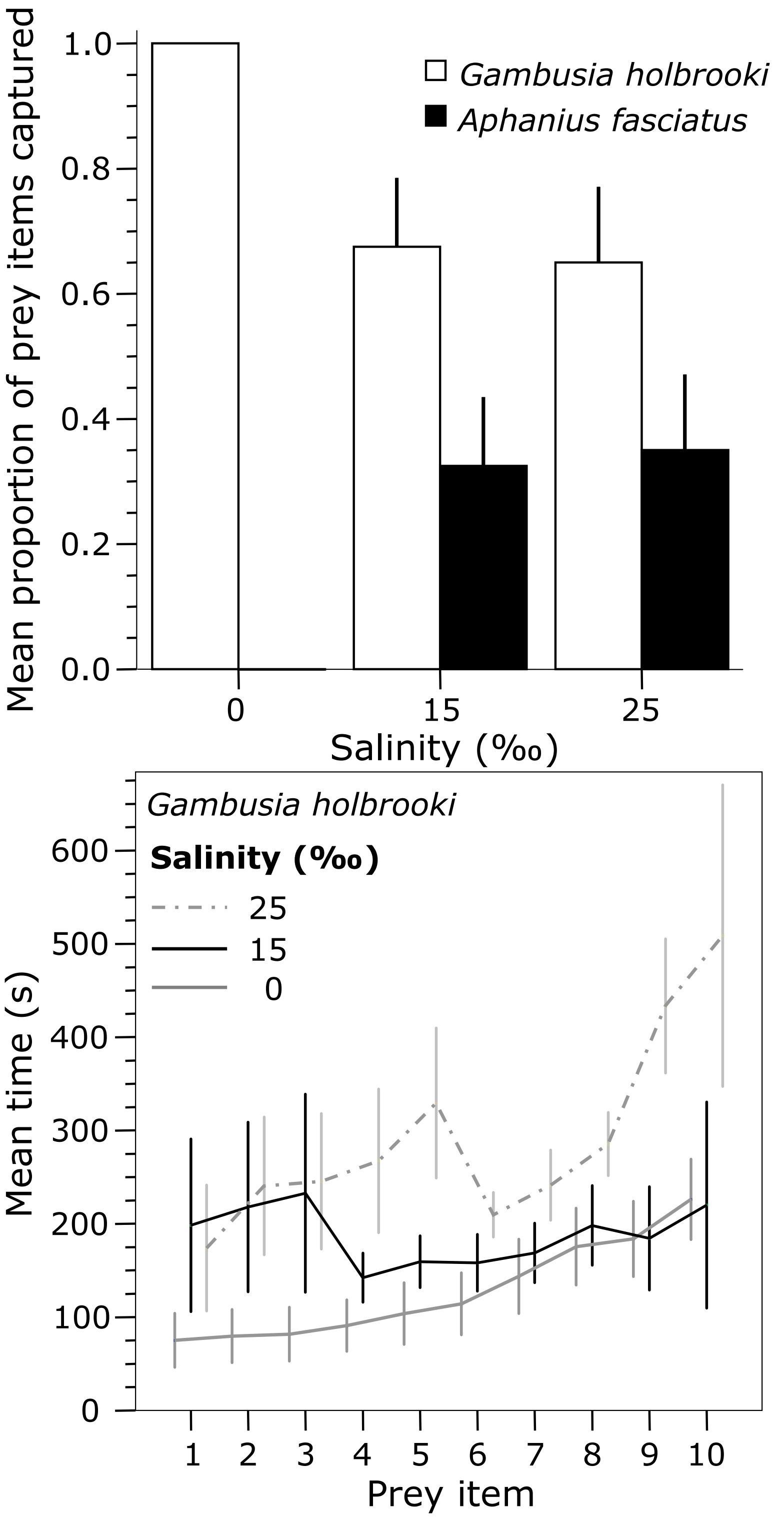

Figure 2. Effects of salinity on the number of prey items captured by mosquitofish and toothcarp (top) and the mean time employed by mosquitofish to capture the ten prey items (bottom). Bar are standard errors.

DISCUSSION

We present the first experimental evidence that the competitive interactions between introduced mosquitofish (*Gambusia holbrooki*) and the native toothcarp (*Aphanius fasciatus*) are mediated by salinity: the mosquitofish decreased their aggressiveness towards the toothcarp and captured less prey as salinity increased. In contrast, the toothcarp did not change their aggressive behavior towards mosquitofish with salinity, except perhaps for increased defensive acts, and captured more prey in higher salinity because of the reduced mosquitofish efficiency. Several points indicate that these results are not artifactual: (i) the speed and range of salinity changes in our experiment are well below those often experienced by both species in their natural environments; (ii) the two intraspecific relationships and the interspecific agonistic behavior of toothcarp towards mosquitofish did not change with salinity, and the only significant result was aggressive mosquitofish behavior towards the toothcarp; and (iii) salinity × species interactions were not significant. It is particularly noteworthy that, although the orientations of mosquitofish on mosquitofish and the orientations of toothcarp on mosquitofish were also frequent, unlike the number of aggressive acts of mosquitofish towards the toothcarp, they did not vary with salinity, suggesting that what was mainly affected was the relative performance of mosquitofish versus toothcarp. Note also that, in contrast to the mosquitofish, the toothcarp suffered two acclimation processes (from polysaline water to fresh water and then acclimation to experimental salinity) and yet did not change their aggressive behavior with salinity treatments.

It has often been thought that salinity limits the invasive success of mosquitofish, displacing native cyprinodonts to saltier waters, which serve as a refuge from mosquitofish (Bianco 1995, Nordlie & Mirandi 1996, Doadrio 2002). There were no previous data on the mechanisms by which salinity mediates the competitive interaction between these species. Both toothcarp and mosquitofish are secondary freshwater, euryhaline fish species. The only previous studies on experimental mosquitofish salinity tolerance limits were done by Chervinski (1983) and Nordlie & Mirandi (1996). Nordlie & Mirandi (1996) reported an upper tolerance limit under 30‰ in *G. holbrooki*, whereas Chervinsky (1983) showed that *G. affinis,* a very close species, was able to tolerate direct transfer to 19.5‰ salinity and that 65% of the fishes survived in sea water (39‰) while 50% survived in water at 58.5‰ salinity. However, records of mosquitofish in eusaline or hypersaline waters are very scarce. There are no similar data available for *A. fasciatus,* but experiments in similar species such as *A. dispar* (Plaut 2000) and *A. iberus* (Oltra & Todoli 2000), field observations of *A. fasciatus* in hypersaline lagoons with four to five times the sea salinity (Bianco 1995, Leonardos & Sinis 1998), and our findings of no modification of *A. fasciatus* behavior with salinity treatments support that the upper salinity tolerance limit for *A. fasciatus* is higher than for mosquitofish. Therefore, the toothcarp is more euryhaline than the mosquitofish, and tolerates a wider range of salinity. At our experimental salinities the toothcarp was still within its optimum.

Salinity adaptation by euryhaline teleosts is a complex process involving a suite of physiological and behavioral responses to environments with differing osmoregulatory requirements (Swanson 1998). Salinity is expected to affect metabolism through changes in energy expenditure for osmoregulation and energy costs are expected to increase in water that deviates from the isosmotic level because fish must impose regulatory energy costs for active ion transport and create a water flow in the opposite direction to the passive flow imposed by the osmotic gradient (Swanson 1998, Plaut 2000). The metabolism response to an increase over the salinity tolerance limit is similar in all euryhaline cyprinodontiform fishes. A decrease in the routine metabolic rate and an increment of plasma osmotic concentration (a measure of stress) (Nordlie 1987, Nordlie *et al.* 1992, Haney *et al.* 1999) has been previously reported for several cyprinodontiform fishes. This decrease of metabolism has substantial ecological effects, since swimming capacity and activity rate are expected to decrease, reducing the ability of fish to capture prey, to avoid predation, and to perform other critical survival functions (Plaut 2000). A previous study of *Gambusia holbrooki* (Nordlie & Mirandi 1996) reported that the mean plasma osmotic concentration for mosquitofish increased with salinity, showing the best osmotic regulation from freshwater to 10‰ and a loss of regulatory capability near the upper tolerance limit (25-30‰) and exhibiting limited tolerance and limited plasma osmotic regulatory abilities at elevated salinities near to those of the sea. With regard to *A. fasciatus,* there are no data available but a very similar species, *A. dispar,* decreased its oxygen consumption, critical swimming speed and routine activity rate with increasing salinity (at extreme salinities over 105‰) (Plaut 2000). Therefore, since the toothcarp is more euryhaline than the mosquitofish, when both species were exposed to a salinity increase, the overall performance of the mosquitofish was more affected than that of the toothcarp and its overall performance significantly decreased, reducing the number of aggressive acts towards the toothcarp and capturing less prey.

The reduction of activity and behavioral changes experienced by the mosquitofish with increasing salinity stress was determined in our study and the better relative performance of *A. fasciatus* agrees with the current distribution of mosquitofish and Mediterranean native cyprinodontiforms. Mosquitofish are abundant in most Mediterranean lowland waters. Native cyprinodontiforms are usually restricted to lagoons that present a wide range of salinities but are hypersaline in summer, thus not allowing mosquitofish survival. Our study is the first experimental evidence that salinity mediates the competitive interactions between invasive mosquitofish (*Gambusia holbrooki*) and native cyprinodontiforms and confirms the field observations that polysaline, eusaline and hypersaline waters constitute a competitive refuge for the latter. Further conservation implications might emerge if other species and ecosystems affected by mosquitofish also showed similar patterns.

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Conclusions

1. The taxonomic composition of invasive Iberian fish fauna deviated significantly from world freshwater richness and in contrast to native species, invasive species belonged to only five taxonomic orders (Cypriniformes, Salmoniformes, Perciformes, Cyprinodontiformes, and Siluriformes) but to a wide spectrum of families without natives species in the Iberian Peninsula.

2. Without correcting for phylogeny, there were no significant differences on the ecological traits between native and invasive species but after controlling for phylogeny, midpoint of reproductive season, mean latitude and latitude range became different between native and invasive species, because many fish life-history traits are highly dependent on phylogeny.

3. Taxonomy and human use are the main factors differentiating native and invasive fish species in the Iberian Peninsula. Human interests in fish introductions are diverse and thus obscure life-history trait characterisation of invasive fish species.

4. We investigated the role of resource availability and prey electivity in the ecology of the Spanish toothcarp (*Aphanius iberus*). The density of toothcarp was very low in the open water, since toothcarp mostly inhabited vegetated habitats. The habitat that is eventually inundated (glasswort) had more density of mature toothcarp, but immature fish were similarly abundant in glasswort and algal mat habitats. Condition (weight-length relationship) and total food biomass in the gut contents of immature toothcarp were higher in algal mats than in glasswort, the poorer condition of immatures being due to lower ingestion rate in the latter habitat.

5. Toothcarp’s diet was omnivorous, dominated by harpacticoid copepods, copepod nauplii and detritus. However, diet composition strongly varied among habitats, depending on prey availability. Although a benthic feeding habitat has previously been suggested, in our study the diet was based rather on water column organisms for both glasswort and algal mat habitats.

6. There was also an ontogenetic diet shift, with an increase of mean prey length with fish length, clearly linked to a microhabitat change. Smaller fish showed positive electivity on planktonic prey, while larger fish elected more benthic organisms. Among habitats, microhabitat change in diet was more apparent in algal mats than glasswort because the shallowness of the latter habitat.

7. Although it has been previously hypothesized that salinity limits invasive success of mosquitofish (*Gambusia holbrooki*), and constitutes a refuge for native cyprinodontiforms (such as *Aphanius* spp.), there a few studies on how salinity affects the ecology of mosquitofish. In an observational study from fresh (near ca. 0.2‰ of salinity) to polysaline waters (ca. 23‰) we observed that both mosquitofish sexes decreased in density and increased their reproductive investment (higher gonadal weight and GSI values) with salinity, at the cost of somatic condition in the case of females.

8. The increase of reproductive investment in females at higher salinity also implied an increase in total weight, in contrast to the significant decrease of somatic weight, indicating that the latter is a better indicator of condition.

9. Females from higher salinity waters reproduced earlier, since they presented embryos on more advanced developmental stages, and presented heavier embryos (independently of the maturation state, after correction for brood and fish size).

10. We observed a weight gain of the oocytes during the developmental process instead of the previously observed loss of weight; therefore although some North American and Iberian populations of *Gambusia holbrooki* have been shown to be lecithotrophic, our data show that some populations can adopt a matrotrophic strategy (i.e. mothers nourishing the embryos).

11. We have experimentally demonstrated that mosquitofish decreased its aggressive behaviour and captured less prey with increasing salinity. Whereas an endangered cyprinodontiform (*Aphanius fasciatus*) did not change its behaviour with salinity, but benefited from the reduced efficiency of mosquitofish.

12. The observed effects of salinity on mosquitofish life history and the first experimental evidence that salinity mediates the competitive interactions between invasive mosquitofish (*Gambusia holbrooki*) and a native cyprinodontiform confirms previous field observations that salinity limits mosquitofish invasive success, with polysaline, eusaline and hypersaline waters constituting a competitive refuge for native cyprinodontiforms.

Conclusiones

1. La composición taxonómica de la fauna piscícola ibérica se desvió significativamente de la riqueza mundial de aguas continentales y en contraste con las especies nativas, las invasoras pertenecieron tan solo a cinco ordenes taxonómicos (Cypriniformes, Salmoniformes, Perciformes, Cyprinodontiformes, y Siluriformes) pero a un amplio espectro de familias sin representantes nativos en la Península Ibérica.

2. Sin aplicar la corrección filogenética, no hubo diferencias significativas en las características ecológicas de especies nativas e invasoras, pero después de controlar el efecto de la filogenia, el punto medio de la época reproductora, latitud media y el rango latitudinal fueron diferentes entre especies invasoras y nativas, ya que muchas características de la biología reproductiva son altamente dependientes de la filogenia.

3. La taxonomía y los usos por parte del hombre son los principales factores que diferencian las especies de peces nativos e invasores en la Península Ibérica. Los intereses humanos en las introducciones de peces son diversos y esto oscurece la caracterización de la biología reproductiva de las especies de peces invasores.

4. Hemos investigado el papel de la disponibilidad de recursos y la selección de presas en la ecología del fartet (*Aphanius iberus*). La densidad de fartet fue muy baja en aguas abiertas, ya que el fartet mayoritariamente se encontraba en los hábitats con vegetación. El hábitat ocasionalmente inundado (salicornia) tenía mayor densidad de fartet adultos, pero los juveniles presentaron abundancias similares en la salicornia y las algas verdes. La condición (relación peso-longitud) y el contenido total de biomasa en los estómagos de los juveniles de fartet fue más alto en las algas verdes que en la salicornia, esta condición inferior de los juveniles estaba debida a una tasa de ingestión inferior en la salicornia.

5. La dieta del fartet resultó ser omnívora, dominada por copépodos harpacticoides, nauplios de copépodos y detritos. No obstante, la composición de la dieta varió fuertemente entre hábitats, dependiendo de la disponibilidad de presas. A pesar de que previamente se había sugerido un hábito alimentario bentónico, en nuestro estudio la dieta estaba basada mayoritariamente en organismos de la columna de agua en ambos hábitats, tanto en la salicornia como en las algas verdes.

6. También existió un cambio ontogenético en la dieta, con un incremento de la longitud media de las presas con la longitud de los peces, claramente unido a un cambio de microhábitat. Los fartets más pequeños seleccionaron positivamente presas planctónicas, mientras los más grandes seleccionaron organismos más bentónicos. Entre hábitats, el cambio de microhábitat fue más aparente en las algas verdes que en la salicornia por la reducida profundidad de éste.

7. A pesar de que previamente se ha planteado que la salinidad puede limitar el éxito invasor de la gambusia (*Gambusia holbrooki*), y constituye un refugio para los ciprinodontiformes nativos (por ejemplo *Aphanius* spp.), sólo hay unos pocos estudios sobre como afecta la salinidad la ecología de la gambusia. En un estudio observacional desde agua dulce (aprox. 0,2‰ de salinidad) hasta aguas polisalinas (aprox. 23‰), encontramos que tanto los machos como las hembras de gambusia redujeron su densidad e incrementaron el esfuerzo reproductivo (mayor peso gonadal y valores de GSI) con la salinidad, a costa de la condición somática en el caso de las hembras.

8. El incremento del esfuerzo reproductivo en las hembras de salinidades superiores también implicó un incremento en el peso total, en contra de la reducción significativa del peso somático, mostrando que este último es un mejor indicador de la condición.

9. Las hembras de salinidades superiores se reproducen antes, ya que presentaron embriones en estados más avanzados de desarrollo y embriones más grandes (independientemente del estado de maduración y después de eliminar el efecto del tamaño de la puesta y la longitud del pez).

10. Observamos una ganancia de peso de los oocitos durante el proceso de desarrollo en lugar de la pérdida de peso previamente observada; por lo tanto a pesar de que algunas poblaciones norteamericanas e ibéricas de *Gambusia holbrooki* se han mostrado como lecitotróficas, nuestros datos muestran como alguna poblaciones pueden adoptar una estrategia matrotrófica (es decir, la madre nutre los embriones durante su desarrollo).

11. Experimentalmente hemos demostrado que la gambusia reduce su agresividad y captura menos presas con el incremento de salinidad. Mientras un ciprinodóntido en peligro de extinción (*Aphanius fasciatus*) no cambió su comportamiento con la salinidad, pero resultó beneficiado de la reducción en la eficiencia de la gambusia.

12. Los efectos de la salinidad observados en la biología reproductiva de la gambusia y la primera evidencia experimental que la salinidad media la interacción competitiva entre la invasora gambusia (*Gambusia holbrooki*) y un ciprinodontiforme nativo confirma las observaciones previas que la salinidad limita el éxito invasor de la gambusia, constituyendo las aguas polisalinas, eusalinas e hipersalinas un refugio competitivo para los ciprinodontiformes nativos.

Conclusions

1. La composició taxonòmica de la fauna piscícola ibèrica es va desviar significativament de la riquesa mundial de les aigües continentals i en contrast a les espècies natives, les invasores van pertànyer a només cinc ordres taxonòmics (Cypriniformes, Salmoniformes, Perciformes, Cyprinodontiformes, i Siluriformes) però a un ampli rang de famílies sense representants a la Península Ibèrica.

2. Sense aplicar la correcció filogenètica, no hi va haver diferències significatives en les característiques ecològiques d'espècies natives i invasores, però després de controlar l'efecte de la filogènia, el punt mig de l'època reproductiva, la latitud mitjana i el rang latitudinal van ser diferents entre especies invasores i natives, ja que moltes de les característiques de la biologia reproductiva són molt dependents de la filogènia.

3. La taxonomia i els usos per part de l'home són els principals factors que diferencien les espècies de peixos natius i invasors a la Península Ibèrica. Els interessos humans en les introduccions de peixos són diversos i això obscureix la caracterització de la biologia reproductiva de les espècies de peixos invasors.

4. Hem investigat el paper de la disponibilitat de recursos i la selecció de preses en la ecologia del fartet (*Aphanius iberus*). La densitat de fartet va ser molt baixa en aigües obertes, ja que el fartet majoritàriament es trobava en ela hàbitats amb vegetació. L'hàbitat ocasionalment inundat (salicòrnia) tenia major densitat de fartet adults, però els juvenils presentaren abundàncies similars a la salicòrnia i a les algues verdes. La condició (relació pes-longitud) i el contingut total de biomassa en els estómacs dels juvenils de fartet va ser més alt a les algues verdes que a la salicòrnia, aquesta condició inferior dels juvenils estava deguda a una taxa d'ingestió inferior en la salicòrnia.

5. La dieta del fartet va resultar omnívora, dominada per copèpodes harpacticoids, nauplis de copèpodes i detritus. No obstant, la composició de la dieta varia molt entre hàbitats, dependent de la disponibilitat de preses. Encara que prèviament se li havia suggerit un hàbit alimentari bentònic, en el nostre estudi la dieta estava basada majoritàriament en organismes de la columna d'aigua en ambdós hàbitats, tant a la salicòrnia com a les algues verdes.

6. També va existir un canvi ontogenètic a la dieta, amb un increment de la longitud mitjana de les preses amb la longitud dels peixos, clarament unit a un canvi de microhàbitat. Els fartets més petits seleccionaren positivament preses planctòniques, mentre que els més grans van seleccionar organismes més bentònics. Entre hàbitats, el canvi de microhàbitat va ser més aparent en les algues verdes que en la salicòrnia per la reduïda profunditat d'aquest.

7. Tot i que prèviament s'ha hipotetitzat que la salinitat limita l'èxit invasor de la gambúsia (*Gambusia holbrooki*), i constitueix un refugi pels ciprinodontiformes natius (per exemple *Aphanius* spp.), tan sols hi ha uns pocs estudis sobre com afecta la salinitat l'ecologia de la gambúsia. En un estudi observacional des de aigua dolça (aprox. 0,2‰ de salinitat) fins a aigües polihalines (aprox. 23‰), trobem que tant els mascles com les femelles de gambúsia van reduir la seva densitat i van incrementar l'esforç reproductiu (major pes gonadal i valor de GSI) amb la salinitat, a costa de la condició somàtica en el cas de les femelles.

8. L'increment d'esforç reproductiu en les femelles de salinitats superiors també va implicar un increment en el pes total, en contra de la reducció significativa del pes somàtic, mostrant que aquest últim és un millor indicador de la condició.

9. Les femelles de salinitats superiors es reprodueixen abans, ja que van presentar embrions en estats més avançats de desenvolupament, i van presentar embrions més grossos (independentment de l'estat de maduració i després d'eliminar l'efecte de la mida de la posta i de la longitud del peix).

10. Vam observar un guany de pes durant el procés de desenvolupament en lloc de la pèrdua de pes dels oòcits prèviament observada,; per tant, encara que algunes poblacions nord-americanes i ibèriques de *Gambusia holbrooki* s'han mostrat com a lecitotròfiques, les nostres dades mostren que algunes poblacions poden adoptar una estratègia matrotròfica (és a dir, la mare nodreix els embrions).

11. Experimentalment hem demostrat que la gambúsia redueix la seva agressivitat y captura menys preses amb l’increment de salinitat. Mentre que un ciprinodòntid en perill d’extinció (*Aphanius fasciatus*) no va canviar el seu comportament amb la salinitat, però en va resultar beneficiat de la reducció en la eficiència de la gambúsia.

12. Els efectes de la salinitat observats en la biologia reproductiva de la gambúsia i la primera evidència experimental que la salinitat regula la interacció competitiva entre la invasora gambúsia (*Gambusia holbrooki*) i un ciprinodontiforme natiu confirma les observacions prèvies que la salinitat limita l’èxit invasor de la gambúsia, constituint les aigües polihalines, eusalines i hipersalines un refugi competitiu pels ciprinodontiformes natius.