# Universidad de Huelva

## Departamento de Biología Ambiental y Salud Pública

# Ecología, distribución y conservación de peces continentales en el cuadrante suroccidental ibérico

**Memoria para optar al grado de doctor**
**presentada por:**

**Francisco Blanco Garrido**

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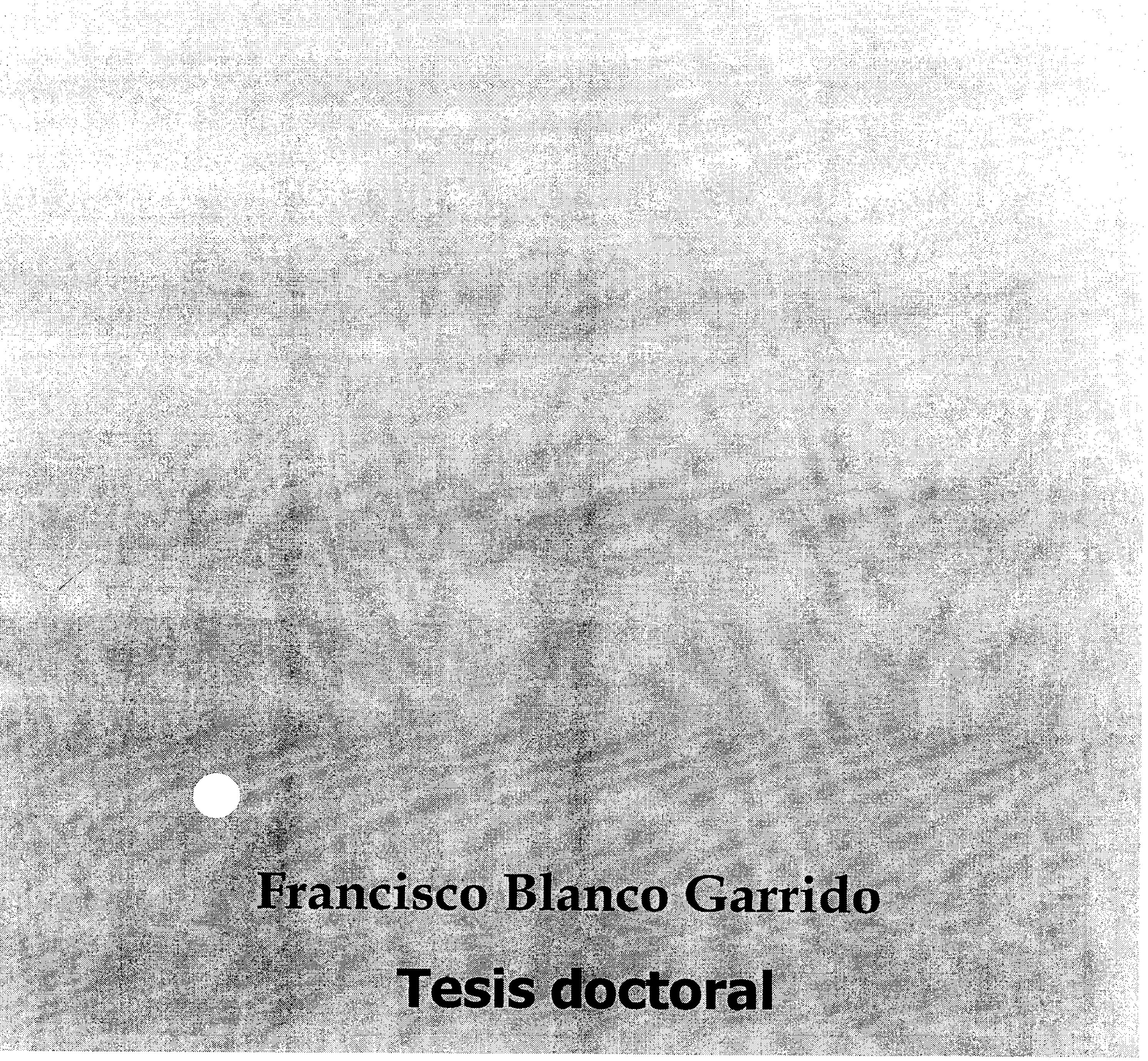

Francisco Blanco Garrido

**Tesis doctoral**

Universidad
de Huelva

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**Memoria presentada por el licenciado Francisco Blanco Garrido para optar al grado de Doctor por la Universidad de Huelva**

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**LICENCIADO**

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**Huelva, de Julio de 2006**

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# CAPÍTULO 1

# SÍNTESIS GENERAL

# INTRODUCCIÓN

Los ecosistemas de aguas continentales, y entre ellos los sistemas de aguas corrientes, se encuentran en la actualidad entre los más amenazados de todos los existentes en el planeta (Saunders et al., 2002). Sufren de forma generalizada contaminación de distinto origen (industrial, agrícola-ganadera y urbana), desecación, eliminación de la vegetación de las orillas, encauzamiento de cursos de agua y regulación de caudales (Dynesius y Nilson, 1994; Saunders et al., 2002). Además, el agua es un factor imprescindible para el desarrollo de las civilizaciones. De hecho, la gran mayoría de las sociedades se han asentado en aquellas zonas con fácil acceso a tan preciado recurso. Por ello, las obras hidráulicas, como embalses, canalizaciones y encauzamientos de ríos son ciertamente una de las más antiguas y extendidas obras de ingeniería (Matheny, 1976; Baxter, 1977).

En la actualidad la mayor parte de la superficie terrestre se encuentra drenada por ríos regulados (Petts, 1984) y se estima que alrededor de la mitad del agua dulce disponible en el planeta está retenida en más de 800.000 embalses (Rosemberg et al., 2000). La regulación de los caudales ha llegado a tal extremo que en algunos grandes ríos del planeta como el Nilo, el Ganges, el Río Amarillo o el Río Colorado, el agua circulante apenas llega al mar en momentos concretos del año (Postel, 1995). A pesar de ello, se estima que aproximadamente dos tercios de la población humana mundial tendrá problemas de abastecimiento de agua para el año 2025 (Szöllosi-Nagy et al., 1998). Estas presiones sobre el medio acuático continental han causado, están causando y causarán una profunda modificación de los hábitats existentes, llevando aparejada una irreversible pérdida de biodiversidad (Moyle y Leidy, 1992; Allan y Flecker, 1993; Pringle et al., 2000).

Mucha de la fauna que depende de las aguas continentales está en serio peligro de desaparecer (Saunders et al., 2002), especialmente los peces continentales. Estos sufren especialmente la degradación de los hábitats acuáticos: aun siendo el grupo de vertebrados más diverso, es el que posee una mayor proporción de especies amenazadas de extinción (Duncan y Lockwood, 2001).

Los cursos de agua de la Península Ibérica son enormemente dependientes de las fluctuaciones típicas del clima mediterráneo. Existe un marcado ciclo anual en la circulación de los caudales, con máximos en invierno y mínimos en la época veraniega, cuando coinciden los máximos de temperatura anuales y el máximo estrés hídrico (Gasith y Resh, 1999). A estas variaciones intra-anuales predecibles habría que sumar otras de carácter más impredecible, relacionadas con la variación en la cantidad de precipitación registrada en distintos años (variaciones inter-anuales, Gasith y Resh, 1999). Esta adversidad ecológica, además de ejercer una profunda influencia sobre las comunidades acuáticas y ser un elemento clave en su configuración (Prenda y Gallardo, 1996; Pires et al., 1999; Magalhães et al., 2002), ha determinado la regulación masiva de la mayoría de los cursos de agua ibéricos. Valga como ejemplo que sólo en España hay construidos más de 1000 grandes embalses, que proporcionan en conjunto más kilómetros de costa interior que de costa exterior o real (MMA, 1998). Al mismo tiempo, la Península Ibérica se caracteriza por la existencia de una compleja red de cuencas fluviales donde las biocenosis acuáticas de cada cuenca permanecen totalmente aisladas de las del resto de cuencas. La combinación de condiciones climáticas adversas junto con este aislamiento natural, ha dado como resultado un marco ecológico y evolutivo en el que ha surgido una ictiofauna con el porcentaje de endemismos más elevado de Europa (Doadrio, 2001). Este marco ecológico excepcional está siendo intensamente alterado por la transformación y destrucción de los hábitats fluviales lo que, además, está favoreciendo la proliferación de especies exóticas (Herbold y Moyle, 1986; Ross, 1991; Clavero et al., 2004). Es de resaltar que en las grandes cuencas ibéricas el promedio de especies de origen foráneo roza el 50% (Elvira, 1995), y este hecho se considera como una de las principales amenazas para la conservación de la ictiofauna nativa (Doadrio, 2001; Elvira y Almodóvar, 2001), aunque existan pocas evidencias documentales de ello.

Está situación ha llevado a que en la actualidad más del 80% de la ictiofauna continental autóctona española esté catalogada como Vulnerable (VU), En Peligro de Extinción (EN) o En Peligro Crítico (CR) (Doadrio, 2001). Por otro lado, existe un profundo desconocimiento sobre aspectos claves y básicos de la ictiofauna ibérica. Buena prueba de ello es que a día de hoy no se conoce con exactitud el número total de especies de peces existentes, su distribución precisa, su ecología, y mucho menos las relaciones existentes entre los peces exóticos y los nativos (Ver Godinho y Ferreira, 1998, 2000; Doadrio, 2001), o entre los depredadores ictiófagos nativos y las especies foráneas, especialmente del posible papel controlador de los primeros sobre los segundos. Despejar estas incógnitas proporcionará un conocimiento básico imprescindible sobre la biología y ecología de nuestras especies, ayudará a comprender los efectos de la degradación ambiental sobre las biocenosis acuáticas, anticipará las consecuencias de nuevos impactos y aportará herramientas útiles para la conservación y gestión de estos ecosistemas tan sensibles.

Los resultados obtenidos en esta Tesis pretenden, por tanto, resolver algunas de las carencias de conocimiento antes referidas. El escenario elegido lo conforman varias cuencas fluviales localizadas en el suroeste ibérico, pertenecientes a una unidad biogeográfica homogénea desde el punto de vista de la distribución de la ictiofauna continental (Doadrio, 1988). Toda la zona sufre las fluctuaciones estacionales propias del clima mediterráneo y en ella tratamos de conocer distintos aspectos relacionados con los patrones de distribución y la ecología de la ictiofauna continental.

En el capítulo 2 analizamos los patrones generales de distribución y uso del hábitat de la ictiofauna continental del área de estudio. En este trabajo se pone de manifiesto que los patrones de distribución y uso del hábitat de las especies nativas están fuertemente condicionados por los peces exóticos. Estos resultados servirán como base e hilo conductor del resto de capítulos presentados en esta memoria. A continuación, en el capítulo 3, se evalúa explícitamente el impacto generado por las especies exóticas sobre la comunidad de peces nativos. En concreto se analiza la influencia ejercida por los centrárquidos (pez sol, *Lepomis gibbosus*, y blacbás, *Micropterus salmoides*), especies originarias de Norteamérica y que constituyen la fracción mayoritaria de las especies exóticas de la zona. En el capítulo 4 se estudia la relación existente entre las características del hábitat, los centrárquidos y la distribución de dos especies nativas muy amenazadas, el jarabugo (*Anaecypris hispanica*) y el pez fraile (*Salaria fluviatilis*). Dado el impacto negativo que provocan los centrárquidos, sería interesante conocer si el principal depredador ictiófago nativo ibérico, la nutria (*Lutra lutra*), puede ejercer algún control sobre los mismos. Por ello, en el capítulo 5 analizamos la importancia de los centrárquidos como presas potenciales para el mustélido. En el capítulo 2 se apunta hacia una posible segregación espacial intragenérica de las especies de *Chondrostoma* y *Squalius*, fenómeno común entre especies congenéricas de peces continentales (Winston, 1995). Este fenómeno se estudia en detalle en el último capítulo de esta memoria en el que describimos los patrones de distribución de las especies de los referidos géneros *Chondrostoma* y *Squalius*.

Con los conocimientos adquiridos durante la realización de este trabajo pretendemos comprender mejor el funcionamiento de los ecosistemas fluviales mediterráneos, así como proporcionar conocimientos básicos que puedan servir de herramientas útiles a la hora de diseñar y/ o proponer medidas de conservación de los mismos.

## OBJETIVOS

1. *Analizar los patrones de distribución y uso del hábitat de la ictiofauna continental de un conjunto amplio de cursos de agua del suroeste de la Península Ibérica, relacionando tales patrones con los principales gradientes ambientales observados en la zona.*

2. *Evaluar la relación existente entre las especies introducidas, principalmente los centrárquidos, y la ictiofauna nativa, a partir del análisis del efecto que los primeros provocan en la distribución y en la estructura de la comunidad de los peces autóctonos.*

3. *Caracterizar el estado de conservación en el área de estudio de dos de las especies de peces ibéricos más desconocidos y amenazados, el jarabugo* (Anaecypris hispanica) *y el pez fraile* (Salaria fluviatilis)*, evaluando su área de distribución, hábitat y abundancia, así como la influencia que puedan ejercer sobre ellas las especies introducidas.*

4. *Determinar la dieta de la nutria* (Lutra lutra) *en el área de estudio para valorar la importancia de los centrárquidos como presa de este depredador ictiófago nativo y su papel potencial como controlador de la expansión de peces introducidos.*

5. *Examinar las relaciones espaciales entre las especies congenéricas de los géneros* Squalius *y* Chondrostoma*, nunca antes estudiadas entre ciprínidos ibéricos.*

6. *Proporcionar conocimientos sobre la historia natural, los factores de amenaza y el estado de conservación de las especies de peces continentales del área de estudio que puedan ser útiles a la hora de promover estrategias de gestión encaminadas a la preservación de sus poblaciones.*

## ÁREA DE ESTUDIO

Aunque en cada uno de los capítulos de esta memoria se describe el área de estudio de manera específica para los contenidos de cada uno de ellos, la amplitud de la zona donde se plantea el trabajo hace conveniente una descripción general de la misma. El área de estudio completa incluye 81 localidades de muestreo, distribuidas por cursos de agua del suroeste peninsular y pertenecientes a las cuencas del Guadiana (28 localidades), Guadalquivir (25), Tinto (9), Odiel (11) y arroyos costeros del Campo de Gibraltar (8) (Figura 1). Cada grupo de localidades pertenecientes a las distintas cuencas posee unas características ambientales propias, estadísticamente diferenciables (Tabla 1, Figura 2). Esta circunstancia permite la existencia de un amplio rango de condiciones ambientales que pueden definir gradientes en los que detectar patrones o tendencias espaciales en la distribución de la ictiofauna.

Entre las cuencas estudiadas, son las del río Tinto y Odiel las que conforman seguramente un escenario más singular. Ambas cuencas drenan la faja píritica ibérica, una zona muy rica en minerales ferrocupríferos que ha sufrido una intensa actividad minera desde hace más de 5.000 años (Davis et al., 2000). Esta actividad junto con el lavado natural de la faja pirítica han provocado un proceso contaminante que ha aportado elevadas cantidades de metales pesados y una extrema acidez a las aguas de los cauces principales (pH~2,5-3), coincidentes con los ríos Tinto y Odiel y con ciertos tramos de algunos tributarios del Odiel (Ruiz et al., 1998; Amaral-Zettler et al., 2002). Como consecuencia de estas condiciones extremas la vida macroscópica es prácticamente inexistente en esta porción de la red fluvial, siendo los microorganismos extremófilos el componente mayoritario de la biocenosis acuática (López-Archilla y Amils, 1999; Amaral-Zettler et al., 2002). Estos tramos contaminados actúan de barrera infranqueable para los peces, que sobreviven aislados en subcuencas sin posibilidad alguna de interconexión entre ellas. El reducido tamaño de algunas de estas subcuencas les hace sufrir de manera especial las fuertes oscilaciones típicas del clima mediterráneo, especialmente la sequía estival.

Otra zona bastante peculiar dentro del área de estudio es la que forman los pequeños arroyos costeros del Campo de Gibraltar. El escarpado relieve es la característica más llamativa de esta zona, donde se alcanzan alturas superiores a los 800 m a menos de 7 km en línea recta de la costa. Este relieve lo forman varios macizos montañosos que dan lugar a una compleja red de drenaje por donde discurren numerosos arroyos. Hacia el norte estas sierras drenan hacia la cuenca del río Almodóvar, afluente del río Barbate, y también hacia la del río Palmones, que desemboca en el mar en la bahía de Algeciras. Además la banda costera incluye numerosos cursos de agua, aunque la mayoría de ellos presentan un régimen estrictamente torrencial. Las cuencas principales de la zona son de Este a Oeste, la del río del Valle, Jara, de la Vega, Guadalmesí, Pícaro y de la Miel. Todos ellos poseen áreas de drenaje bastante reducidas, entre los apenas 16 km$^2$ del río de La Miel y los 58,2 km$^2$ del río Jara. Al igual que ocurre en el Tinto y Odiel, el reducido tamaño de estas cuencas hace que sufran un acusado estiaje en la época veraniega. Este hecho, junto a las extracciones de agua para consumo urbano que se realizan en los tramos altos de las cuencas del Guadalmesí, Pícaro y Miel, han dado como resultado que algunas poblaciones de peces de la zona se hayan extinguido en las últimas sequías (Clavero et al., 2002). De todas formas, el aporte hídrico directo y el efecto atemperador de las nieblas, frecuentes en la zona entre julio y septiembre (Ibarra, 1993; Ojeda et al., 2000), atenúan en gran medida la severidad de la sequía estival, sobre todo en la cuencas que vierten en el Mediterráneo (Guadalmesí, Pícaro y Miel). La zona se encuentra en su mayor parte incluida dentro de los límites de dos espacios protegidos, el Parque Natural de Los Alcornocales y el recientemente creado Parque Natural del Estrecho.

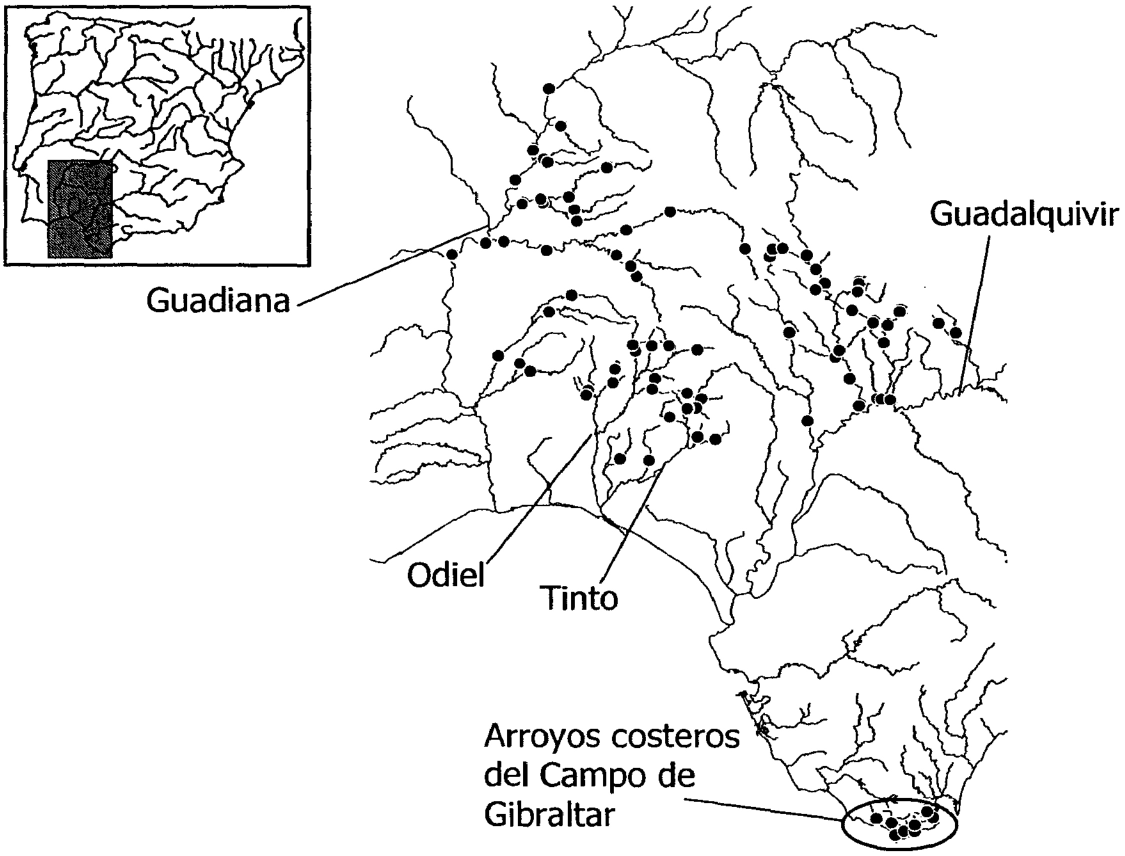

**Figura 1.** Mapa del área de estudio con indicación de las localidades de muestreo.

Los ríos de la cuenca del Guadiana estudiados junto con los del Guadalquivir son los de mayor longitud de todos los del área de estudio, en especial Ardila (180 km, Guadiana), Viar (124 km, Guadalquivir) y Huéznar (65 km, Guadalquivir). Los ríos de estas cuencas discurren en su mayoría por los macizos montañosos de Sierra Morena. El relieve no es muy escarpado con alturas que no suelen superar los 1000 m. Este sector del área de estudio es el que presenta una mayor regulación fluvial, existiendo un total de 8 embalses que superan los 100 $Hm^3$. Entre todos ellos destaca el embalse de Alqueva en el Guadiana, que con sus 3.150 $Hm^3$ inunda una superficie total de 245 $km^2$ y está considerado el mayor lago artificial de Europa (Collares-Pereira et al., 2000). Hay que señalar que el trabajo de campo en esta zona se llevó a cabo antes del llenado de este embalse y del de la recientemente terminada presa del Andévalo, por lo que algunas de las localidades muestreadas se encuentran en la actualidad inundadas. Parte de esta zona está incluida en dos espacios protegidos, el Parque Natural de Sierra Norte de Sevilla y el Parque Natural de Sierra de Aracena y Picos de Aroche. Dentro de los límites del primero se incluye la mayoría de los tramos altos y medios de los ríos estudiados pertenecientes a la cuenca del Guadalquivir. El P.N. de Sierra de Aracena y Picos de Aroche incluye el río Múrtigas, afluente del río Ardila (cuenca del Guadiana), el tramo alto de la rivera de Huelva (cuenca del Guadalquivir) y parte del curso alto de la rivera del Chanza (cuenca del Guadiana). Esta última rivera es el límite sur del parque en parte de su recorrido.

**Tabla 1.** Cuencas del área del estudio. Se incluye una pequeña descripción de las mismas.

| Zonas de estudio | Descripción |
|---|---|
| Cuencas de los ríos Tinto y Odiel | *Cuencas muy peculiares pues se encuentran muy fragmentadas por efecto de la contaminación minera. La ictiofauna sólo sobrevive en pequeños tributarios o zonas libres de contaminación.* |
| Pequeñas cuencas costeras del Campo de Gibraltar | *Pequeñas cuencas aisladas. Parte de ellas desemboca en costa arenosa, mientras que el resto lo hace en costa rocosa con fuertes pendientes.* |
| Ríos y arroyos de las cuencas del Guadiana y Guadalquivir | *Los tramos elegidos de ambas cuencas presentan características similares. Discurren sobre materiales pizarrosos paleozoicos y comparten un régimen de precipitaciones similar. Los tributarios estudiados son de dimensiones también similares* |

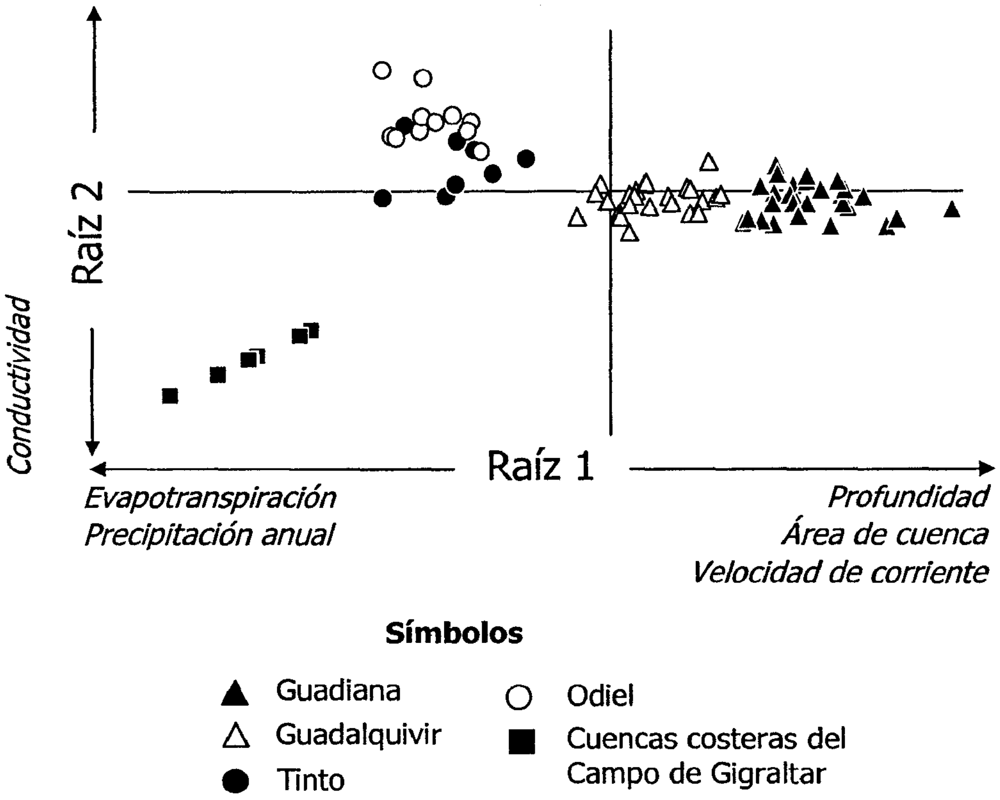

**Figura 2.** Distribución de las localidades de muestreo del área de estudio en el espacio definido por las dos primeras raíces de un Análisis Discriminante, aplicado a una matriz de variables ambientales (ver capítulos siguientes). El significado de cada raíz se presenta bajo flechas. Sólo aparecen aquellas variables que presentaron una correlación con las raíces de r>±0.55.

## LOS PECES CONTINENTALES DEL ÁREA DE ESTUDIO

La ictiofauna del área de estudio se puede considerar bastante rica en el contexto ibérico, con un total de 16 especies estrictamente dulceacuícolas, de las cuales 10 son autóctonas y 6 de origen exótico (Tabla 2). Este elevado número de especies autóctonas hace que en el área de estudio habite un alto porcentaje (27%) del total de especies dulceacuícolas descritas en España (37 especies, Doadrio, 2001; Doadrio y Madeira, 2004). De todas las cuencas estudiadas, es sin duda la del río Guadiana la que contiene un mayor número de especies (Tabla2).

**Tabla 2.** Listado de especies de peces del área de estudio. Se indican las cuencas donde han sido capturadas y su carácter autóctono o exótico. Los códigos de las cuencas son: D- Guadiana, Q- Guadalquivir, T- Tinto, O- Odiel y S- pequeñas cuencas del Campo de Gibraltar.

| Especies | Cuencas en las que se ha capturado | Origen |
|---|---|---|
| Cyprinidae | | |
| *Barbus sclateri* | D,Q,T,O,S | Autóctono |
| *Barbus comizo* | D | Autóctono |
| *Barbus microcephalus* | D | Autóctono |
| *Chondrostoma willkommii* | D,Q,T | Autóctono |
| *Chondrostoma lemmingii* | D,Q,T,O | Autóctono |
| *Squalius pyrenaicus* | D,Q,T,O,S | Autóctono |
| *Squalius alburnoides* | D,Q,O | Autóctono |
| *Anaecypris hispanica* | D | Autóctono |
| *Cyprinus carpio* | D,Q,T | Exótico |
| *Carassius auratus* | D | Exótico |
| Cobitidae | | |
| *Cobitis paludica* | D,Q,T,O,S | Autóctono |
| Blenniidae | | |
| *Salaria fluviatilis* | D | Autóctono |
| Poeciliidae | | |
| *Gambusia holbrooki* | D,Q,T,O,S | Exótico |
| Cichlidae | | |
| *Herichthys facetum* | D | Exótico |
| Centrarchidae | | |
| *Micropterus salmoides* | D,Q,T,O | Exótico |
| *Lepomis gibbosus* | D,Q,T,O | Exótico |

Entre todas las especies son las del género *Barbus* las más ampliamente distribuidas (Figura 3). En la zona de estudio existen tres especies de barbos, dos de ellos, el picón (*B. comizo*) y el cabecicorto (*B. microcephalus*), son exclusivos de la cuenca del Guadiana, mientras que el barbo común (*B. sclateri*) se distribuye por la práctica totalidad del área de estudio (Figura 3). Tan sólo falta en algunos tributarios del Guadiana y en varias cuencas costeras del Campo de Gibraltar. En esta última zona hemos podido constatar que durante la fuerte sequía del período 92-95 se extinguieron los barbos que habitaban en las cuencas de los ríos del Valle, Jara y Guadalmesí (Clavero et al., 2002). Las abusivas tomas de agua en los tramos altos de algunos de estos ríos, como el Guadalmesí, donde tradicionalmente los efectos de la sequía veraniega eran amortiguados por la humedad de las nieblas, probablemente desecaron las últimas pozas donde subsistían esta especie.

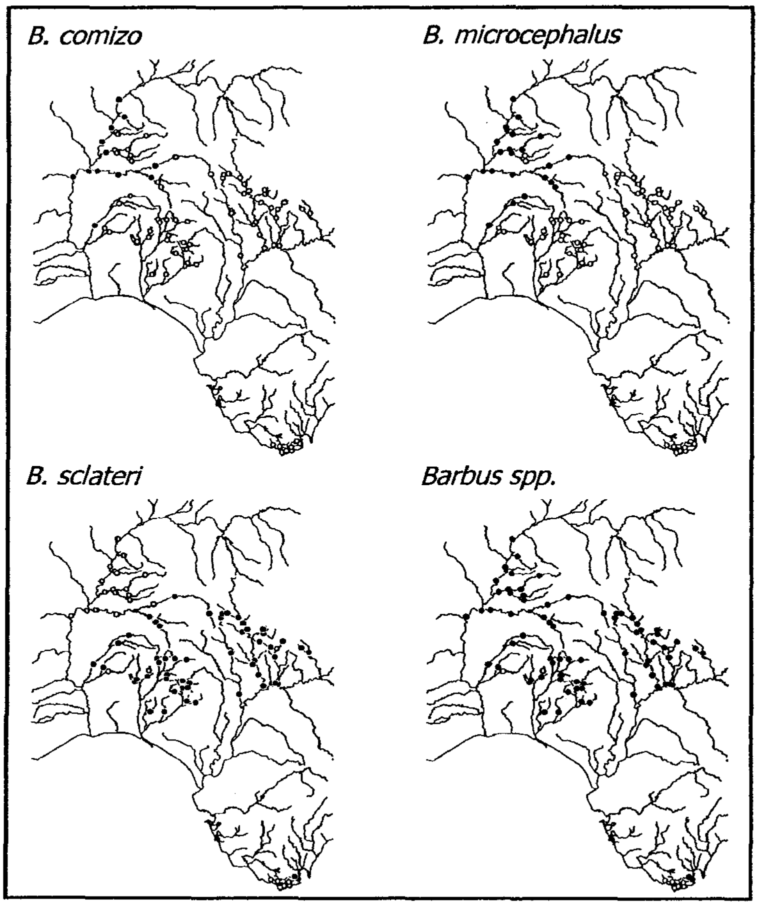

**Figura 3.** Distribución de las especies del género *Barbus* en el área de estudio. Se representan con puntos negros las localidades donde se capturaron individuos con características claras de *B. comizo, B. microcephalus* y *B. sclateri*. Se acompaña también un mapa con la distribución completa del género.

En las localidades del Guadiana donde coexistieron las tres especies de barbos, la diferenciación entre ellas fue muy complicada debido a la gran plasticidad morfológica del barbo picón. Esta circunstancia obligó a que en los análisis estadísticos se agruparan y fueran consideradas como una sola especie.

Otros géneros con distribución amplia en la zona de estudio son *Chondrostoma* y *Squalius* (Figura 4). Los cachuelos (*S. pyrenaicus*) ocupan ríos y arroyos de toda la zona de estudio (cuencas de los ríos Guadiana, Guadalquivir, Tinto, Odiel y algunas cuencas costeras del Campo de Gibraltar). Las pardillas (*Ch. lemmingii*), bogas (*Ch. willkommii*) y calandinos (*S. alburnoides*) se distribuyen por las cuencas de los ríos Guadiana, Guadalquivir y Odiel. Además, las primeras también aparecen en algunos tributarios del Tinto. Pardillas y bogas, aunque coexisten en varias localidades, tienden a ocupar hábitats distintos en el área de estudio (ver capítulos 2 y 6). Ésto mismo se repite para los cachuelos y calandinos.

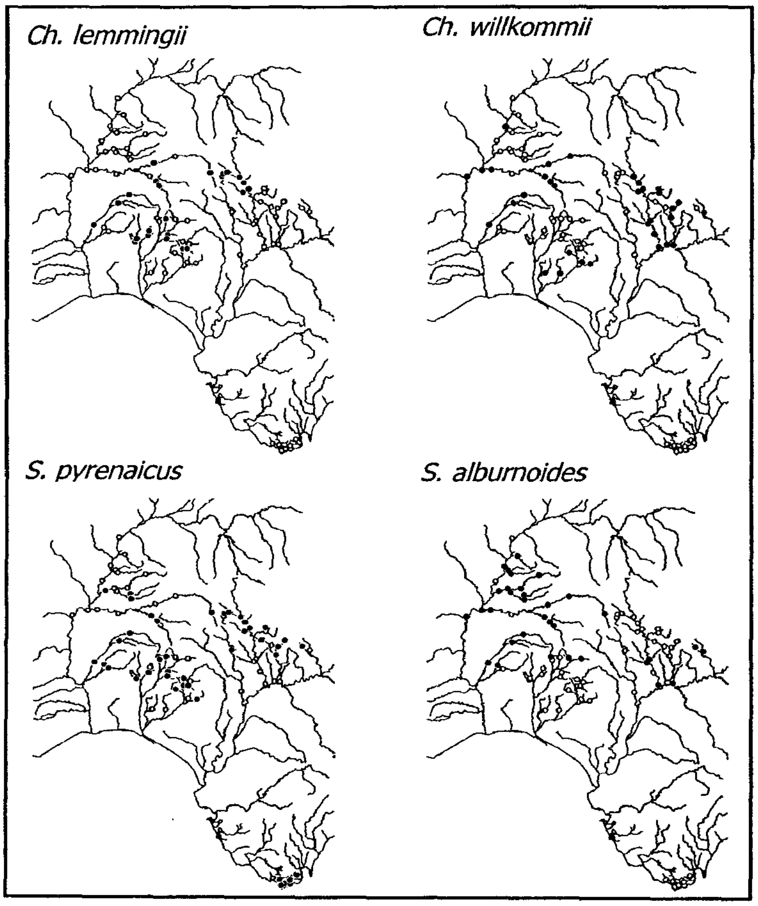

**Figura 4.** Distribución de las especies del género *Chondrostoma* y *Squalius* en el área de estudio.

Tanto pardillas como cachuelos habitan preferentemente en arroyos de cauces estrechos, poco profundos y con abundante vegetación acuática sumergida, normalmente zonas de tramo alto o pequeñas cuencas independientes (pardillas y cachuelos -tributarios de los ríos Tinto y Odiel-, y cachuelos -cuencas del Campo de Gibraltar-). Por el contrario, las bogas y calandinos, aún siendo bastante más generalistas, suelen ocupar cauces más amplios y profundos, con velocidad de corriente moderada-elevada y con mayor superficie de drenaje. Estas zonas coinciden con los tramos medios-bajos de los ríos o con las subcuencas de los tributarios mayores del Tinto (en el caso de las bogas) y algunos tributarios del Odiel (para los calandinos).

La colmilleja (*Cobitis paludica*) también se distribuye por la práctica totalidad de las cuencas estudiadas (Figura 5). Habita en tributarios de las cuencas del Guadiana, Guadalquivir, Tinto, Odiel y arroyos costeros del Campo de Gibraltar. En esta última zona solo está presente en los arroyos que vierten a la vertiente atlántica (Valle, Jara y Vega).

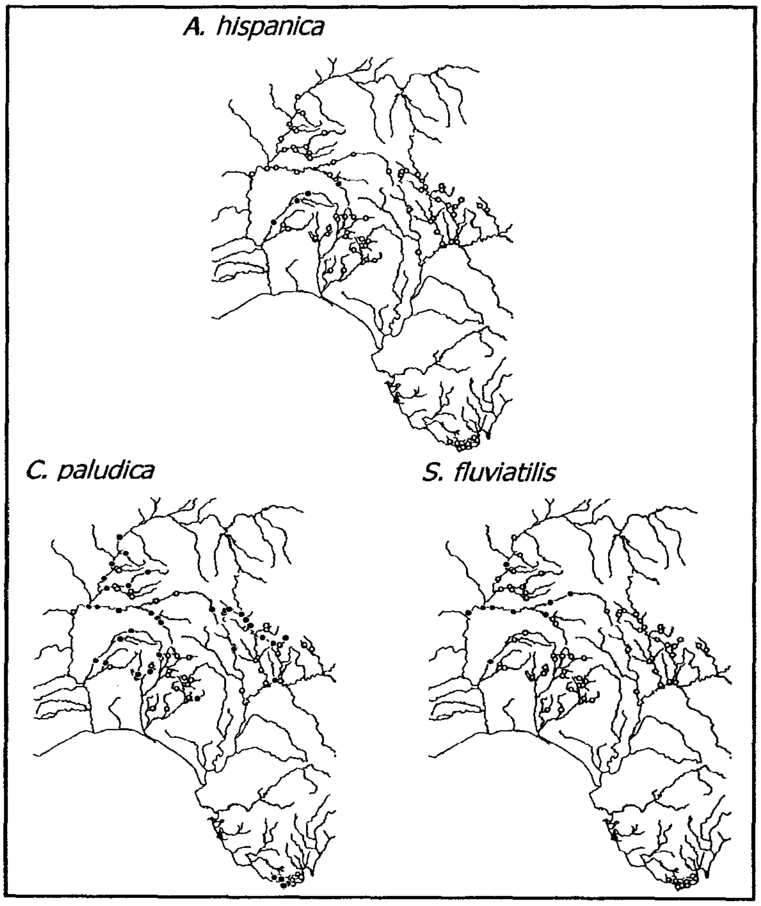

**Figura 5.** Distribución del jarabugo (*A. hispanica*), la colmilleja (*C. paludica*) y el pez fraile (*S. fluviatilis*) en el área de estudio.

Estos pequeños arroyos, con poca corriente y sustratos finos, representan hábitats más idóneos para la especie que los que desembocan en la vertiente mediterránea (Guadalmesí, Pícaro y Miel) (Doadrio, 2001; Clavero et al., 2002).

El jarabugo (*Anaecypris hispanica*) y el blenio de río o pez fraile (*Salaria fluviatilis*) son las especies nativas que muestran una distribución más restringida en la zona (Figura 5). El primero es un endemismo del Guadiana, el segundo es una especie con distribución circum-mediterránea que en la Península Ibérica se distribuye por las cuencas del Ebro, Fluvià, Bullén, Júcar, río Verde y Guadiana (Doadrio, 2001). Los blenios del Guadiana son, por tanto, los que forman la única población atlántica de la especie en la Península Ibérica. Los jarabugos habitan preferentemente en arroyos pequeños con abundante vegetación acuática sumergida. Esta especie parece bastante sensible a las especies exóticas hasta el punto que sólo se ha capturado en las pocas localidades libres de centrárquidos (ver capítulo 4). El pez fraile habita generalmente en los tramos bajos de ríos con cauces amplios y elevada velocidad de corriente, donde la estabilidad hidrológica es mayor (Capítulo 4). Al contrario que el jarabugo, esta especie parece indiferente a la presencia de especies exóticas.

Las especies exóticas presentan en general una distribución más restringida que las nativas. Las más extendidas y más abundantes son el pez sol (*Lepomis gibbossus*) y el blacbás (*Micropterus salmoides*) (ver capítulos 2 y 3). Estas especies están bien distribuidas por los tributarios del Guadiana y por los tramos bajos de los afluentes del Guadalquivir (Figura 6). La carpa (*Cyprinus carpio*) también está presente en estas dos cuencas, coincidiendo con zonas de aguas remansadas y en tramos bajos. La gambusia (*Gambusia holbrookii*) ocupa localidades repartidas por todas las cuencas estudiadas, mientras que el carpín (*Carassius auratus*) y el chanchito (*Herichthys facetum*) sólo aparecen en ciertas localidades de la cuenca del Guadiana. El grado de invasión de las especies exóticas es mínimo en las pequeñas cuencas aisladas del Tinto, Odiel y Campo de Gibraltar (Figura 6). Probablemente la escasa regulación de estas cuencas y su gran inestabilidad hidrológica impiden el asentamiento de poblaciones estables de estas especies (Prenda et al., 2002; Clavero et al., 2004).

A pesar de la escasa importancia cuantitativa de las especies exóticas en el área de estudio, ejercen un fuerte impacto sobre los peces nativos, influyendo en sus patrones de distribución, en el uso que hacen del hábitat y en la estructura de sus comunidades (capítulos 2 y 3).

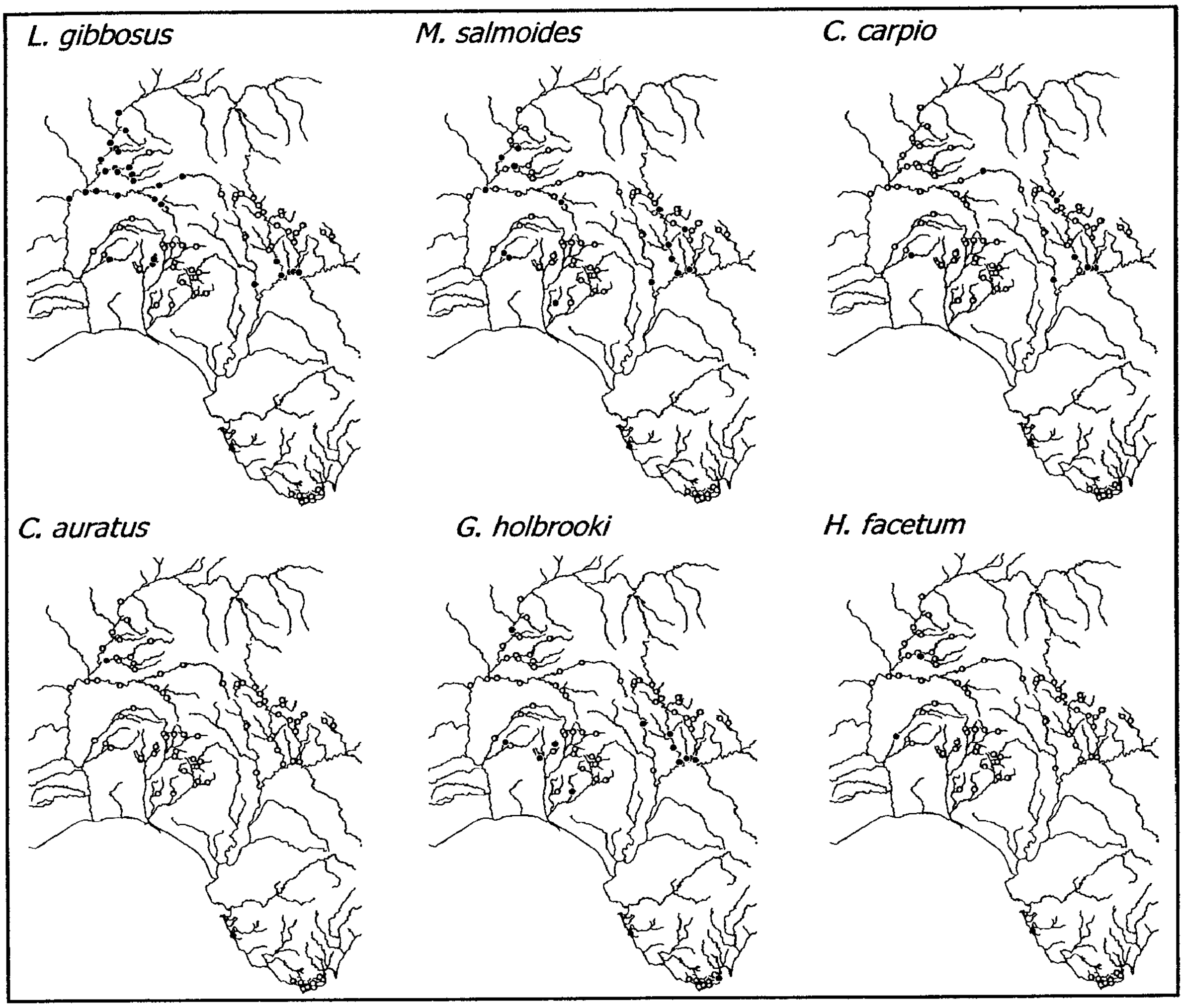

**Figura 6.** Distribución de las especies exóticas presentes en el área de estudio.

## RESUMEN DE RESULTADOS

Según lo establecido en el procedimiento para la presentación, autorización y evaluación de la Tesis doctoral en la Universidad de Huelva, las memorias escritas en un idioma distinto al castellano, como es este caso, deberán contener un resumen en este idioma donde se incluyan los elementos de la misma necesarios para su comprensión integral. Por este motivo, se presenta a continuación un resumen de los resultados obtenidos.

### LAS ESPECIES EXÓTICAS DETERMINAN LOS PATRONES DE DISTRIBUCIÓN Y EL USO DEL HÁBITAT DE LA ICTIOFAUNA NATIVA.

La ictiofauna continental ibérica se encuentra entre los grupos de vertebrados peor conocidos. De hecho, aspectos claves como el número total de especies existentes, la distribución o la ecología de muchas de las especies son a día de hoy una incógnita. Existen trabajos en ríos de zonas templadas donde se establecen relaciones entre la ictiofauna que albergan y determinadas características del hábitat (p. ej. Prenda et al., 1997). Sin embargo, estos patrones son desconocidos para las especies ibéricas. Además, dada la variabilidad intrínseca del clima mediterráneo, es evidente que el marco de conocimiento desarrollado en otras zonas de clima templado no es directamente aplicable a los peces ibéricos (Magalhães et al., 2002). A esto hay que añadir que las cuencas ibéricas están siendo invadidas por especies exóticas (Doadrio, 2001), que pueden interferir en los patrones naturales de distribución y uso del hábitat de las especies nativas.

En este primer capítulo se analizan los patrones de distribución y el uso del hábitat por parte de un amplio grupo de peces continentales. Parar ello se muestrearon 80 localidades repartidas por las cuencas de los ríos Guadiana, Guadalquivir, Tinto, Odiel y por pequeños arroyos costeros del Campo de Gibraltar (Figura 1). Los peces de cada localidad se muestrearon con pesca eléctrica, expresando la abundancia de cada especie como n capturas*100 $m^{-1}$ de pesca*1 $h^{-1}$ de pesca (CPUE). Todas ellas están bajo la influencia del clima mediterráneo y pertenecen a una unidad biogeográfica homogénea, el sector sur-ibérico, definido por Doadrio (1988) en función de la distribución de la ictiofauna. Además, en ciertas zonas, como en las cuencas del Guadiana y Guadalquivir, están proliferando peces exóticos (Doadrio, 2001; Bernardo et al., 2003) que pueden actuar como un potente agente perturbador de las comunidades de peces nativos.

En el conjunto del área de estudio se capturaron al menos 20 especies de peces (una estima a la baja, ya que la familia *Mugilidae* pudo incluir a varias especies, Tabla

3). Sólo las especies estrictamente dulceacuícolas y con una frecuencia de aparición superior al 5% se incluyeron en los análisis estadísticos (Tabla 3). Un análisis de componentes principales (ACP), aplicado a una matriz de abundancias de especies (en CPUE transformadas $lg_{10}$) por localidad, separó claramente las especies nativas de las exóticas (Figura 7A). Esto indica que ambos grupos de especies tienden a ocupar hábitats distintos. Este patrón se repitió al analizar la distribución espacial de las clases de talla de las distintas especies: la mayoría de las clases de tallas de las especies nativas se segregaron del grupo de especies exóticas, excepto las tallas mayores de barbos (*Barbus* spp.) (>200 mm), bogas (*Chondrostoma willkommii*) (>150 mm) y pez fraile (*Salaria fluviatilis*) (>80 mm) (Figura 7B).

En base a un análisis de uso-disponibilidad del hábitat (test de chi-cuadrado, Figura 8) y de correlación se definieron cuatro gremios de hábitat (Tabla 4), que ocuparon distintas posiciones a lo largo de un gradiente de cabecera-tramo bajo. Este gradiente quedó definido al aplicar un nuevo ACP a una matriz de variables de hábitat (Figura 8, ver Capítulo 2 para una descripción más detallada).

En las localidades con presencia de exóticas (n=35), la abundancia de los gremios llamados "generalistas-tramo medio" y "nativas de tramo bajo" diminuyó significativamente al aumentar la abundancia de las primeras (r=-0.47, p=0.005 y r=-0.38, p=0.025; respectivamente). Este resultado indica que el patrón de distribución de estas especies nativas está influenciado por las especies exóticas. Probablemente estas especies foráneas estén enmascarando el patrón natural de distribución y uso del espacio de las especies nativas, expulsándolas o eliminándolas principalmente de los tramos bajos, el hábitat preferido por las especies exóticas. Por esta razón, gran parte de las especies nativas muestran un uso del hábitat muy laxo, tal y como se desprende de la Figura 8. Este hecho implica que las especies nativas queden relegadas a hábitats subóptimos, sobre todo aquellas especies con tendencia a ocupar los tramos bajos, como es el caso del gremio denominado "nativas de tramo bajo".

**Tabla 3.** Listado de especies capturadas en 80 localidades del suroeste de la Península Ibérica. Se indica el código utilizado para cada especie, las clases de tallas, la frecuencia de aparición (FA %), la abundancia media (CPUE) y las cuencas donde se capturó cada especie (D-Guadiana, Q-Guadalquivir, T-Tinto, O-Odiel y S-cuencas del Campo de Gibraltar). Especies no incluidas en los análisis: [1]*FA<5%*; [2]*especies no estrictamente continentales.* * *B. microcephalus* y *B. comizo* son exclusivas de la cuenca del Guadiana. Los datos de abundancia sólo se aportan para las especies capturadas con pesca eléctrica. El resto de especies se han capturado con nasas holandesas y/o trampas de botellas.

| | | **Clases de talla (mm)** | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Especies** | **Código** | **1** | **2** | **3** | **4** | **5** | **FA (%)** | **abundancia (media±DS)** | **Cuenca** |
| **Especies nativas** | | | | | | | | | |
| Cyprinidae | | | | | | | | | |
| *Barbus microcephalus**. | | | | | | | | | |
| *Barbus comizo** | Bsp | <50 | 50-100 | 100-150 | 150-200 | >200 | 86.3 | 229.9±350.6 | D,Q,T,O,S |
| *Barbus sclateri.* | | | | | | | | | |
| *Chondrostoma willkommii* | Cw | <50 | 50-100 | 100-150 | >150 | | 38.8 | 44.1±194.5 | D,Q,T |
| *Chondrostoma lemmingii* | Cl | <50 | 50-80 | >80 | | | 27.5 | 32.4±102.6 | D,Q,T,O |
| *Squalius pyrenaicus* | Sp | <50 | 50-80 | >80 | | | 50.3 | 50.5±128.7 | D,Q,T,O,S |
| *Squalius alburnoides* | Sa | <50 | 50-80 | >80 | | | 40.0 | 101.6±251.4 | D,Q,O |
| *Anaecypris hispanica* | Ah | <50 | >50 | | | | 5.0 | 7.4±61.5 | D |
| Cobitidae | | | | | | | | | |
| *Cobitis paludica* | Cp | <50 | 50-70 | >70 | | | 45.0 | 11.5±43.1 | D,Q,T,O,S |
| Blenniidae | | | | | | | | | |
| *Salaria fluviatilis* | Sf | <60 | 60-80 | >80 | | | 11.3 | 0.5±2.2 | D |
| Cyprinodontidae | | | | | | | | | |
| *Aphanius baeticus*[2] | | | | | | | | | S |
| Atherinidae | | | | | | | | | |
| *Atherina boyeri*[2] | | | | | | | | | S |
| Anguillidae | | | | | | | | | |
| *Anguilla anguilla*[2] | | | | | | | | | S |
| Mugilidae[2] | | | | | | | | | S |
| **Especies exóticas** | | | | | | | | | |
| Cyprinidae | | | | | | | | | |
| *Cyprinus carpio* | Cc | <100 | 100-150 | 150-200 | >200 | | 12.5 | 0.9±4.0 | D,Q,T |
| *Carassius auratus*[1] | Ca | | | | | | 1.3 | 0.03±0.3 | D |
| Poeciliidae | | | | | | | | | |
| *Gambusia holbrooki* | Gh | | | | | | 15.0 | 3.3±21.2 | D,Q,T,O,S |
| Cichlidae | | | | | | | | | |
| *Herichthys facetum*[1] | Hf | | | | | | 2.5 | 0.1±0.7 | D |
| Centrarchidae | | | | | | | | | |
| *Micropterus salmoides* | Ms | <100 | 100-200 | >200 | | | 17.5 | 6.6±40.1 | D,Q,T,O |
| *Lepomis gibbosus* | Lg | <50 | 50-100 | >100 | | | 35.0 | 10.4±26.2 | D,Q,T,O |

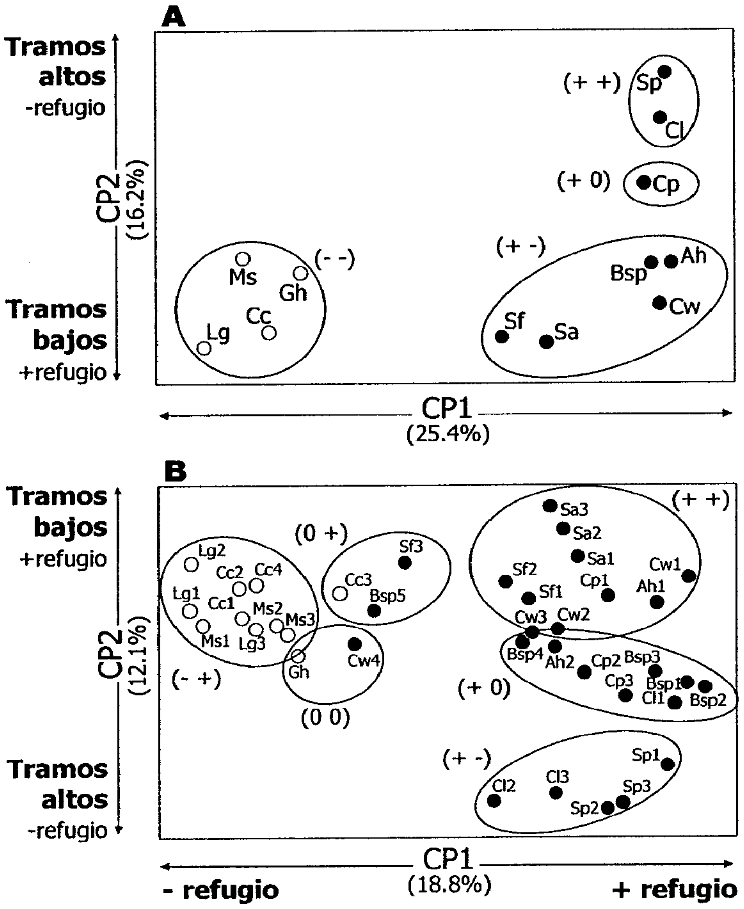

**Figura 7.** Distribución de los factores de carga de las especies (A) y de las clases de talla de cada especie (B) al aplicar un ACP una matriz de abundancia (log-CPUE) x localidad. Los círculos engloban a las especies y a las tallas de cada especie que presentaron el mismo tipo de correlación con los componentes (CP1 y CP2) y que, por tanto, ocupan hábitats similares. Los símbolos 0, + o – en la primera posición dentro de paréntesis indican ausencia de correlación, correlación positiva ($p<0.05$) o correlación negativa ($p<0.05$), respectivamente con el CP1. En la segunda posición la correlación es con el CP2. Debajo de cada CP aparece su significado ecológico (ver Capítulo 2). Los números entre paréntesis debajo de CP1 y CP2 representan el porcentaje de varianza explicado por cada componente. Los códigos de las especies y de las clases de talla están en la Tabla 3.

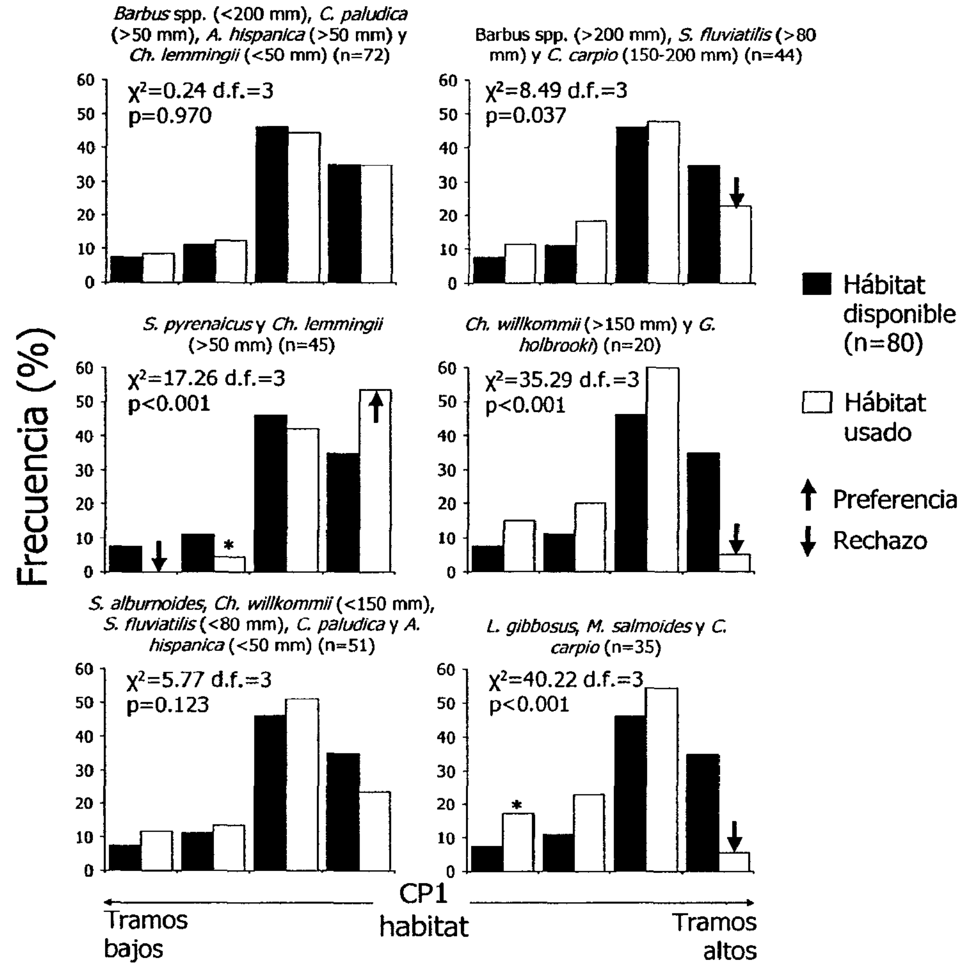

**Figura 8.** Comparación entre la porción de hábitat disponible y la usada por cada grupo de especies mediante un test Chi-cuadrado. (ver Capítulo 2 para una descripción detallada). El CP1 representa un gradiente cabecera-tramo bajo obtenido al aplicar un ACP a una matriz de variables de hábitat.

Los resultados de este primer trabajo muestran la fuerte influencia que ejercen las especies exóticas en los patrones de distribución y uso del hábitat de la ictiofauna nativa. Esta clara influencia hace imposible conocer la distribución original y el uso que hacían estas especies del hábitat en ausencia de exóticas. Por tanto, los patrones aquí descritos pueden deberse a la combinación de la influencia ejercida por las especies exóticas y a los propios requerimientos de hábitat de las especies nativas. Estos resultados nos han servido como base para desarrollar el resto de capítulos que se presentan en esta memoria.

**Tabla 4.** Gremios de hábitat identificados en el área de estudio y comentarios sobre sus preferencias de hábitat. Los gremios se han definido mediante ACP y análisis de uso de hábitat-disponibilidad y de correlación (ver Capítulo 2 para una descripción detallada).

| Gremios de hábitat | Especies | Preferencias de hábitat |
|---|---|---|
| "Cabeceras" | *Squalius pyrenaicus* y *Chondrostoma lemmingii* (>50 mm) | Preferencia por arroyos pequeños, normalmente de orden ≤3, con abundante vegetación sumergida. Se trata, en general, de tramos de cabeceras o de pequeñas cuencas aisladas. |
| "Generalistas-tramo medio" | *Barbus* spp.<200 mm, *Cobitis paludica* (>50 mm), *Anaecypris hispanica* (>50 mm) y *Ch. lemmingii* (<50 mm) | Uso del hábitat generalizado, pero con tendencia a ocupar tramos medios con vegetación acuática y refugio. |
| "Nativas de tramo bajo" | *S. alburnoides*, *Ch. willkommii* (<150 mm), *Salaria fluviatilis* (<80 mm), *C. paludica* (<50 mm) y *A. hispanica* (<50 mm) | Se hacen más abundantes hacia los tramos bajos de los ríos estudiados y en zonas con elevado refugio. |
| "Exóticas, grandes ciprínidos, y blenios de tramos bajo" | *Cyprinus carpio*, *Gambusia holbrooki*, *Micropterus salmoides*, *Lepomis gibbosus*, *Barbus* spp.>200 mm, *Ch. willkommii*>150 mm and *S. fluviatilis*>80 mm | Rechazan claramente los tramos bajos y aparecen principalmente en zonas de tramo bajo, caracterizadas por tener orden elevado y cauces amplios y profundos. |

## EL IMPACTO DE LOS CENTRÁRQUIDOS SOBRE LAS COMUNIDADES DE PECES NATIVOS EN RÍOS MEDITERRÁNEOS.

A partir de los resultados del trabajo anterior, donde se observa una fuerte segregación existente entre las especies exóticas y las nativas, en este capítulo analizamos de forma específica la relación existente entre las especies introducidas y la comunidad de peces nativos. En concreto se analiza el impacto ejercido por los centrárquidos (pez sol y blacbás), especies que componen la fracción mayoritaria de las especies exóticas del área de estudio. Los centrárquidos, peces originarios de Norteamérica, están proliferando en los ríos de la Península Ibérica (Doadrio, 2001) y de forma especial en la cuenca del Guadiana (Bernardo et al., 2003). Esta expansión en la distribución junto con el carácter ictiófago y depredador de huevos y alevines de peces (García-Berthou y Moreno-Amich, 2000; García-Berthou, 2001) los convierte en una seria amenaza para la conservación de la ictiofauna nativa. No obstante, apenas existen datos en la literatura científica sobre la interacción entre especies exóticas y nativas, ni sobre los mecanismos específicos que emplean las primeras para desplazar a las segundas.

Para el propósito de este trabajo se eligieron 50 localidades distribuidas por las cuencas de los ríos Guadiana y Guadalquivir (Figura 1). Estas cuencas conforman un buen escenario para evaluar el impacto de los centrárquidos en la comunidad de peces nativos, ya que poseen zonas o tramos fluviales invadidos por estas especies junto con áreas donde la comunidad original está libre de especies exóticas.

En general, la importancia cuantitativa de los centrárquidos fue escasa para el área de estudio. A pesar de ello, la abundancia de centrárquidos mostró una clara relación negativa con los descriptores de la comunidad de peces: la abundancia (medida como capturas por unidad de esfuerzo, CPUE), la biomasa (expresada como biomasa por unidad de esfuerzo, BPUE), la riqueza y la diversidad (H') de especies nativas (Figura 9)

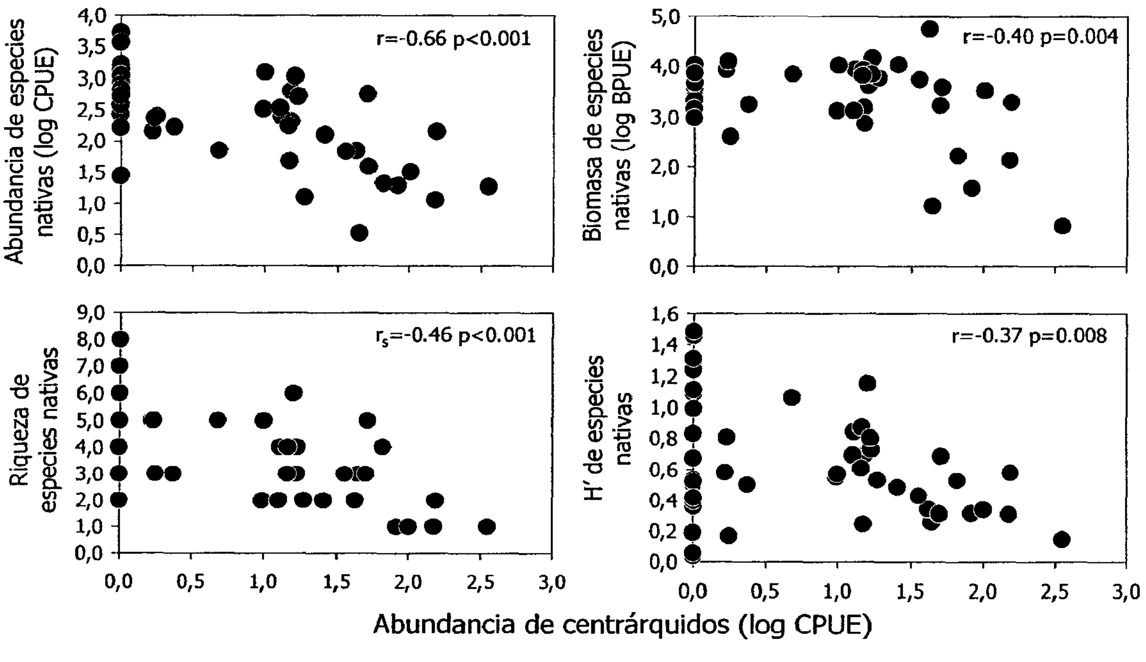

**Figura 9.** Correlaciones entre descriptores de la comunidad de peces nativos y la abundancia de los centrárquidos. Los datos de abundancia y biomasa están expresados en CPUE y BPUE respectivamente. Estos datos están transformados logarítmicamente ($\log_{10}$).

Esta relación inversa se mantuvo al correlacionar la abundancia de cada una de las especies nativas de forma individual con la abundancia de los centrárquidos. Todas las especies nativas, excepto el calandino (*Squalius alburnoides*), disminuyeron sus abundancias en respuesta al aumento de centrárquidos (Tabla 5). El resto de especies exóticas de la zona, carpas (*Cyprinus carpio*) y gambusias (*Gambusia holbrooki*), mostraron, no obstante, una correlación positiva con la abundancia de los centrárquidos (Tabla 5).

Los patrones de abundancia y riqueza de especies nativas variaron siguiendo el gradiente longitudinal de tramos altos-tramos bajos, medido éste como la distancia de cada localidad al río principal (Guadiana o Guadalquivir). Sin embargo, este cambio se produjo de forma inversa a la esperable. El patrón natural consiste en un aumento progresivo de la abundancia y del número de especies desde los tramos más altos hacia los tramos bajos (Schlosser, 1990; Magalhães et al. 2002), pero los resultados obtenidos en este trabajo mostraron justamente el patrón contrario: la abundancia y la riqueza de especies nativas disminuyeron progresivamente hacia los tramos bajos, (Figura 10).

**Tabla 5.** Correlación de Pearson (r) entre la abundancia de cada una de las especies (CPUE) y la abundancia de los centrárquidos (CPUE). Los valores de abundancia están transformados logarítmicamente (log10). *p<0.05, **p<0.01 and ***p<0.001. (E): Especies exóticas.

| Especies | r |
|---|---|
| *Barbus sp.* | -0.57*** |
| *Cyprinus carpio* $^{(E)}$ | 0.39** |
| *Chondrostoma lemmingii* | -0.49*** |
| *Chondrostoma willkommii* | -0.32* |
| *Squalius pyrenaicus* | -0.49*** |
| *Squalius alburnoides* | -0.12 |
| *Cobitis paludica* | -0.39** |
| *Gambusia holbrooki* $^{(E)}$ | 0.35* |

De aquí se desprende que tanto la abundancia de centrárquidos como el gradiente tramo alto-tramo bajo fueron factores influyentes en la abundancia y riqueza de las especies nativas. Sin embargo, sólo la abundancia de centrárquidos se incluyó de forma significativa en un análisis de regresión múltiple donde ambos parámetros (abundancia de centrárquidos y gradiente tramo alto-tramo bajo) fueron predictores de la abundancia, biomasa, riqueza y diversidad de especies nativas (Tabla 6). Todo ello apunta a los centrárquidos como el principal factor responsable de la inversión del patrón natural de abundancia y riqueza de peces nativos a lo largo del gradiente ambiental tramo alto- tramo bajo.

Por último, otro efecto atribuible a los centrárquidos estuvo relacionado con diferencias en la estructura de tallas de la comunidad. Las localidades con presencia de centrárquidos tuvieron comparativamente una menor proporción de peces de talla pequeña y un mayor porcentaje de tallas grandes que aquellas donde faltaron estas especies (Figura 11). Esto se explica por el hecho de que los peces de talla pequeña son generalmente más sensibles que los de talla grande a especies ictiófagas o agresivas, como los centrárquidos (MacRae y Jackson, 2001).

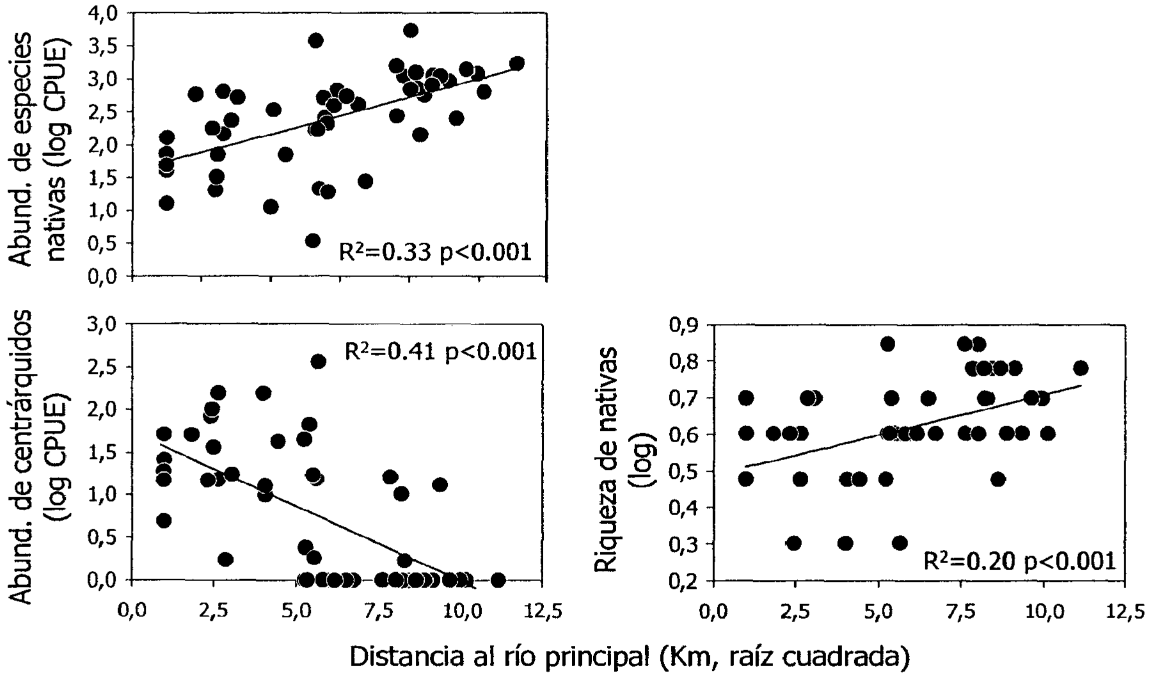

**Figura 10.** Relación entre el gradiente tramo alto-tramo bajo, medido como la distancia al río principal (Guadiana o Guadalquivir), y la abundancia de las especies nativas, su riqueza y la abundancia de centrárquidos en 50 localidades de las cuencas del Guadiana y Guadalquivir (SO de la Península Ibérica).

**Tabla 6.** Modelo de regresión múltiple saturado que analiza el efecto de la abundancia de centrárquidos y el gradiente tramo alto-tramo bajo (medido como distancia al río principal) sobre la abundancia, riqueza, biomasa y diversidad de especies nativas. Los valores de abundancia, riqueza y biomasa están transformados logarítmicamente ($\log_{10}$). Las distancias están transformadas mediante la raíz cuadrada.

| Variable dependiente | | g.l.. | F | p | R² | Variable independiente | g.l. | p | β |
|---|---|---|---|---|---|---|---|---|---|
| Abundancia de especies nativas | Modelo | 2 | 21.5 | <0.001 | 0.48 | Ordenada origen | 1 | <0.001 | |
| | Error | 47 | | | | Abund. centrárquidos | 1 | <0.001 | -0.51 |
| | Total | 49 | | | | Dist. al río | 1 | 0.08 | 0.24 |
| Riqueza de especies nativas | Modelo | 2 | 15.1 | <0.001 | 0.39 | Ordenada origen | 1 | <0.001 | |
| | Error | 47 | | | | Abund. centrárquidos | 1 | <0.001 | -0.58 |
| | Total | 49 | | | | Dist. al río | 1 | 0.637 | 0.07 |
| Biomasa de especies nativas | Modelo | 2 | 4.3 | 0.018 | 0.15 | Ordenada origen | 1 | <0.001 | |
| | Error | 47 | | | | Abund. centrárquidos | 1 | 0.010 | -0.47 |
| | Total | 49 | | | | Dist. al río | 1 | 0.430 | -0.14 |
| Diversidad de especies nativas (H′) | Modelo | 2 | 3.9 | 0.028 | 0.14 | Ordenada origen | 1 | <0.001 | |
| | Error | 47 | | | | Abund. centrárquidos | 1 | 0.059 | -0.34 |
| | Total | 49 | | | | Dist. al río | 1 | 0.774 | 0.05 |

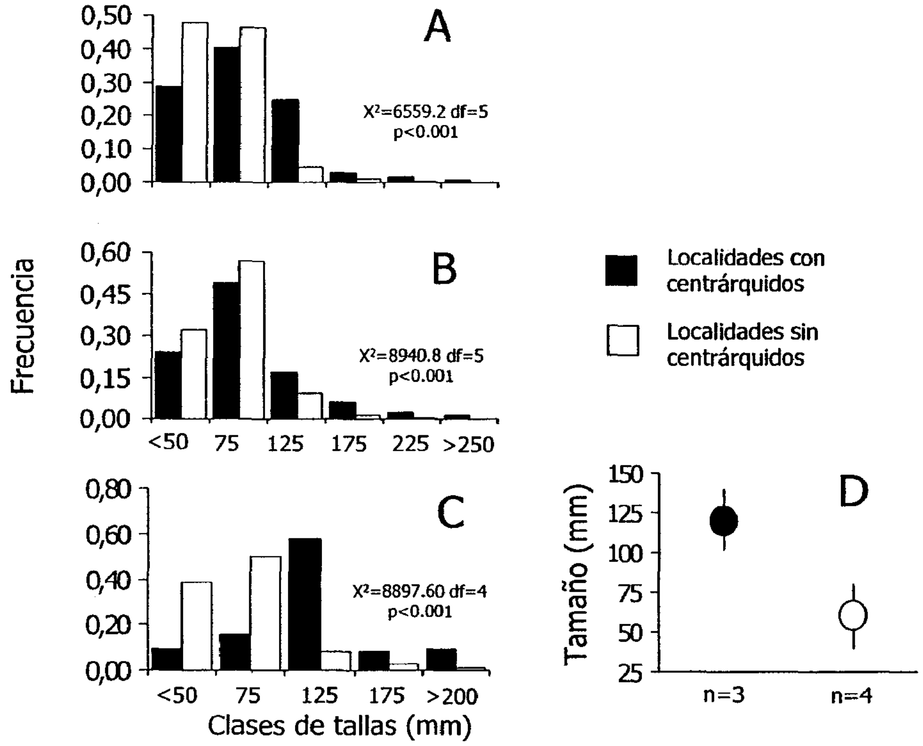

**Figura 11.** Distribución de clases de talla de las especies nativas (A, B y C) comparando localidades con y sin centrárquidos. A) Distribución de tallas para el conjunto de la comunidad (sólo nativas) en localidades incluidas en el

sector de 40-20 km al río principal, B) Idem para el sector de más de 40 km, C) Idem sólo para barbos (*Barbus* spp.) en sector de 40-20 Km al río principal. La comparación se ha realizado con un test chi-cuadrado. Ver capítulo correspondiente para una descripción detallada de los sectores. D) Diferencias de los tamaños de barbos (longitud total, mm) en localidades con y sin centrárquidos para el mismo sector. Los círculos son las medianas y las barras los cuartiles 25% y 75%. Las diferencias son significativas (U de Mann Whitney $p<0.05$)

Estos resultados demuestran que, a pesar de la mínima importancia de los centrárquidos en términos de abundancia y biomasa, éstos ejercen un fuerte impacto sobre las comunidades que invaden. Prevenir su expansión hacia los lugares que están aún sin invadir y evitar la llegada de nuevas especies exóticas es fundamental para la conservación de la ictiofauna nativa ibérica.

## INFLUENCIA DEL HÁBITAT Y LOS PECES EXÓTICOS EN LA DISTRIBUCIÓN Y CONSERVACIÓN DE DOS ESPECIES IBÉRICAS AMENAZADAS: EL JARABUGO (*ANAECYPRIS HISPANICA*) Y EL PEZ FRAILE (*SALARIA FLUVIATILIS*).

A partir de los resultados anteriores, se estudia en este trabajo la influencia que ejercen las características del hábitat y los peces exóticos en los patrones de distribución del jarabugo (*Anaecypris hispanica*) y del pez fraile (*Salaria fluviatilis*), la primera endémica de la cuenca del Guadiana. Ambas especies se encuentran entre los peces continentales más amenazados y a su vez más desconocidos de la Península Ibérica (Collares-Pereira et al., 1999; Doadrio, 2001).

El estudio se centró en 28 localidades de la cuenca del Guadiana (Figura 12), donde los peces se muestrearon mediante el empleo de pesca eléctrica antes del llenado de los embalses de Alqueva y Andévalo. También se llevó a cabo una caracterización del hábitat fluvial a dos escalas distintas: 1) escala de mesohábitat, en la que se midieron o estimaron in situ 15 variables relacionadas con la estructura de los cauces y las características físico-químicas del agua; 2) escala de macrohábitat, donde se calcularon mediante mapas topográficos e hidrológicos variables relacionadas con las características de las cuencas (Tabla 7).

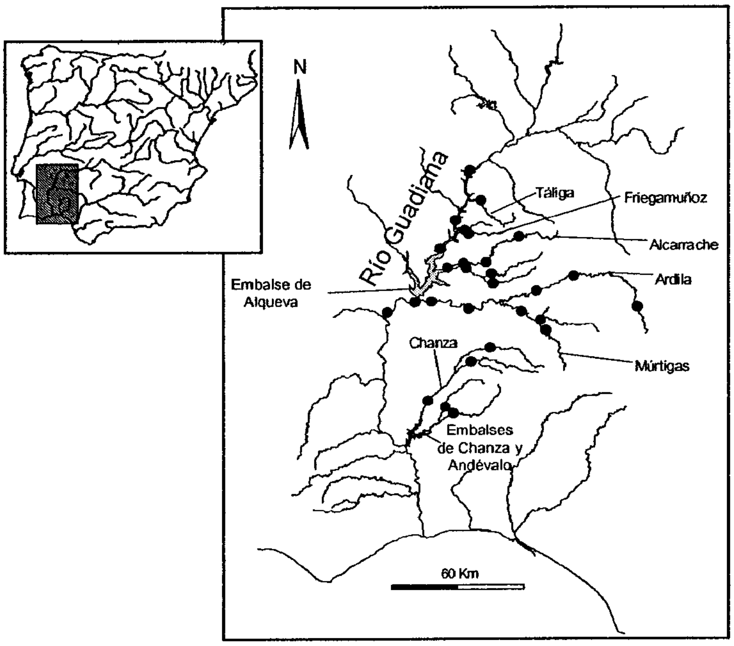

**Figura 12**. Mapa del área de estudio. Se indican las localidades de muestreo, los tributarios estudiados y la localización de los embalses principales.

Para poder detectar cambios recientes en la distribución de ambas especies se compararon nuestros datos con los publicados en el "Atlas y Libro Rojo de los peces continentales de España" (Doadrio, 2001). Las preferencias de hábitat de cada especie se analizaron mediante ACP y test de Chi-cuadrado. La posible influencia de las especies exóticas en la distribución del jarabugo y el pez fraile se analizó mediante modelos probabilísticos basados en un test de Monte Carlo.

La situación del jarabugo fue muy precaria, con una distribución muy restringida, limitada al 14,3% de las localidades prospectadas y unos valores de abundancia muy bajos. De la comparación con estudios previos se desprende que el jarabugo ha desparecido del 86% de su rango original dentro del área de estudio (Figura 13). El pez fraile, por el contrario, fue capturado en el 32,1% de las localidades y con ello se amplía notablemente su rango de distribución conocido respecto a estudios previos (Figura 13). La abundancia de esta especie fue igualmente muy baja.

El jarabugo mostró preferencias por arroyos pequeños, con poca velocidad de corriente y con abundante vegetación acuática sumergida (Figura 14). El pez fraile en cambio seleccionó los tramos bajos de los tributarios o el propio río Guadiana (zonas caracterizadas por caudal elevado, cauces amplios y gran velocidad de la corriente) (Figura 14).

**Tabla 7.** Variables del hábitat utilizadas para caracterizar las localidades de muestreo.

| **MACROHABITAT** | **MESOHABITAT** | |
|---|---|---|
| **Variables** | **Variables** | **Método** |
| Área de la cuenca ($Km^2$) | Temperatura (ºC) | Equipo portátil |
| Orden | Conductividad (µs/cm) | Equipo portátil |
| Distancia al Guadiana (Km) | Turbidez (FTU) | Equipo portátil |
| Altitud (m) | $O_2$ disuelto (mg/l and %) | Equipo portátil |
| | pH | Equipo portátil |
| | Velocidad de la corriente | Objeto flotante, 3 replicas |
| | Profundidad (cm) | Metro rígido |
| | Anchura cauce (m) | Metro flexible |
| | Granulometría del sustrato | Escala Wentworth |
| | Vegetación acuática sumergida (%) | Estima visual, 2-3 observadores |
| | Vegetación acuática emergente (%) | Estima visual, 2-3 observadores |
| | Herbáceas de orillas (%) | Estima visual, 2-3 observadores |
| | Arbustos de orillas (%) | Estima visual, 2-3 observadores |
| | Árboles (%) | Estima visual, 2-3 observadores |
| | Refugio para peces | Estima visual, 2-3 observadores |

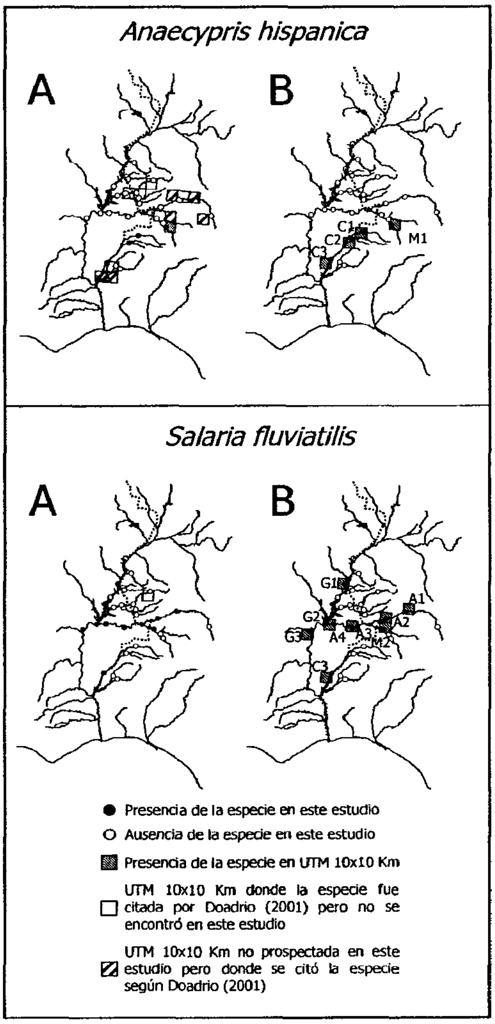

**Figura 13.** Distribución del jarabugo y el pez fraile en el área de estudio. A) Información publicada en Doadrio (2001), B) datos de este estudio. Los círculos negros son las presencias y los blancos las ausencias. Los cuadrados son cuadriculas UTM de 10x10 km. Los códigos de las localidades comienzan con la primera letra del nombre del río y se refieren a las localidades donde las especies estuvieron presentes.

La incidencia de las especies exóticas sobre los patrones de distribución de las nativas fue distinto para cada especie analizada. Mientras que en el caso del pez fraile no ejercieron efecto aparente, para el jarabugo fue un factor que limitó claramente su distribución. Los jarabugos nunca coexistieron en las mismas localidades con los centrárquidos, aun siendo la probabilidad de que coexistieran por puro azar extremadamente alta (Figura 15).

Los grandes embalses del área de estudio, que transforman el hábitat fluvial original y crean ambientes idóneos para la proliferación de especies exóticas, se postularon como una grave amenaza para la conservación de ambas especies, sobre todo para el jarabugo.

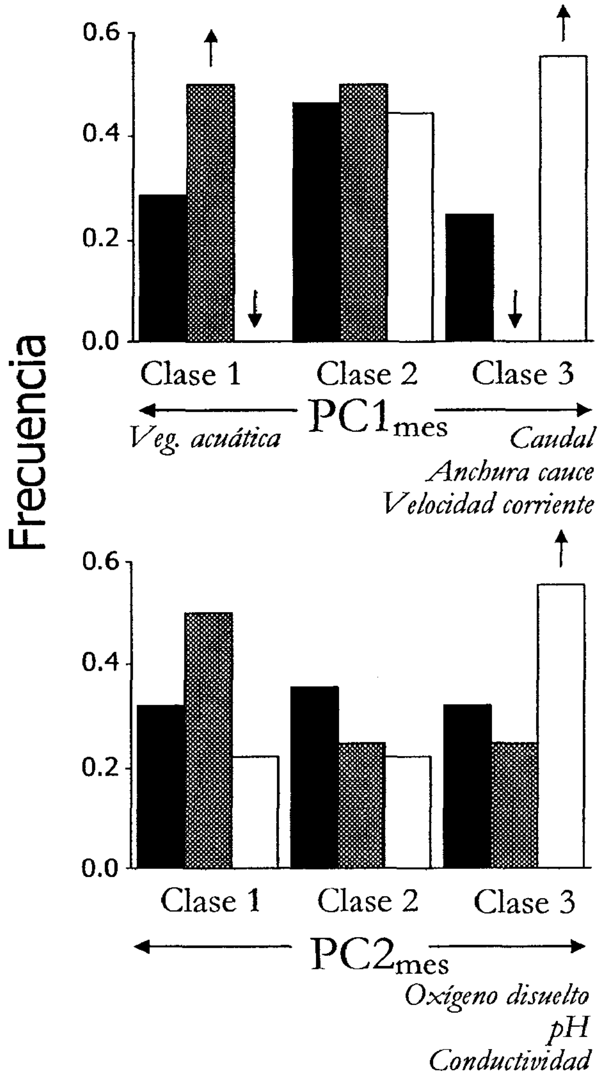

**Figura 14.** Preferencias de hábitat del jarabugo (*Anaecypris hispanica*) y el pez fraile (*Salaria fluviatilis*) en el Guadiana medio. Las barras negras se corresponden con el hábitat disponible, las grises con la frecuencia de uso del jarabugo y las blancas con la frecuencia de uso del pez fraile. $CP1_{mes}$ y $CP2_{mes}$ son los gradientes de hábitat. La interpretación de cada gradiente se representa bajo flechas. Los tipos de hábitat (clases) preferidos o rechazados se indican con flechas verticales ($\chi^2$-subdividido, $p<0,05$). Jarabugo n=4 localidades, pez fraile n=9 localidades.

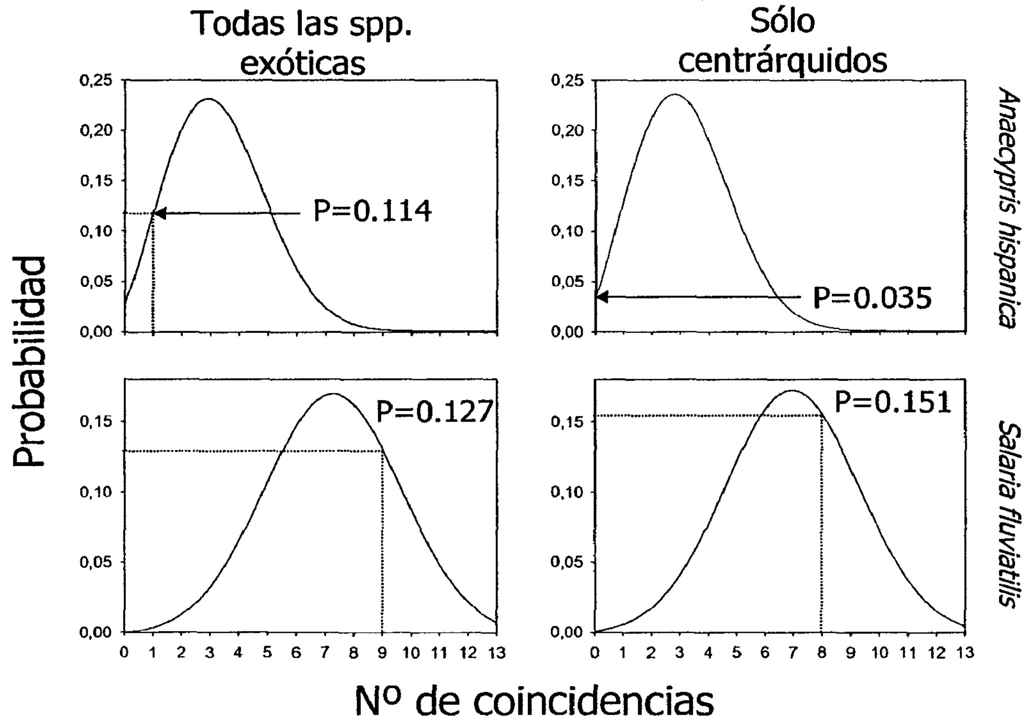

**Figura 15.** Modelos de probabilidad de coincidencias entre jarabugo/ pez fraile con todas las especies exóticas y los centrárquidos, generadas a partir de un test de Monte Carlo. Los valores de P calculados representan la probabilidad de coincidencia esperable por azar entre cada par de comparaciones para el número de localidades observadas en el campo en que realmente coinciden. Ver capítulo 4 completo para una descripción más detallada del análisis.

## DIETA Y SELECCIÓN DE PRESAS DE LA NUTRIA (*LUTRA LUTRA*) EN RÍOS MEDITERRÁNEOS INVADIDOS POR CENTRÁRQUIDOS.

¿En que medida consume centrárquidos un depredador ictiófago como la nutria?, en caso afirmativo ¿podría ser la nutria un controlador natural eficiente de las poblaciones de estas especies exóticas invasoras? Con este trabajo pretendemos responder a estas cuestiones, analizando la dieta del mustélido en el área de estudio y estudiando su selección de presas.

La dieta de la nutria se describió analizando un total de 1633 excrementos recolectados en 71 localidades de muestreo repartidas por las cuencas de los ríos Guadiana (28 localidades), Guadalquivir (28 localidades) y Tinto (15 localidades) (metodología de análisis descrita por Beja, 1996, 1997). La selección de presas se llevó a cabo en las localidades del Guadiana (547 excrementos, Figura 16), puesto que es esta zona la que sufre en mayor medida la invasión de los centrárquidos. Este análisis se realizó teniendo en cuenta únicamente peces, comparando las proporciones consumidas por la nutria con las disponibles en el medio (datos obtenidos con pesca eléctrica) mediante el índice de Jacobs (1974).

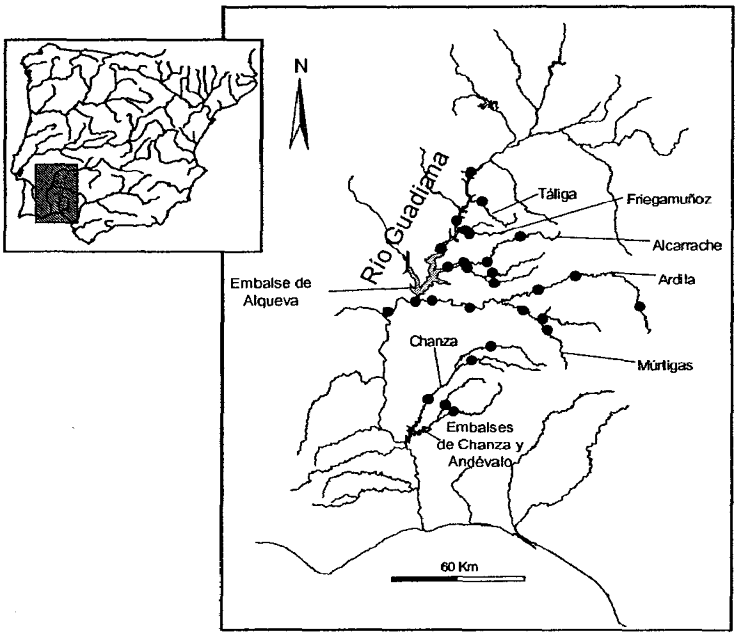

**Figura 16.** Situación de las localidades donde se realizó el estudio de selección de presas de la nutria.

**Tabla 8.** Dieta de la nutria en el Guadiana expresada como frecuencia relativa de aparición (FRA), porcentaje de individuos (% Ind), biomasa (% Bio) y la aparición, el porcentaje de individuos y la biomasa de cada especie de pez presente en el medio. Número total de excrementos analizados n=547. *Especies de peces excluidas del análisis de selección de presa.

| | En la dieta | | | En el medio | | |
|---|---|---|---|---|---|---|
| | FRA n=1013 | % Ind n=1480 | % Bio (g) n=36768.4 | Aparición | % Ind n=31197 | % Bio (g) n=166420.4 |
| ***Peces*** | *57.8* | *62.1* | *81.1* | | | |
| Familia Cyprinidae | | | | | | |
| *Barbus* spp. | 29.2 | 29.5 | 67.7 | 92.9 | 23.8 | 69.7 |
| *Chondrostoma lemmingii* | 0.3 | 0.5 | 0.01 | 25.0 | 4.1 | 0.9 |
| *Chondrostoma willkommii* | 1.7 | 1.3 | 2.8 | 42.9 | 7.3 | 1.5 |
| *Chondrostoma sp.* | 1.1 | 1.4 | 0.06 | | | |
| *Squalius alburnoides* | 12.9 | 19.2 | 2.2 | 85.7 | 54.1 | 16.0 |
| *Squalius pyrenaicus* | 0.6 | 0.5 | 0.4 | 39.3 | 2.3 | 1.0 |
| *Anaecypris hispanica** | - | - | - | 14.3 | 1.4 | 0.2 |
| Familia Cobitidae | | | | | | |
| *Cobitis paludica* | 5.5 | 4.4 | 0.7 | 64.3 | 4.0 | 1.6 |
| Familia Blenniidae | | | | | | |
| *Salaria fluviatilis** | 0.2 | 0.1 | 0.02 | 32.1 | 0.2 | 0.2 |
| Familia Anguillidae | | | | | | |
| *Anguilla anguilla** | 0.1 | 0.1 | 1.5 | - | - | |
| Especies exóticas | | | | | | |
| Familia Cyprinidae | | | | | | |
| *Carassius auratus** | 0.7 | 0.9 | 0.4 | 3.6 | 0.02 | 0.02 |
| *Cyprinus carpio** | 0.3 | 0.2 | 0.4 | 7.1 | 0.1 | 4.7 |
| Familia Centrarchidae | | | | | | |
| *Micropterus salmoides* | 0.8 | 0.8 | 0.4 | 25.0 | 1.1 | 1.1 |
| *Lepomis gibbosus* | 2.7 | 1.9 | 0.9 | 75.0 | 1.5 | 3.0 |
| Familia Poeciliidae | | | | | | |
| *Gambusia holbrooki** | - | - | - | 7.1 | 0.05 | 0.01 |
| Familia Cichlidae | | | | | | |
| *Herichthys facetum** | - | - | - | 7.1 | 0.03 | 0.1 |
| *Pez indeterminado** | 1.7 | 1.3 | 3.6 | | | |
| ***Otras presas*** | *42.2* | *37.9* | *18.9* | | | |
| *Procambarus clarkii* | 24.2 | 21.5 | 8.2 | | | |
| *Insectos* | 1.6 | 1.3 | 0.04 | | | |
| *Rana perezi* | 12.4 | 11.9 | 4.6 | | | |
| *Bufo bufo* | 0.3 | 0.2 | 0.2 | | | |
| *Anfibios sin identificar* | 0.9 | 1.0 | 0.4 | | | |
| *Natrix sp.* | 2.2 | 1.5 | 0.9 | | | |
| *Mauremys leprosa* | 0.4 | 0.3 | 2.7 | | | |
| *Aves* | 0.1 | 0.1 | 1.1 | | | |
| *Mamíferos* | 0.1 | 0.1 | 0.8 | | | |

Los valores de este índice oscilan entre –1 y 1, donde –1 representa un rechazo total de la presa considerada, 0 indica que la presa se consume en la medida en que está disponible y 1 es una preferencia total. Para interpretar el índice de forma objetiva comparamos hipótesis alternativas, donde la hipótesis nula correspondía con valor del índice igual a cero (sin selección). El valor obtenido del índice se comparó con una t-Student (Revilla et al., 2000).

La dieta de la nutria se expresó como frecuencia relativa de aparición (FRA), porcentaje de individuos y porcentaje de biomasa ingeridos. Los peces fueron la presa fundamental de la nutria, representado más del 60% del total de individuos consumidos y aportando más del 80% de la biomasa total ingerida (Capítulo 5, Tabla 8) . En las localidades del Guadiana la nutria consumió la mayoría de los taxones de peces presentes, siendo los barbos (*Barbus* spp.) la presa más importante (25,9% del total de individuos y 67,7% de la biomasa total), seguida del cangrejo rojo americano (*Procambarus clarkii*) (21,5% de los individuos y 8,2% de la biomasa total). El resto de presas ocupó un papel secundario (Tabla 8). El consumo de ambas especies de centrárquidos (pez sol y blacbás) fue bajo. De hecho, la aplicación del índice de Jacobs mostró un claro rechazo por ambas especies, teniendo en cuenta tanto los individuos consumidos como la biomasa ingerida para ambas categorías de presa (Figura 17).

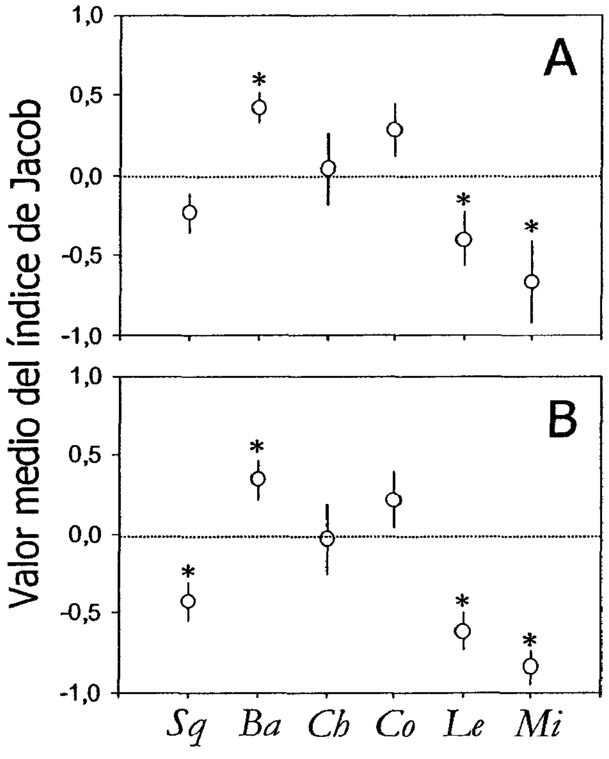

**Figura 17.** Valores medios (± error estándar) del índice de Jacobs para los peces consumidos por la nutria (*L. lutra*). Los valores positivos y negativos indican

"preferencia" y "rechazo", respectivamente. A) selección de presas sobre individuos, B) Idem para biomasa. Sq-*Squalius* sp., Ba-*Barbus* sp., Ch-*Chondrostoma* sp., Co-*C. paludica*, Le-*L. gibbosus* y Mi-*M. salmoides*. * media significativamente distinta de cero (t-Student, $p<0.05$).

El consumo por parte de la nutria de especies nativas de los géneros *Squalius*, *Barbus* y e incluso *Chondrostoma* aumentó a medida que lo hacían en el medio. Sin embargo, el aumento progresivo del pez sol en el medio no reflejó un aumento paralelo en la dieta, siendo prácticamente ignorado por la nutria (Figura 18). A la luz de estos resultados es posible afirmar que, de momento, la nutria no está consumiendo centrárquidos en la medida en que éstos están disponibles en el medio. Por tanto, este depredador nativo no está actuando como un controlador natural eficiente de las poblaciones de centrárquidos.

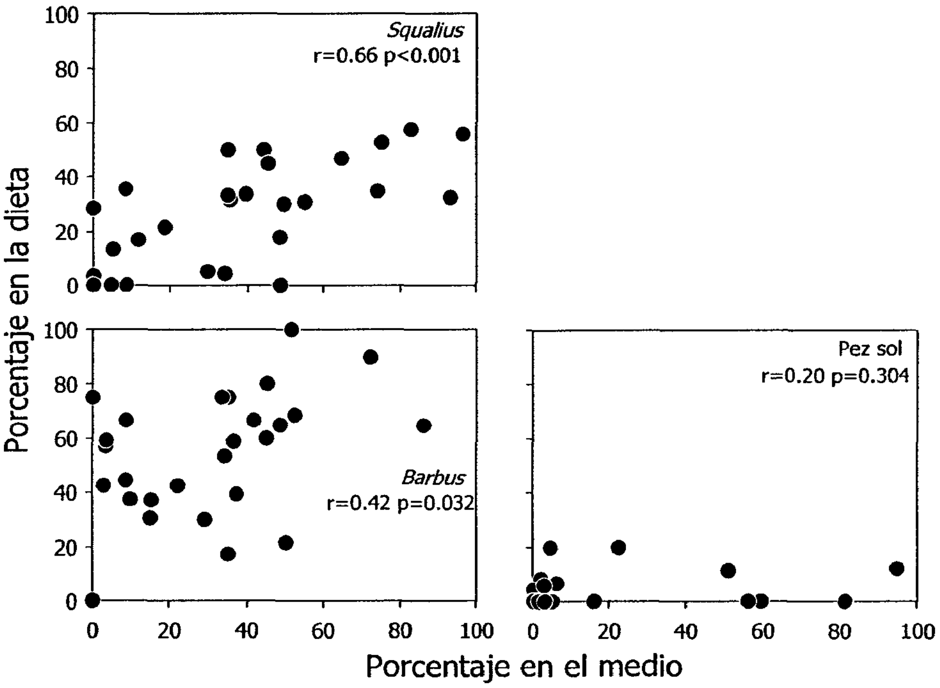

**Figura 18.** Relación entre el porcentaje de individuos de *Squalius* sp., *Barbus* sp. y pez sol (*L. gibbosus*) presentes en la dieta y en el medio

El declive generalizado de los peces continentales ibéricos (Aparicio et al., 2000; Doadrio, 2001), tradicionalmente las presas preferidas de la nutria, junto con la expansión de peces introducidos (centrárquidos y otros) puede forzar al mustélido a consumir estas presas en una mayor proporción que la actual. Sin embargo, el mantenimiento de poblaciones estables y saludables de este depredador amenazado no sólo dependerá de que consuma estas nuevas presas, sino también de que el aporte energético proporcionado por éstas sea similar al proporcionado por las especies de peces nativos.

## SEGREGACIÓN ESPACIAL ENTRE ESPECIES CONGENÉRICAS A LO LARGO DE UN GRADIENTE AMBIENTAL EN RÍOS MEDITERRÁNEOS.

La segregación espacial es un fenómeno común entre las especies de peces continentales y ocurre normalmente siguiendo los gradientes ambientales observados en los ríos (Gilliam et al., 1993; Winston, 1995; Taylor, 1996). Los mecanismos que generan tales patrones pueden ser debidos a factores abióticos, bióticos e incluso aleatorios (Jackson et al., 2001). En el primer trabajo se observa una segregación espacial dentro de las especies del género *Chondrostoma* y entre las del género *Squalius*, al no pertenecer en ningún caso las dos especies del mismo género a la misma asociación.

En este estudio analizamos en profundidad los patrones de distribución de las especies del género *Chondrostoma* (*Ch. lemmingii* y *Ch. willkommii*) y *Squalius* (*S. alburnoides* y *S. pyrenaicus*), con el fin de examinar la segregación espacial entre tales especies congenéricas. El estudio se centró en las localidades pertenecientes a las cuencas del Guadiana, Guadalquivir, Tinto y Odiel (n=73 localidades).

Los resultados confirmaron la existencia de una clara segregación espacial entre estas especies congenéricas. Un ACP de las variables ambientales separó las localidades exclusivamente ocupadas por *Ch. lemmingii* de las que lo hacían en exclusiva por *Ch. willkommii*, a lo largo de un gradiente tramo alto –tramo bajo. El mismo resultado se obtuvo para ambas especies de *Squalius*, indicando que las especies pertenecientes al mismo género ocuparon hábitats o tramos fluviales distintos (Figura 19). *S. pyrenaicus* y *Ch. lemmingii* aparecieron fundamentalmente en tramos altos, mientras que *S. alburnoides* y *Ch. willkommii* ocuparon sobre todo tramos medios-bajos (Figura 20). Aquellas localidades en las que las especies congenéricas coexistieron o estuvieron ausentes, ocuparon posiciones intermedias en la ordenación de las localidades a lo largo del gradiente ambiental (Figura 19).

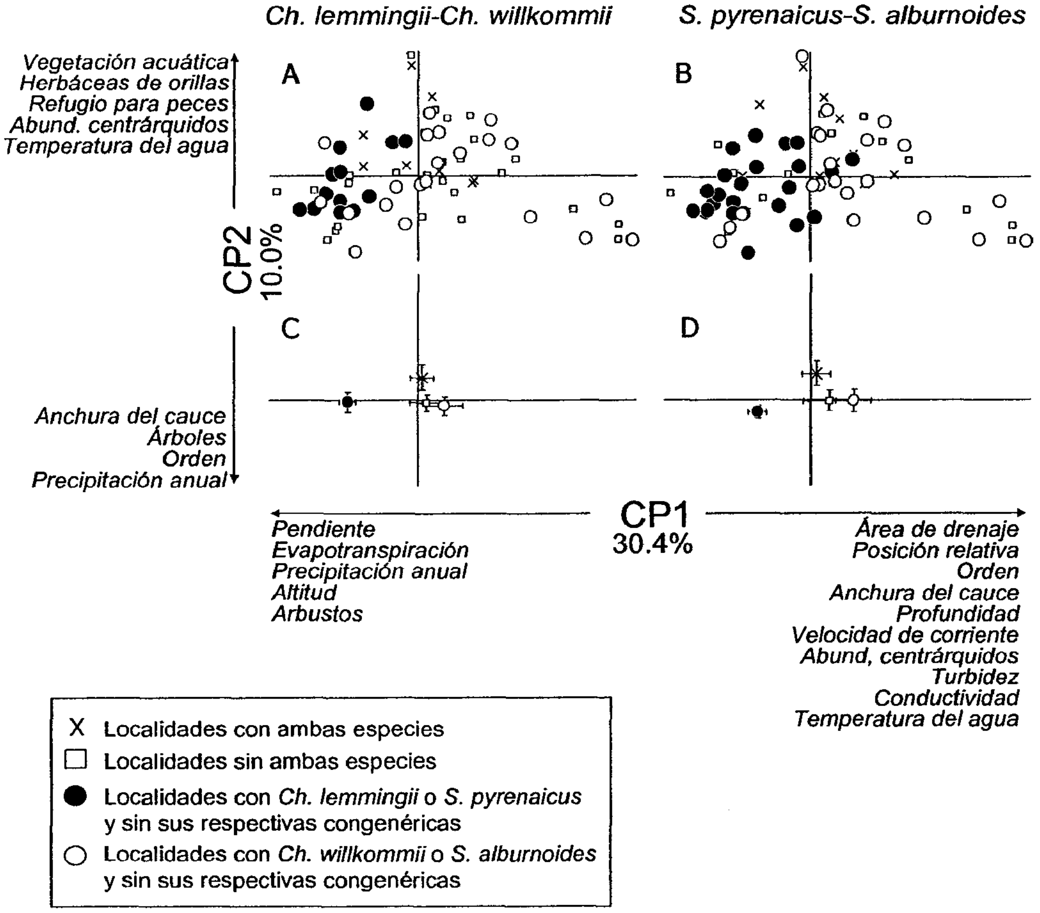

**Figura 19.** Distribución de las localidades de muestreo en el espacio definido por los dos componentes principales (CP1 y CP2) de un ACP de variables del hábitat. A) distribución de *Chondrostoma* y B) distribución de *Squalius*. C) y D) representan las medias de los valores de cada grupo y las barras error estándar. Los símbolos representan cuatro grupos que indican distintas combinaciones de presencia-ausencia de las especies congenéricas La interpretación de cada gradiente se indica debajo de las flechas.

Por otra parte, las especies del género *Chondrostoma* no coexistieron en las pequeñas subcuencas aisladas de los ríos Tinto y Odiel. *Ch. lemmingii* habitó las subcuencas más pequeñas, mientras que *Ch. willkommii* lo hizo en las de mayor tamaño.

En general, esta segregación espacial puede explicarse por diferencias en las preferencias de hábitat de cada especie. Otra posible explicación, no excluyente con la anterior, estaría relacionada con factores bióticos de distinto tipo. De hecho, existen trabajos donde se pone de manifiesto que la segregación entre especies morfológicamente similares, como aquéllas que pertenecen al mismo género, pueden ser debidas a interacciones bióticas (Winston, 1995)

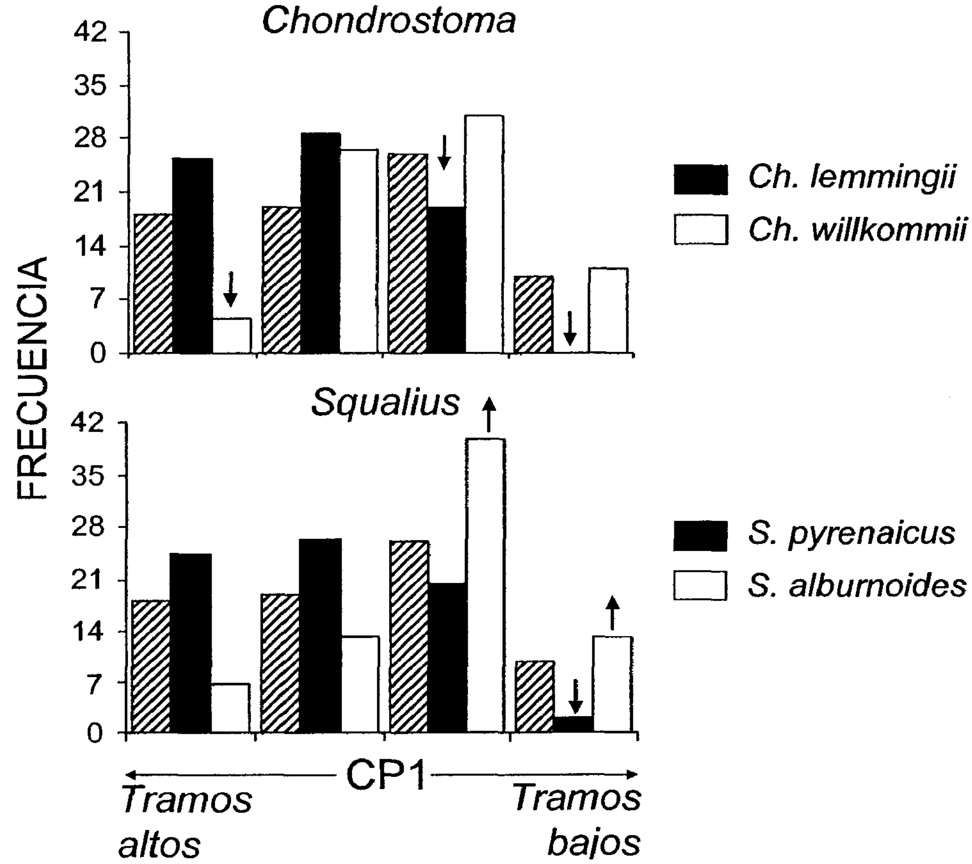

**Figura 20.** Preferencias de hábitat de los géneros *Chondrostoma* y *Squalius* a lo largo del gradiente ambiental tramo alto-tramo bajo representado por el CP1. Las barras rayadas representan el hábitat disponible. El hábitat disponible y el usado por cada especie se comparó con un análisis chi-cuadrado. Las porciones del gradiente preferidas o rechazadas se representan con flechas verticales (análisis chi-cuadrado subdividido, $p<0.05$).

## CONCLUSIONES

1. En el área de estudio, constituida por el sector medio-bajo del Guadiana, una franja del Guadalquivir medio, las cuencas de los ríos Tinto y Odiel, y varias pequeñas cuencas del entorno de Tarifa (Cádiz), se capturaron 16 especies de peces estrictamente dulceacuícolas, de las cuales 10 fueron autóctonas y 6 exóticas. Este elevado número de especies nativas representa el 27% del total de especies dulceacuícolas descritas en España. De todas las cuencas estudiadas, fue la del río Guadiana la que contuvo un mayor número de especies

2. Los peces continentales del área de estudio se agruparon en cuatro gremios de hábitat en función de las similitudes de sus patrones de distribución y del uso del hábitat: "Cabeceras", formado por *Squalius pyrenaicus* y *Chondrostoma lemmingii* (>50 mm); "Generalistas-tramo medio", constituido por Barbus spp.<200 mm, *Cobitis paludica* (>50 mm), *Anaecypris hispanica* (>50 mm) y *Ch. lemmingii* (<50 mm); "Nativas de tramo bajo", con *S. alburnoides*, *Ch. willkommii* (<150 mm), *Salaria fluviatilis* (<80 mm), *C. paludica* (<50 mm) y *A. hispanica* (<50 mm); "Exóticas, grandes ciprínidos, y blenios de tramos bajo", conformado por *Cyprinus carpio*, *Gambusia holbrooki*, *Micropterus salmoides*, *Lepomis gibbosus*, *Barbus* spp.>200 mm, *Ch. willkommii*>150 mm and *S. fluviatilis*>80 mm. Estos gremios definen una sectorización fluvial desde los tramos de cabecera hasta las zonas más bajas.

3. Se observó una clara segregación espacial dentro de las especies del género *Chondrostoma* y entre las del género *Squalius*. En general las especies congenéricas no coexistían. Pardillas (*Ch. lemmingii*) y cachuelos (*S. pyrenaicus*) ocuparon normalmente tramos altos o pequeñas cuencas independientes, mientras que bogas (*Ch. willkommii*) y calandinos (S. *alburnoides*) habitaron en tramos medios-bajos.4. Entre las especies exóticas destacaron, por su abundancia y amplitud de distribución, los centrárquidos pez sol y blacbás. Ambas especies fueron relativamente abundantes en los ríos y arroyos estudiados de las cuencas del Guadiana y Guadalquivir.

4. Las especies exóticas y las nativas tendieron a aparecer espacialmente segregadas. Además, en las zonas donde solaparon, la abundancia de las nativas disminuía como respuesta al aumento de la abundancia de las exóticas. Ello parece enmascarar el patrón natural que mostrarían las especies autóctonas en ausencia de exóticas.

5. Entre las especies exóticas destacaron, por su abundancia, amplitud de distribución y potencial de impacto, los centrárquidos pez sol (*Lepomis gibbosus*) y

blacbás (*Micropterus salmoides*). Ambas especies fueron relativamente abundantes en los ríos y arroyos estudiados de las cuencas del Guadiana y Guadalquivir.

6. Los centrárquidos ejercieron un fuerte impacto en las comunidades de peces nativos. El aumento en la abundancia del pez sol y el blacbás produjo un descenso en la abundancia, biomasa, riqueza y diversidad de la comunidad de peces autóctonos.

7. La abundancia de pez sol y blacbás aumentó hacia los tramos bajos y zonas cercanas a los cauces principales de los ríos Guadiana y Guadalquivir. Este hecho invertía el patrón de distribución natural esperable de las especies nativas, al aumentar la abundancia y riqueza de estas especies hacia los tramos más altos y alejados de los cauces principales de ambos ríos.

8. La estructura de tallas de la comunidad de especies autóctonas se vio afectada por la presencia de centrárquidos. Las localidades con centrárquidos mostraron una menor proporción de tallas pequeñas que las zonas sin pez sol y/o blacbás. Las tallas menores pueden ser fácilmente depredadas/ desplazadas por estas especies exóticas, provocando esta anomalía en la estructura de tamaños. Esto puede producir problemas de reclutamiento de las poblaciones de peces autóctonos.

9. Los ciprínidos autóctonos de pequeño tamaño, como el jarabugo (endemismo del Guadiana), estuvieron especialmente afectados por el pez sol y el blacbás. El jarabugo, especie gravemente amenazada, sólo se capturó en localidades sin centrárquidos (tramos medios-altos especialmente). En cambio, no se observó un impacto significativo de los centrárquidos sobre el pez fraile, que convivió con centrárquidos y otras especies exóticas en los tramos bajos de los ríos y arroyos de la cuenca del Guadiana.

10. La nutria mostró una dieta basada principalmente en peces, siendo la presa básica *Barbus* spp. seguido del cangrejo rojo americano (*Procambarus clarkii*). No obstante, la nutria rechazó significativamente a los centrárquidos como recurso trófico. Por tanto, su papel como potencial controlador natural de las poblaciones de estas especies exóticas parece poco relevante.

11. Los centrárquidos se erigen como un factor clave de conservación de la ictiofauna nativa. La prevención de la expansión de los centrárquidos y otras especies exóticas hacia los lugares que están aún sin invadir es fundamental para la conservación de la ictiofauna nativa ibérica.

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# CAPÍTULO 2

## EXOTIC FISH SPECIES DETERMINE THE DISTRIBUTION PATTERNS AND HABITAT USE OF NATIVE FISH ASSEMBLAGES IN IBERIAN MEDITERRANEAN RIVER SYSTEMS

### *Las especies exóticas determinan los patrones de distribución y uso del hábitat de la ictiofauna nativa de ríos ibéricos mediterráneos*

Enviado a Conservation Biology

# EXOTIC FISH SPECIES DETERMINE THE DISTRIBUTION PATTERNS AND HABITAT USE OF NATIVE FISH ASSEMBLAGES IN IBERIAN MEDITERRANEAN RIVER SYSTEMS

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## ABSTRACT

We examined the distribution patterns of freshwater fish fauna in various basins of the southwest Iberian Peninsula to identify habitat use and assemblage organization. The 80 studied sites are located along a typical headwater-downstream river gradient and a fish-shelter-related gradient according to a PCA based on 21 environmental variables. At least 20 fish species were collected, 16 of them primary freshwater species, including six exotics. The non-native species were spatially segregated from the native ones. Based on PCA techniques and habitat use-availability analysis, we identified four habitat guilds on the basis of observed similarities in distribution and habitat use patterns. The distribution of these guilds determines the following river zonation from the headwaters to the river mouth. 1) "Headwaters"- chub (*Squalius pyrenaicus*) and medium-large (>50 mm) pardilla nase (*Chondrostoma lemmingii*): preference for small streams (headwaters or small isolated basins) with abundant submerged vegetation. 2) "Middle reaches-generalists"- small-medium (<200 mm) barbels (*Barbus* spp.), medium-large (>50 mm) loach (*Cobitis paludica*), large (>50 mm) jarabugo (*Anaecypris hispanica*), and small (<50 mm) pardilla nase: generalized habitat use, although more abundant in sites with high fish shelter and aquatic vegetation cover, occupying an intermediate river position between headwaters and habitats of the lower reaches. 3) "Lower reaches natives"- roach (*S. alburnoides*), small-medium (<150 mm) nase (*Ch. willkommii*), small-medium (<80 mm) freshwater blenny (*Salaria fluviatilis*), and small loach and jarabugo (<50 mm): apparent generalized pattern of habitat use but more abundant toward the lower reaches and sheltered zones. 4) "Lower reaches exotics and very large cyprinids and blenny"- exotics (*Lepomis gibbosus, Micropterus salmoides, Cyprinus carpio*, and *Gambusia holbrooki*), very large barbels (>200 mm), nase (>150 mm), and blenny (>80 mm): reject headwaters and preferentially use lower reaches (high-order streams with wide watercourses and deep channels). In sites where the exotics sunfish (*L. gibbosus*), largemouth bass (*M. salmoides*), and carp (*C. carpio*) were present (n=35), the abundance of the "Middle reaches-generalists" and "Lower reaches natives" guilds decreased as exotic abundance increased. This trend indicates the exclusion of these native species from the lower stream reaches because of the presence of exotics. Although others factors such as habitat disturbance could be involved, the results show that exotic species determine the native species' distribution patterns and habitat use. This makes it impossible to determine the original distribution and habitat use of native species in the absence of exotic species. The described fish assemblages and their habitat use patterns may reflect a combination of the exotic species' influence, their own habitat requirements, and the nature of the habitat itself.

Key words: Iberian endemisms, biotic interactions, habitat preference, Mediterranean streams, Guadiana basin, conservation, small basins, organization

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## INTRODUCTION

Faunal distribution patterns and habitat use are a consequence of adaptive, biotic, and even random factors. Stream fishes are not an exception to this trend, and the characteristics of their assemblages are determined by both abiotic and biotic processes that operate at multiple spatial and temporal scales (Matthews, 1998). Many fish species exhibit well-defined spatial zonation (Huet, 1959; Copp, 1989; Schlosser, 1987 and 1991). In temperate streams, some fish species show affinities for certain habitat features; consequently, different habitat guilds can be defined according to their similarities in terms of both fish distribution and habitat use (Prenda et al., 1997). However, other studies note that most temperate stream fishes display relatively generalized macrohabitat use-patterns (Grossman et al., 1985). This suggests that the effect of density-independent factors (i.e. environmental variability) is the main force that structures fish communities or the adaptation to particular habitat conditions (upstream versus downstream: Cowx and Welcomme, 1998; floodplains versus mid-channel habitats: Welcomme, 1985). Given the above, few habitat specializations should be promoted in highly variable environments (Poff and Allan, 1995) such as Mediterranean stream ecosystems (Gasith and Resh, 1999).

Understanding these patterns is of special relevance not only from a species conservation perspective (Filipe et al., 2002 and 2004), but also for the assessment of the ecological status of rivers. This is the underlying philosophy of the Index of Biotic Integrity, which evaluates the "health" of streams and rivers based on descriptive metrics of the fish community (habitat guilds, trophic guilds, species richness, etc) (see for example Karr, 1981; Oberdorff et al., 2002; Pont et al., 2006, and references therein). However, habitat use and distribution patterns remain poorly understood for Iberian freshwater fishes (see for example Godinho et al., 1997; Godinho and Ferreira, 2000) because the Iberian freshwater fish fauna is generally poorly understood. Key aspects such as the total number of species and the basic ecological features of many species remain uncertain (Doadrio, 2001). As a result, a formal classification of Iberian fish into habitat guilds or any other form of ecological grouping has yet to be undertaken.

Iberian fish fauna inhabit highly variable Mediterranean streams, and are currently being invaded by multiple new exotic species whose impact on natural patterns has not yet been sufficiently evaluated. The characteristics of Mediterranean streams are strongly linked to those of the Mediterranean climate, which results in seasonal events of flooding (autumn, winter, and early spring) and strong droughts (summer) that vary markedly between years (Gasith and Resh, 1999). Invasion by introduced species is a widespread phenomenon in Iberian rivers. On average, 32.4% of the species in the southern Iberian basins were introduced, and in the largest basins of this area the mean proportion is 52% (Clavero et al., 2004). These values are clearly higher than those presented by Gido and Brown (1999) for 125 North American drainages, where the mean proportion of non-native species was 14.2%. All these features combined (streams regime and exotic species) must strongly influence the structure and organization of Mediterranean fish communities (Godinho et al., 1997; Pires et al., 1999; Godinho and Ferreira, 2000; Magalhães et al., 2002b; Morán-López et al., 2005). Given this scenario, it is doubtful that the knowledge framework developed for fish from other areas could be directly applied to the Iberian Peninsula (Magalhães et al., 2002a).

In this work, we studied the distribution of fish across a wide area of the southwest Iberian Peninsula. All studied basins feature a Mediterranean climate across all of their surface areas, and are part of a homogeneous biogeographic unit, the south Iberian sector, as defined by Doadrio (1988) on the basis of freshwater fish distribution. In the light of an extensive but spatially variable presence of exotic fish species (Doadrio, 2001), our main purposes were to assess three questions: (i) What are the main freshwater fish distribution patterns in such hydrologically fluctuating stream habitats? (ii) which species assemblages can be defined according to observed similarities in their distribution patterns? and finally (iii) is it possible to define fish habitat guilds based on habitat preference patterns?

## METHODS

### Study area

The study area comprised several streams and rivers from the southwest Iberian Peninsula (Figure 1). Sampling sites (n=80) were distributed along the Guadiana, Guadalquivir, Tinto, Odiel river basins plus six small isolated basins located upon the southern tip of the Iberian Peninsula (Campo de Gibraltar, Figure 1). The area is characterised by a typical Mediterranean climate with most rainfall occurring between October and March, and with summer drought being a common feature. However, some zones of the southern tip of the peninsula benefit from humid summertime SE winds that reduce to some extent the impact of summer drought on these small coastal streams (Ojeda et al., 2000).

The Tinto and Odiel river basins drain the Iberian Pyrite Belt, a region that contains massive sulphide deposits. Mining activity and natural alteration of the mineral masses have long been a source of pollution in these rivers. The extreme water acidity (pH 2–3) and the high concentration of dissolved heavy metals (Amaral-Zettler et al., 2002) do not enable the existence of macroscopic life. In this environment, fishes remain isolated within unpolluted tributaries or sub-basins, without the possibility of interconnection between populations (Figure 1) (Clavero et al., 2004).

The unpredictable climate regime, typical of the Mediterranean area, results in great pressure on the exploitation of water resources (Collares-Pereira et al., 2000). Most of the studied drainage networks, especially a number of tributaries within the large Guadiana and Guadalquivir Basins, are subjected to strict water regulations. In fact, the Alqueva dam (3150 Hm3 capacity), which impounds the middle reaches of the

Guadiana Basin, is the largest artificial lake in Europe. This dam directly affects various studied tributaries (Táliga, Friegamuñoz, and Alcarrache), but this was not the case during fieldwork (fish sampling in Guadiana Basin during April–June 2001, dam closed in February 2002). A further seven large dams (> 100 Hm3) are located in the studied area.

The coastal streams of the Campo de Gibraltar are almost completely free from damming (only a single small dam that is only closed during the summer months), and human impacts are mainly related to water extraction, which is probably responsible for some freshwater fish population extinctions in the area during recent times (Clavero et al., 2002).

In general, the human population density in the study area is moderate to low (mean 43 inhabitants/km2, range 3–320 inhabitants/km2). The main economic activities of the area are agriculture and farming, and large areas are still covered by pastures and natural forests. Other impacts on rivers, apart from damming, are associated with punctual domestic sewage effluent, mining activities (Tinto and Odiel river basins), diffuse pollution from agriculture, and sand and gravel extraction. Exotic fish species are spreading throughout the study area (e.g., Doadrio, 2001; Bernardo et al., 2003), mostly in the largest Guadalquivir and Guadiana Basins. Nonetheless, the mean conservation status of most of the watercourses in the area can be considered moderate to high.

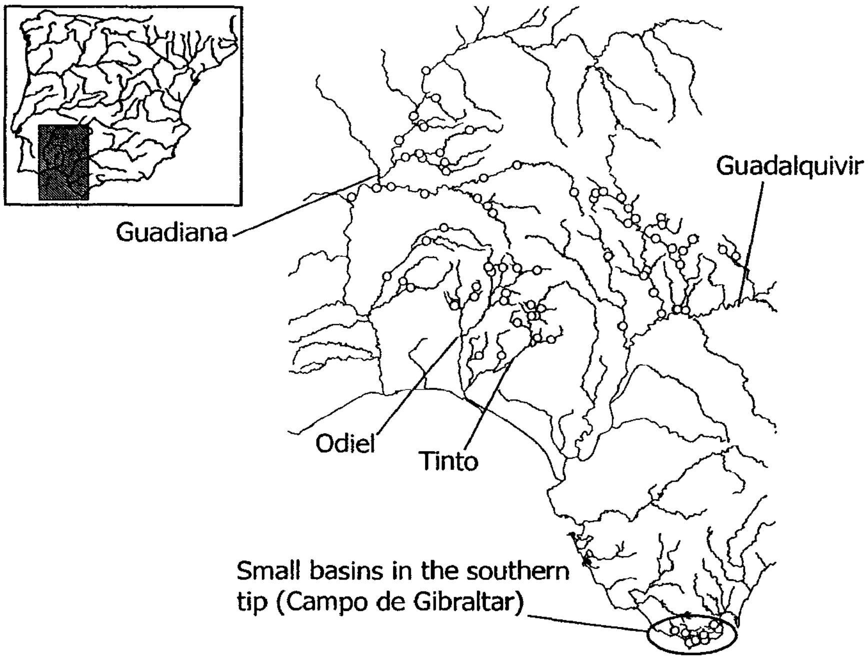

**Figure 1.** Map of the study area showing sampling sites.

### Fish sampling

Fish fauna were sampled during the same season (Guadiana Basin: April and June 2001; Guadalquivir Basin: July 2002; Tinto Basin: April 2001; Odiel Basin: April 2003; and Campo de Gibraltar coastal streams: June 2002) using electrofishing techniques. Although fish sampling was conducted over three different years, the climatic conditions were very similar during this period (the average rainfall for the 3 years recorded at five stations randomly distributed over the study area was 677, 640, and 732 mm, Kruskal-Wallis, $\chi2=3.75$ df=2, p=0.15; and the mean temperature at the same sites for 2001, 2002, and 2003 was 17.3, 17.1, and 17.4 °C, K-W, $\chi2=0.53$, df=2, p=0.77). Sampling was conducted once at each location, covering all available habitats and running along a length of between 100 and 250 m. Fishing time was noted to calculate captures per unit effort (CPUE) as number of captures*100 m-1*1 h-1. All collected fish were identified to the species level and total length was measured to the nearest mm. At least three species of *Barbus* inhabit the study area (*B. microcephalus*, *B. comizo*, and *B. sclateri*), and in many cases it was difficult to distinguish between species; consequently, all individuals were included in the same group (barbels hereafter) and treated as a single taxon for statistical analysis.

### Habitat characterisation

Habitat was characterised using two different approaches. First, we measured or estimated in situ 15 habitat parameters that were recorded from at least 5–10 points that were evenly distributed along the surveyed sites. These variables included physico-chemical water parameters and stream structure parameters (Table 1). Fish shelter was estimated as the area ($m^2$) occupied by certain structures of wetted channel (submerged roots and branches, cavities between bedrocks, etc) that could serve as effective fish refuge in a 4-m-wide band oriented perpendicular to the stream channel (Prenda et al., 1997). The average of all points was used to summarize the site. Secondly, physiographical and climatic parameters were compiled using GIS (Table 1). The variable named "relative position" at a given site was calculated as the ratio between its distance to the headwater point to the total length of the stream. The higher this value, the more downstream is the site location.

### Statistical analysis

To partition the fish community into groups of species with similar distributions (fish assemblages), we applied a PCA to a matrix of fish taxa (Barbus genus plus the remaining species) abundance (CPUE x sites). The correlation between each species/taxa and both PC1 and PC2 (+: "significant positive", –: "significant negative", or 0: "no correlation") determines the fish assemblages (Prenda et al., 1997). As fishes are known to display differential habitat use during ontogeny (e.g., Kováč et al., 2006), a new matrix of taxa–size combinations (CPUE x sites, Table 2) was subjected to the aforementioned multivariate and correlation analysis (extracted components are hereafter referred to as $PC_{abund\text{-}size}$). A PCA was also applied to habitat variables to extract independent components (hereafter $PC_{habitat}$) that could be interpreted as gradients that describe patterns of habitat variation within the study area.

**Table 1.** Environmental variables used to characterise the habitat were fish sampling was performed in 80 localities from south-western Iberia. Both physico-chemical and stream structure parameters were measured or estimated in 5-10 points per site (see methods).

| **Physiographical and climatic variables** | **Physico-chemical parameters** | | **Stream structure parameters** | |
|---|---|---|---|---|
| Variable | Variable | Method | Variable | Method |
| Stream order (Strahler, 1964) | Temperature (ºC) | YSI model 55 | Current velocity (m/s) | Floating object, 3 replicates |
| Basin area (Drainage surface in each site, Km$^2$) | Conductivity (µS/cm) | Crison CM 35 | Depth (cm) | Rigid meter, 6 measures |
| Altitude (m) | Turbidity (FTU) | Hanna Instrum. HI 93703 | Width (m) | Flexible meter |
| Slope ($^{0}/_{00}$) | Dissolved oxygen (mg/L and %) | YSI model 55 | Substrate coarseness (1-9) | Wentworth scale, 6 measures |
| Relative position (dist. to the most headwater point/total length of the stream) | pH | Crison pH-meter 507 | Submerged veget. cover (%) | Visual estimate, 2-3 observers |
| Evapotranspiration (mm) | | | Emergent veget. cover (%) | Visual estimate, 2-3 observers |
| Annual rainfall (mm) | | | Herbaceous cover (%) | Visual estimate, 2-3 observers |
| | | | Shrub cover (%) | Visual estimate, 2-3 observers |
| | | | Tree cover (%) | Visual estimate, 2-3 observers |
| | | | Fish shelter (m2 in a 4 m band) | Visual estimate, 2-3 observers |

Fish habitat guilds were defined following two different but complementary approaches. The first approach was based on an analysis of habitat use-availability using presence–absence data. For this purpose, the habitat gradients represented by the extracted $PC_{habitat}$ were equally divided into four classes of equal amplitude based on PC scores (Grossman and Freeman, 1987; Prenda et al., 1997). We calculated the frequency distribution of each principal component score (sampling sites) in these classes to provide the relative availability of specific habitat configurations. To test the preferences of each fish assemblage for any particular habitat class, the previously described frequency distribution was compared to the frequency distribution of the sites where each fish assemblage was present (Grossman and Freeman, 1987; Prenda et al., 1997). A Chi-square analysis was conducted to determine whether significant differences existed between the two frequency distributions. If fish assemblages occupied sampling sites randomly, then the distributions for habitat availability and expected frequency should not significantly differ. When a significant result was obtained, a partitioned Chi-square analysis was conducted to determine those classes that contributed to the statistical significance, i.e., in which habitat class the species was over- or under-represented (Prenda et al., 1997). The second approach was based on the relationship (Pearson correlation) between the habitat gradients and the abundance of each fish assemblage (Prenda et al., 1997). We assumed that the previously defined fish assemblages become more abundant towards their preferred habitats and decrease in abundance in avoided habitats.

Prior to statistical analysis, those variables that departed significantly from a reasonable normal distribution were log-transformed (continuous variables) or arcsine transformed (percentages).

## RESULTS

### Community composition

At least 20 fish species were caught in the study area (Table 2). This is a minimal estimation because grey mullets (Mugilidae) include various species (*Mugil cephalus*, *Liza ramada*, *Liza aurata*, and *Chelon labrosus*). Neither non-primary freshwater fish (*Aphanius baeticus*, *Atherina boyeri*, *Anguilla anguilla*, and grey mullets) nor freshwater species *sensu stricto* that occur within less than 5% of sites (Table 2) were included in further statistical analysis. Barbels were the most common and abundant taxa (mean CPUE 229.9±350.6 SD), being present in most of the studied sites (86.3%, Table 2). Chub (*Squalius pyrenaicus*), roach (*S. alburnoides*), nase (*Chondrostoma willkommii*), and pardilla nase (*Ch. lemmingii*) were also relatively widespread and abundant species (Table 2). Loach (*Cobitis paludica*) was common (45.0% of sites) but not very abundant (mean CPUE 11.5±43.1 SD). Jarabugo (*Anaecypris hispanica*, Guadiana's endemism) and freshwater blenny (*Salaria fluviatilis*) were restricted to the Guadiana Basin. Both species, considered "endangered" following IUCN criteria (Doadrio, 2001), displayed a very restricted distribution and very low densities (Table 2).

In general, exotic species were of secondary importance in terms of abundance and distribution extent (Table 2). Centrarchids (pumpkinseed sunfish *Lepomis gibbosus* and largemouth bass *Micropterus salmoides*) were the most widespread (35.0 and 15.7%, respectively) and abundant (mean CPUE 10.4±26.2 and 6.6±40.1 SD, respectively). These exotic species were mainly captured in tributaries of the Guadiana and Guadalquivir Basins. Two rare exotics were exclusively captured at a few sites within the Guadiana Basin: goldfish (*Carassius auratus*) and chameleon cichlid (*Herichthys facetum*) (Table 2).

**Table 2.** Freshwater fish species captured in 80 sites in south-western Iberia with indication of species codes, size classes, frequency of occurrence (FO %), mean abundance (CPUE) and basins where each species was present (D-Guadiana, Q-Guadalquivir, T-Tinto, O-Odiel and S-Small basins located in Campo de Gibraltar). Species excluded from the analysis: 1 FO<5; 2 not primary freshwater fish species. * B. microcephalus and B. comizo were exclusive from the Guadiana basin. Abundance data are only given for species captured by electrofishing techniques.

| | | **Size clasess (mm)** | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Species** | **Code** | **1** | **2** | **3** | **4** | **5** | **FO (%)** | **abundance (mean±SD)** | **basin** |
| **Native species** | | | | | | | | | |
| Cyprinidae | | | | | | | | | |
| *Barbus microcephalus*.*<br>*Barbus comizo**<br>*Barbus sclateri.* | Bsp | <50 | 50-100 | 100-150 | 150-200 | >200 | 86.3 | 229.9±350.6 | D,Q,T,O,S |
| *Chondrostoma willkommii* | Cw | <50 | 50-100 | 100-150 | >150 | | 38.8 | 44.1±194.5 | D,Q,T |
| *Chondrostoma lemmingii* | Cl | <50 | 50-80 | >80 | | | 27.5 | 32.4±102.6 | D,Q,T,O |
| *Squalius pyrenaicus* | Sp | <50 | 50-80 | >80 | | | 50.3 | 50.5±128.7 | D,Q,T,O,S |
| *Squalius alburnoides* | Sa | <50 | 50-80 | >80 | | | 40.0 | 101.6±251.4 | D,Q,O |
| *Anaecypris hispanica* | Ah | <50 | >50 | | | | 5.0 | 7.4±61.5 | D |
| Cobitidae | | | | | | | | | |
| *Cobitis paludica* | Cp | <50 | 50-70 | >70 | | | 45.0 | 11.5±43.1 | D,Q,T,O,S |
| Blenniidae | | | | | | | | | |
| *Salaria fluviatilis* | Sf | <60 | 60-80 | >80 | | | 11.3 | 0.5±2.2 | D |
| Cyprinodontidae | | | | | | | | | |
| *Aphanius baeticus*² | | | | | | | | | S |
| Atherinidae | | | | | | | | | |
| *Atherina boyeri*² | | | | | | | | | S |
| Anguillidae | | | | | | | | | |
| *Anguilla anguilla*² | | | | | | | | | S |
| Mugilidae² | | | | | | | | | S |
| **Exotic species** | | | | | | | | | |
| Cyprinidae | | | | | | | | | |
| *Cyprinus carpio* | Cc | <100 | 100-150 | 150-200 | >200 | | 12.5 | 0.9±4.0 | D,Q,T |
| *Carassius auratus*¹ | Ca | | | | | | 1.3 | 0.03±0.3 | D |
| Poeciliidae | | | | | | | | | |
| *Gambusia holbrooki* | Gh | | | | | | 15.0 | 3.3±21.2 | D,Q,T,O,S |
| Cichlidae | | | | | | | | | |
| *Herichthys facetum*¹ | Hf | | | | | | 2.5 | 0.1±0.7 | D |
| Centrarchidae | | | | | | | | | |
| *Micropterus salmoides* | Ms | <100 | 100-200 | >200 | | | 17.5 | 6.6±40.1 | D,Q,T,O |
| *Lepomis gibbosus* | Lg | <50 | 50-100 | >100 | | | 35.0 | 10.4±26.2 | D,Q,T,O |

### Major distribution patterns and species assemblages

Four assemblages were defined following a PCA of fish taxa abundance (CPUE) x sites (Figure 2A): (I) chub and pardilla nase, (II) loach, (III) remaining native taxa (barbels, nase, roache, jarabugo, and blennie), and (IV) exotic species. The PC1 (eigenv.=3.04, 25.4% of total expl. variance) clearly separated exotic species from native ones (Figure 2A). The same pattern was maintained when analysing taxa–size class data (Figure 2B). Most native species–size classes appeared clearly segregated from exotic species, with the exception of large barbels (>200 mm), large nase (>150 mm), and large blenny (>80 mm) that occupied a position beside the exotics. Here, six taxa–size class assemblages were defined. Different life stages of the same species could have been assigned to different assemblages (Figure 2B).

It is noticeable that a clear intrageneric spatial segregation is observed within *Chondrostoma* and *Squalius* genus (Figure 2). Neither of the two species that make up each genus belong to the same assemblage.

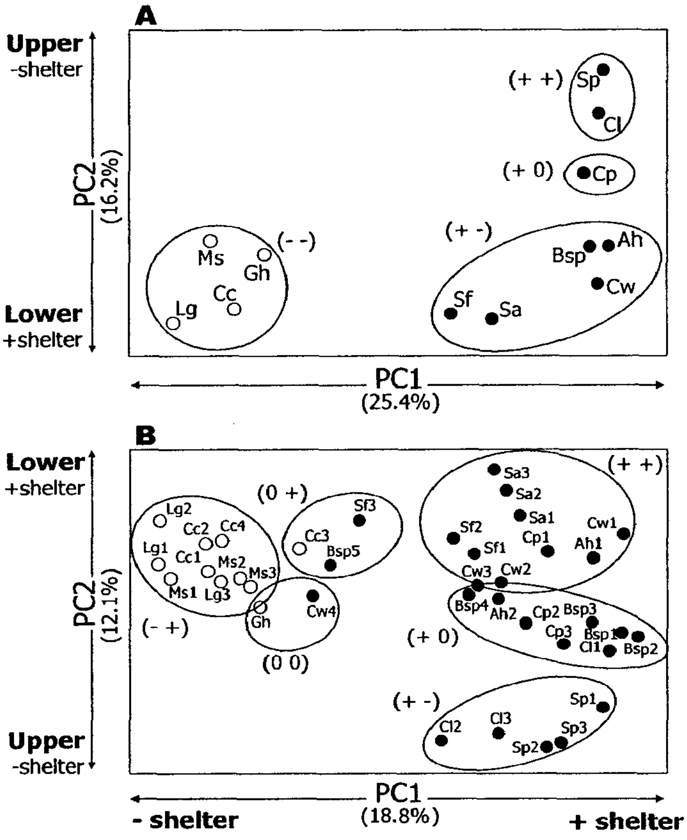

**Figure 2.** Distribution of the factor loadings of the taxa (A) and taxa-size classes (B) after a PCA applied to a matrix of fish abundance (log-CPUE) x sites from south-western Iberia. Circles include those species/taxa or size classes that present the same degree of correlation with PC1 and PC2 and consequently tend to occupy similar habitats. The symbols 0, +, and - in the first position within brackets indicate no correlation, significant ($p<0.05$) positive, and

significant negative correlation, respectively, with respect to PC1; while the same symbols in the second position represent the correlation with respect to PC2. The ecological meaning of each PC is presented following the correlation of each PC with PChabitat. Numbers in brackets below PC1 and PC2 indicate their explained percentage of original variance. Codes for fish and fish size-classes are the same as those used in Table 2.

### Habitat use and habitat guilds

Two habitat gradients were defined in the study area following the PCA of habitat variables (Table 3). The $PC1_{habitat}$ (eigenv.=5.5 26.1% of variance), an upstream–downstream gradient, runs from sites with high slopes, evapotranspiration, annual rainfall, and altitude (headwaters) to sites characterized by large drainage areas, a high degree of order, and wide watercourses (lower reaches) (Table 3). The $PC2_{habitat}$ (eigenv.=2.4 11.3% of variance) represents a shelter-related gradient, being the shelter mainly provided by aquatic vegetation cover and large-size substratum (Table 3).

To classify the six assemblages shown in Figure 2B into habitat guilds, we undertook habitat use–availability and correlation analysis. The $PC1_{abund\text{-}size}$ was positively correlated to the shelter gradient ($PC2_{habitat}$, r=0.25, p<0.05, n=80), and the $PC2_{abund\text{-}size}$ was negatively correlated with $PC1_{habitat}$ (r=–0.56, p<0,001, n=80). Thus, the six assemblages identified in Figure 2B can be ordered along both habitat gradients: upstream–downstream ($PC1_{habitat}$) and shelter availability ($PC2_{habitat}$). The three native assemblages (++, +0, and +-) occupy the most sheltered reaches. The assemblages -+, 0+, and ++ are associated with the lower reaches, while +- inhabit the most upper reaches (Figure 2B).

On the basis of habitat use–availability data (presence–absence data), two out of six fish assemblages identified in Figure 2B apparently display random habitat use on PC1habitat: the +0 (barbels <200 mm, loach >50 mm, jarabugo >50 mm, and pardilla nase <50 mm) and the ++ (roach, nase <150 mm, blenny <80 mm, loach <50 mm, and jarabugo <50mm) assemblages. The four remaining assemblages displayed some selectivities: the assemblages that include exotics (0+, 00, and -+) reject most upstream reaches (Figure 3). Both 0+ and -+ are also negatively correlated with PC1habitat (Table 4), i.e., they are more abundant in the lower reaches. The chub and pardilla nase assemblage displayed the opposite pattern: it rejected lower reaches and clearly preferred headwaters/low-order streams, being significantly more abundant in these stretches of river (Table 4).

Although the ++ assemblage showed a random habitat use following the use–availability analysis (Figure 3), it tends to occupy lower reaches, as inferred from its negative correlation with $PC1_{habitat}$ (Table 4). Therefore, this assemblage occupies reaches with similar typology to that of reaches preferred by exotic species, although overlapping was not frequently observed (Figure 2B). In addition, in sites where the -+ assemblage (sunfish, bass, and carp) is present (n=35), the abundance of natives +0 and ++ decreases (r=–0.47, p=0.005, n=35, and r=–0.38, p=0.025 for the +0 and ++ assemblages, respectively). These results indicate exclusion promoted by the exotics over the natives. To overcome the effect of the exotics, an use–availability test was performed for the ++ assemblage while excluding sites with exotic species. The result is now clear; as expected, ++ assemblage prefers lower stretches and reject upper ones ($X^2$=9.0, d.f.=2, p=0.011). In addition, they are significantly more abundant in sheltered reaches (Table 4).

**Table 3.** Habitat gradients observed at several river basins in south-western Iberia after a PCA. r: Pearson correlation between the variables included in the PCA and the two principal components. In brackets the percentage of the original variance explained by each component. †0.10>p>0.05; *p<0.05; **p<0.01; ***p<0.001.

| | PC1 (26.1%) | PC2 (11.3%) |
|---|---|---|
| **Physico-chemical parameters** | | |
| Water temperature | -0.30** | 0.17 |
| Conductivity | -0.19† | -0.46*** |
| Dissolved oxygen | -0.14 | 0.35** |
| Turbidity | -0.48*** | -0.18 |
| **Channel structure and flow descriptors** | | |
| Current velocity | -0.62*** | 0.11 |
| Depth | -0.61*** | 0.003 |
| Channel width | -0.80*** | -0.16 |
| Substratum coarseness | -0.003 | 0.28** |
| Fish shelter | -0.34** | 0.26* |
| Submerged vegetation cover | 0.22* | 0.43*** |
| Emergent vegetation cover | -0.24* | 0.71*** |
| Herbaceous cover | 0.02 | 0.45*** |
| Shrub cover | 0.24* | -0.04 |
| Tree cover | 0.29** | -0.26* |
| **Physiographical and climatic parameters** | | |
| Stream order | -0.85*** | -0.06 |
| Catchment area | -0.91*** | 0.11 |
| Relative position | -0.60*** | -0.65*** |
| Altitude | 0.34** | 0.49*** |
| Slope | 0.78*** | -0.12 |
| Evapotranspiration | 0.64*** | -0.29** |
| Annual rainfall | 0.54*** | -0.21† |

It is interesting to note the distribution of the smallest size classes of several native taxa. The smallest barbels, nase, chub, jarabugo, and pardilla (size class 1) tend to occupy the most positive extreme of PC1abund-size, which coincides with the highest sheltered sites for fishes.

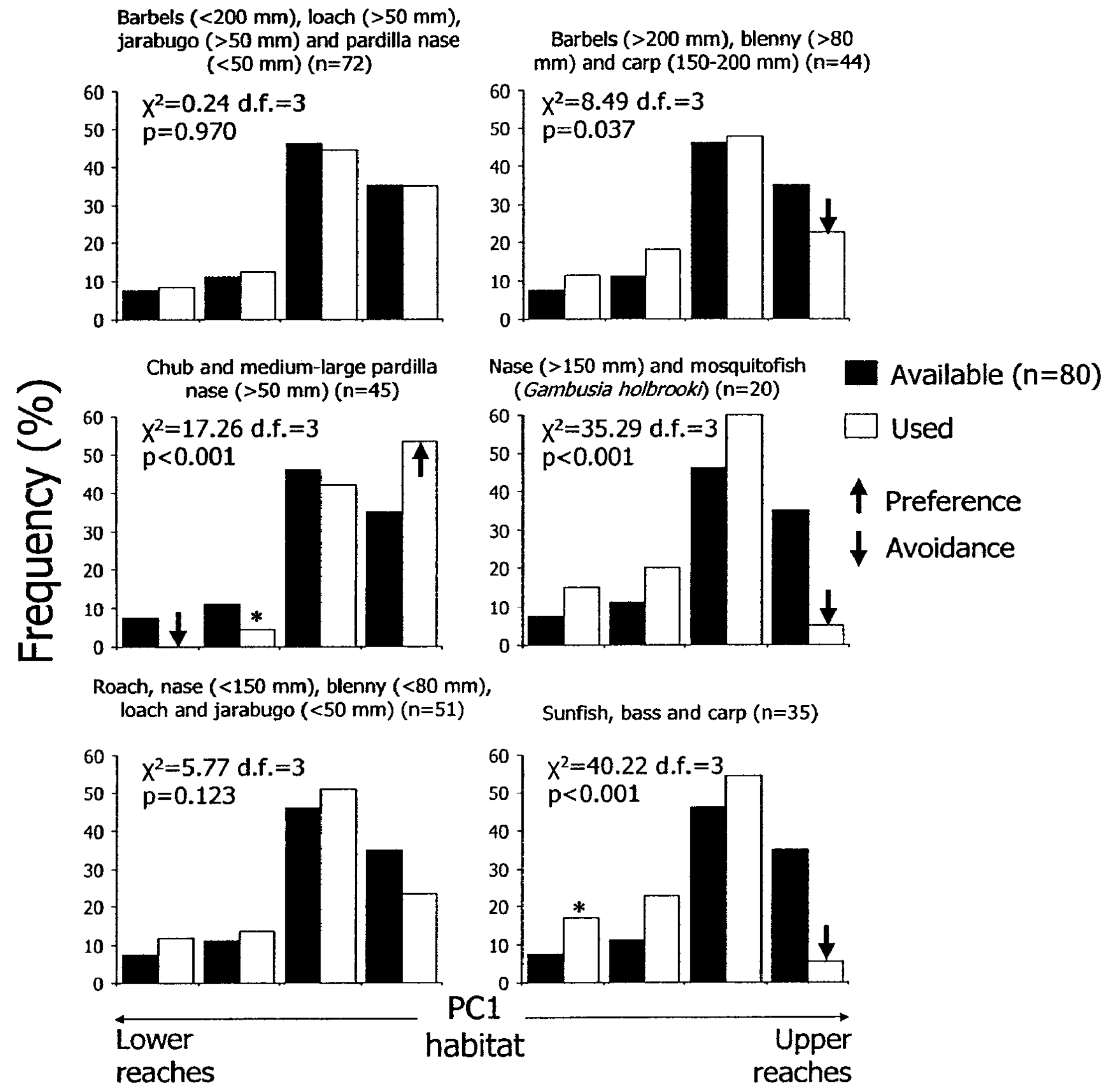

**Figure 3.** Species size-class preferences in the study area comparing habitat availability (black bars) and habitat used (white bars) with a chi-square analysis (see Methods section for a detailed description of the analysis). PC1 represent the upstream-downstream gradient defined in Table 3. Vertical arrows represent the portions of the gradients preferred or rejected by each fish assemblage.

These enable us to classify the six assemblages into the following four habitat guilds (Table 5) that constitute a river zonation from the headwaters to the river mouth. 1) "Headwaters" (chub and medium-large pardilla nase): preference for low-order streams ($\leq 3^{rd}$) ($X^2$=15.8, df=4, p=0.003, n=80), typical in the headwaters or small isolated basins with abundant submerged vegetation. 2) "Middle reaches-generalists" (Small-medium barbels, medium-large loach, large jarabugo, and small pardilla nase): minor habitat preferences; fish distribution is apparently not influenced by major habitat gradients, although they are more abundant in sites with high fish shelter and aquatic vegetation cover (Table 4) and occupy an intermediate position between the typical headwater assemblage and the lower reaches assemblage (Figure 2B). 3) "Lower reaches natives" (roach, small-medium nase, small-medium blenny, small loach, and jarabugo): tendency to occupy downstream and sheltered zones. Their abundance increases towards lower habitats. Sensitive to exotics. 4) "Lower reaches exotics and very large cyprinids and blenny" (exotics, very large barbels, nase, and blenny): reject headwaters and preferentially use high-order streams (wide watercourses and deep channels). The members of this guild are also more abundant toward downstream habitats (Table 4).

**Table 4.** Pearson correlations (r) between habitat gradients (PC1habitat and PC2habitat) and fish assemblages abundance (CPUE) in several river basins in south-western Iberia (n=80 sampling sites). †0.10>p>0.05; *p<0.05; **p<0.01; ***p<0.001.

| | Upstream-downstream gradient ($PC1_{habitat}$) | Fish shelter gradient ($PC2_{habitat}$) |
|---|---|---|
| Barbels (<200mm), loach (>50mm), jarabugo (>50mm) and pardilla nase (<50mm) (+0) | 0.13 | 0.28* |
| Pardilla nase (>50mm) and chub (+-) | 0.46*** | 0.14 |
| Roach, nase (<150mm), blenny (<80mm), loach and jarabugo (<50mm) (++) | -0.25* | 0.40*** |
| Barbels (>200mm), blenny (>80mm) and carp (150-200mm) (0+) | -0.46*** | 0.06 |
| Nase (>150mm) and mosquitofish (00) | -0.09 | 0.04 |
| Sunfish, bass and carps (-+) | -0.55*** | 0.21† |

## DISCUSSION

The general distribution pattern of native ichthyofauna in the study area is strongly influenced by exotic species, although they are relatively unimportant in terms of abundance and distribution extent. Native and exotic species are spatially segregated. Exotic species exclude native species from the lowermost reaches. This process drives some species to display a deviated habitat preference pattern with respect to their expected optimal habitat use. The species that are most affected by exclusion by exotics are those that occupy lower reaches, mostly the "lower reaches natives" and to a lesser extent the "middle reaches-generalist" guilds. The former guild displays an apparently inconsistent pattern of habitat use (random use indicate by X2-analysis), but this could be a direct consequence of interaction with exotics. It must be expected that these species should preferentially occupy the most downstream habitats in a hypothetical situation that involves the absence of exotic species. Unfortunately, it is almost impossible to determine the original distribution pattern of these species because the lower reaches of all large Iberian basins are now invaded by exotic species (Doadrio, 2001).

An alternative explanation to this exclusion hypothesis is that the lower habitats suffer from human-induced disturbances that favour exotics (Ross et al., 2001) and displace the native species, which are usually more sensitive to environmental changes. It is not possible to test this hypothesis from our current data, and accordingly we cannot rule out the possibility that this factor affects the observed fish habitat-use and distribution patterns.

**Table 5.** Species assemblages-habitat guilds and comments on their main habitat preferences. Habitat guilds were defined using PCA techniques, habitat use-availability (Chi-square) and correlation analysis (Pearson) (see methods for a detailed description).

| Habitat guilds | Fish Assemblage | Species/ Species size classes | Habitat preferences |
|---|---|---|---|
| "Headwaters" | Chub and medium-large pardilla nase | *S. pyrenaicus* and *Ch. lemmingii* (>50 mm) | Preference for low order streams ($\leq 3^{rd}$) with narrow and shallow channels and abundant submerged vegetation. In general headwaters and small isolated basins. |
| "Middle reaches-Generalists" | Small-medium barbels, medium-large loach, large jarabugo and small pardilla nase. | *Barbus* spp.<200 mm, *C. paludica* (>50 mm), *A. hispanica* (>50 mm) and *Ch. lemmingii* (<50 mm) | Fish distribution is not influenced by major habitat gradients. They tend to occupy middle reaches with high aquatic vegetation cover and fish shelter. |
| "Lower reaches-natives" | Roach, small-medium nase (<150 mm), small-medium blenny (<80 mm) and small loach and jarabugo (<50 mm) | *S. alburnoides*, *Ch. willkommii* (<150 mm), *S. fluviatilis* (<80 mm), *C. paludica* (<50 mm) and *A. hispanica* (<50 mm) | Tendency to occupy lower reaches with high fish shelter in terms of cover availability (aquatic vegetation and large boulders). Their abundance increase towards downstream habitats. Sensitive to exotics. |
| "Lower reaches exotics and very large native cyprinids and blenny" | Exotics-very large native cyprinids (barbels and nase) and large freshwater blenny. | *C. carpio*, *G. holbrooki*, *M. salmoides*, *L. gibbosus*, *Barbus* spp.>200 mm, *Ch. willkommii*>150 mm and *S. fluviatilis*>80 mm | They clearly reject headwaters and appear preferentially in high order streams (wide watercourse, deep channels) |

The only natives that commonly share habitat with exotic species are the largest cyprinids (barbels >200 mm and nase >150 mm) and blenny (>80 mm). This result is in agreement with previously published observations. Godinho et al. (1997) and Godinho and Ferreira (2000), in studying the Raia River and lower Guadiana, found that the fish community changed from assemblages dominated by small native species to assemblages formed by exotic centrarchids (*M. salmoides* and *L. gibbosus*) and larger size classes of Cyprinidae (barbel and the nase *Ch. polylepis*). The interacting capacity of a fish is dependent on its size (Prenda et al., 1997), and large fish are usually less vulnerable to predation than small ones (Tonn et al., 1992). Adult barbels and nase attain a size that is large enough to enable escape from predation by gape-limited piscivorous fish such as largemouth bass; this enables its coexistence with exotic species.

Despite the nature of the habitat preferences observed for each guild, most of the native species do not show strong linkages to any particular habitat features, at least at the scale studied here, and they tend to occupy broad portions of the observed habitat gradients except for the headwater guild. This "headwater" guild (chubs and pardilla nase) shows clear relationships to certain habitat configurations. Despite the fact that chub is frequently cited as a ubiquitous species (Magalhães, 1993; Doadrio, 2001), both chub and pardilla preferentially appear in small, low-order shallow reaches. This fits in well with previous punctual observations that describe both species as occurring frequently in intermittent and/ or small Iberian streams (Pires et al., 1999; Filipe et al., 2002).

The generalist strategy may be a response of organisms to highly variable habitat conditions (Angermeier and Schlosser, 1989), as with those that occur in Mediterranean rivers. One of the most important features of the Mediterranean climate is the high intra- and inter-annual variability in rainfall that results in highly unstable river systems (Gasith and Resh, 1999). Under these circumstances, the capacity to adapt to the majority of the available habitat conditions is favoured within Iberian freshwater fish fauna (Prenda et al., 2002).

Spatial segregation is a common phenomenon within cyprinid species (Winston, 1995), and strong parapatry has been described for congeneric cyprinids (Taylor and Lienesch, 1996) in other areas. To date, these patterns have not been described for Iberian freshwater fishes. However, our results show that neither of the two species belonging to the *Chondrostoma* and *Squalius* genus are grouped in the same assemblage, indicating a spatial segregation within both genus in the study area; this observation warrants further study. This segregation is also sometimes observed within species. In general, different size classes of the same species belong to different assemblages (all species except both *Squalius*). This indicates that for these species it is necessary to preserve a wide range of habitats—ideally the full gradient of habitats—to assure an adequate life-cycle fulfilment. The alteration of any portion of the natural stream gradient can severely affect the natural development of many fish species, even if other stream sectors remain pristine.

The current study describes the distribution patterns and habitat use of freshwater fishes living across a wide geographical area. It is possible that the distribution pattern displayed by microhabitat-dependent species is not adequately described here, as they must select specific microhabitat configurations (Grossman et al., 1987a,b; Santos et al., 2004; Clavero et al., 2005b) and are probably less influenced by broad-scale gradients. This is possibly also the case for loach, a species that is dependent on the presence of sandy substratum (Perdices and Doadrio, 1997). The necessity to pool all *Barbus* species into a single group does not allow an adequate classification of the species into different habitat guilds. However, *B. comizo* and *B. microcephalus* are known to preferentially occupy lower reaches (Morán-López et al., 2005) and can be included in "lower reach natives" assemblage, while *B. sclateri* is assumed to be a "middle reaches-generalist" member. A further limitation of our results is related to species that display a narrow distribution. Jarabugo and blenny present a very restricted distribution in the study area, and some of their habitat preferences were probably overlooked. Specific studies are required to determine the habitat preferences of these two endangered species.

This study represents the first time that a number of Iberian fish species have been assigned to distinct assemblages and habitat guilds. This information will be helpful for the implementation of river management strategies, fish species conservation projects, and the design of indices used to calculate the biotic integrity of Iberian stream ecosystems, among other uses. In addition, the obtained results highlight the influence of exotic species on the distribution patterns and assemblage organization of native species. These strong influences make it impossible to determine the original distribution and habitat use of native species in the absence of exotic species. The described fish assemblages and their habitat-use patterns may reflect a combination of the influence of exotic species, their own habitat requirements, and the extent and nature of habitat disturbance.

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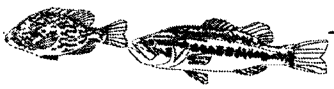
# CAPÍTULO 3

## IMPACT OF INTRODUCED CENTRARCHIDS ON NATIVE FISH COMMUNITIES IN MEDITERRANEAN RIVERS

*Impacto de los centrárquidos sobre las comunidades de peces nativos en ríos mediterráneos*

Enviado a Biological Conservation



# IMPACT OF INTRODUCED CENTRARCHIDS ON NATIVE FISH COMMUNITIES IN MEDITERRANEAN RIVERS

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## ABSTRACT

The impact of exotic species on native communities becomes more severe when they are introduced into isolated systems such as river basins. Many exotic fish species are increasing their distribution range and abundance in Iberian freshwaters, becoming a potential threat to the conservation of native fish biodiversity. In this work, we analyse the effect of pumpkinseed (*Lepomis gibbosus*) and largemouth bass (*Micropterus salmoides*), North American centrarchids widespread in Iberian rivers, on native fish communities, based on their distribution and abundance relationships. The study was carried out in 50 sites distributed along two large river basins of the South West Iberian Peninsula. The importance of centrarchids was low, in terms of abundance and biomass (16.0% and 10.3% on average, respectively), but significantly impacted native fish communities at three levels: 1) Native fish community structure. Fish community attributes (abundance, biomass, species richness and Shannon diversity) were negatively correlated to centrarchid abundance. 2) Native fish distribution patterns. Centrarchid species reversed the expected natural pattern of distribution of native fish, the abundance, richness and diversity of which increased upstream. 3) Native fish age structure. Sites with centrarchids lacked small size classes and had a higher proportion of large individuals compared with sites without them. All of these results indicate that, despite the lesser importance of centrarchids in terms of abundance and biomass, they exert a strong negative impact on the native fish communities they invade. To prevent the establishment of species of this invasive fish family is fundamental for the conservation of native fish fauna.

Key words: Mediterranean streams; fish conservation; exotic fish; biotic interactions; invasive species; Spain

## INTRODUCTION

One of the most extensive human-induced disturbances is the alteration of native ranges of species. Introduction of species out of their native range usually has unpredictable effects on the ecosystems they invade (Moyle et al., 1987). Although changes in species distribution are a natural phenomenon, human activities are accelerating and increasing the scale of these movements. Many species of plants and animals are being spread, replacing autochthonous organisms and resulting in a net loss of biodiversity (Mooney, 1998). The impact of exotic species on native communities becomes more severe when they are introduced into isolated systems (Primack, 1993) such as islands, lakes and river basins. River basins are true islands for the freshwater fauna they contain (Clavero et al., 2004). Freshwater fish are highly differentiated and isolated, and these ecological characteristics make them extremely vulnerable to biological invasions (Taylor et al., 1984; Courtenay and Robbins 1989, Di Castri, 1991; Kaufman, 1992; Courtenay, 1993; Goldschmidt et al., 1993; Lever 1996; Moyle 1997).

The Iberian freshwater fish fauna is characterized by a low number of families, with most species belonging to the family Cyprinidae, but with the greatest proportion of endemic species in Europe (Doadrio, 2001). This fact makes the native Iberian fish fauna very vulnerable to the introduction of new species. More than 80% of Spanish species has been classified as vulnerable, endangered or critically endangered by Doadrio (2001), following IUCN criteria. In contrast, many exotic fish species are expanding their range in Iberian rivers (Elvira and Almodovar, 2001). This is the case for two centrarchids, the pumpkinseed sunfish (*Lepomis gibbosus*) and the largemouth bass (*Micropterus salmoides*), which are native to the USA. Both species are considered a great threat to the conservation of native freshwater fish species (Doadrio, 2001). The pumpkinseed is a generalist feeder (Godinho and Ferreira, 1998b; García-Berthou and Moreno-Amich, 2000; Copp et al., 2002) preying on eggs and spawn of other fish. The largemouth becomes a piscivorous feeder as it grows (García-Berthou, 2001) and is among the '100 of the world's worst invasive alien species' (ISSG, 1999).

Given the potential threat generated by exotic species, the virtual absence of knowledge about their relationship with Iberian native freshwater fishes is surprising. We have observed that the general fish distribution pattern is determined by exotic species (Capítulo 2.), and the most important exotics are centrarchids. Others have obtained similar results (e.g. Godinho and Ferreira, 1998a; Godinho and Ferreira, 2000).

If the impact of centrarchids on Iberian native fish is really important, we might expect perceptible changes in various attributes of fish communities. Is the impact of centrarchids strong enough to affect community structure in terms of abundance, number of species and diversity? ($Q_1$)

The fish fauna shifts in a predictable way with progression downstream. Fish abundance and richness tend to increase following the stream gradient (upstream-downstream) in response to changes in river features (Horwitz, 1978; Magalhães et al., 2002a). Do native fish maintain this natural pattern despite the presence of centrarchids, or is the impact of centrarchids strong enough to alter it? ($Q_2$)

If an impact of centrarchids on the native fish community is evident, are all fish size classes equally affected, or is any particular class especially sensitive to the presence of centrarchids? ($Q_3$)

To answer these questions, we examined 50 sites in streams of the Guadiana and Guadalquivir basins (South West Iberian Peninsula), which have a Mediterranean climate. This area is appropriate to test the hypothesis because there are zones of streams affected by centrarchids together with stretches that exotics have not yet invaded. In this study, we try to clarify the effects of centrarchids on the native fish community based on their distribution relationships.

## METHODS

### Study area

The study area was located in the South West of the Iberian Peninsula, including the middle reaches of the Guadiana and Guadalquivir basins (Figure 1). Most of the studied rivers and streams in the Guadalquivir basin flow through a protected area (Sierra Norte de Sevilla Natural Park). The Guadiana and Guadalquivir rivers are neighbouring drainages with similar characteristics regarding topography, climate and hydrology. The altitude ranges from 10 to 900 masl, with slightly more pronounced slopes in the streams and rivers of the Guadalquivir basin. The whole area has a typical Mediterranean climate. The mean annual rainfall is about 650 mm, falling mainly between October and March. A drought occurs during summer. In an average year, flow persists in the main river channels and downstream areas during summer, but most of the tributaries become a succession of pools, with little or no water flow.

The unpredictable climate produces a high pressure for exploitation of water resources (Collares-Pereira et al., 2000), and eight large dams (more than 100 hm$^3$) are situated in the area. The Alqueva dam (3150 hm$^3$), which impounds the middle reaches of the Guadiana basin, is considered the largest artificial lake in Europe.

Mediterranean sclerophyllous and evergreen scrub with open forests of holm oak, *Quercus rotundifolia*, and/or cork oak, *Q. suber*, (Dehesas) dominate the landscape. There is also dry cereal cultivation, mainly in the Guadiana sector. In some areas, there is a well-conserved riparian vegetation of ash, *Fraxinus angustifolia*, and alder, *Alnus glutinosa*, but in others it is scarce and fragmented.

### Fish sampling

The field work was carried out between April and July 2001 and 2002, after the rainy season but before the summer drought, when river flows were stabilized. The fish community was sampled in 50 locations by electrofishing (27 Guadiana and 23 Guadalquivir, Figure 1). Sampling was done before the filling of the Alqueva and Melonares dams, so many sites are now underwater. Sampling sites were selected based on their distance to the main river channels in each basin (Guadiana or Guadalquivir river), to cover the upstream-downstream gradient in the structure and composition of fish community. Sampling was conducted once in each location along a length of 100–250 m. Fishing time was noted to calculated captures per unit effort (CPUE) as the numbers of captures$*100\ m^{-1}*1\ h^{-1}$ fishing. CPUE was used as an index of species abundance. All fish were identified to species level, measured for total length (to the nearest mm) and then returned to the water. At least three species of *Barbus* inhabit in the study area (*B. microcephalus*, *B. comizo* and *B. sclateri* in the Guadiana basin, and *B. sclateri* in the Guadalquivir basin) and in many cases it was difficult to distinguish between species, so they were included in the same group (barbels hereafter) and treated as a single species for statistical analysis.

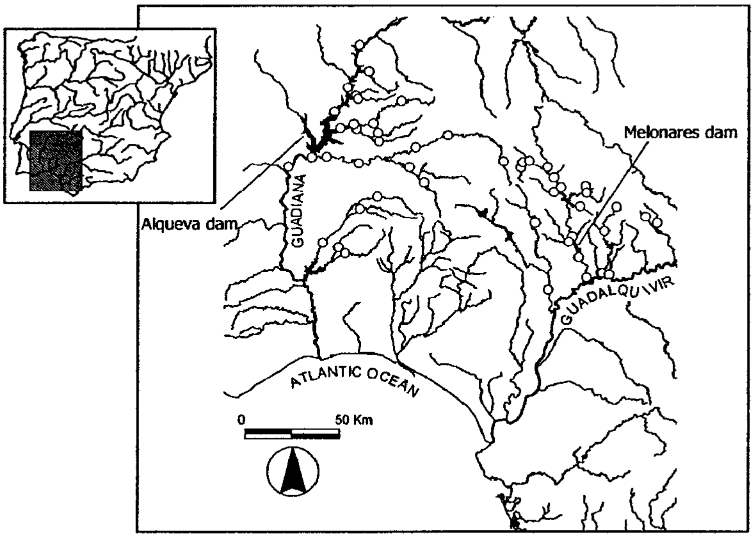

**Figure 1.** Location of the 50 sampling sites distributed in the Guadiana and Guadalquivir river basins (South West Iberian Peninsula).

### Statistical procedures

Native fish community attributes were described by species abundance (CPUE), biomass per unit effort (BPUE), number of species and diversity (Shannon index). Number of native species was the maximum number of species captured in a given site.

To answer $Q_1$, a Pearson correlation analysis was run between centrarchid abundance and the descriptors of the native fish community (abundance, biomass, number of species and diversity). In previous work (Capítulo 2), we observed that the two species of centrarchid (sunfish and bass) had similar distribution patterns, so in this study they were grouped. When data did not meet the assumptions required for parametric statistics, we used non-parametric correlations (Spearman rank correlation).

The relationships between the upstream-downstream gradient (here expressed as distance to the main river channels in km) and the abundance of native species, richness of native species and centrarchid fishes were assessed through linear regressions. These analyses allow us to describe changes in the abundance and richness of species along the upstream-downstream gradient and thus to answer $Q_2$.

To answer $Q_3$, we analysed the size class distribution of native species (altogether), comparing sites with and without centrarchids. To avoid possible bias in species size related to site position along the catchments (Magalhães, 2002a), we divided the streams and rivers studied into three sections based on their distance to the main river (Guadiana or Guadalquivir) (0–20 km, 20–40 km and >40 km) and analysed separately sites belonging to the same section. Chi-square analysis was used to assess differences between the frequency distributions of size classes in sites with and without centrarchids. When significant results were obtained, partitioned chi-square analysis was conducted to determine which size classes contributed to the statistical significance; that is, which size classes were over- or under-represented in sites with and without centrarchids.

## RESULTS

A total of 16 fish species were captured in the study area (Table 1). Native species were much more abundant (82.1% CPUE on average) and widespread (44.5% mean frequency) than exotics (17.9% CPUE on average, 19.3% mean frequency). Barbels were the most abundant and common taxa, followed by the Iberian roach (*S. alburnoides*). In contrast, centrarchid were scarce (16.0% CPUE on average) and had a low to intermediate distribution (Table 1), appearing in 29 of 50 sites (17 pumpkinseed sunfish, three largemouth bass and nine both species). Other exotics, such as the common carp (*Cyprinus carpio*) and the mosquito fish (*Gambusia holbrooki*), were even scarcer. Thus, centrarchids were of low quantitative importance (abundance, biomass and size) in relation to the native species.

### The impact of centrarchids on native fish community attributes

Centrarchid abundance had a strong negative influence on the attributes of the native fish community (Figure 2). Overall native fish abundance and biomass decreased significantly when the abundance of centrarchids increased; this was accounted for by the decline in abundance of most native species, except the Iberian roach (Table 2). Small-bodied cyprinids such as *Chondrostoma lemmingii* and the Iberian chub (*Squalius pyrenaicus*) (Table 1) typically disappeared where centrarchids were abundant. The endangered jarabugo never coexisted with sunfish and/or bass (Blanco-Garrido et al., submitted). This resulted in a dramatic reduction in the number of native species as centrarchid abundance increased (Figure 2). Also, a negative relationship was observed between native community diversity, as measured with Shannon's H', and centrarchid abundance (Figure 2).

### The impact of centrachids on the upstream-downstream fish distribution pattern

The upstream-downstream gradient, measured as distance to the main river channel, was related to the abundance and richness of native fish species, and centrarchid abundance. However, the patterns displayed by native and centrarchid species were the inverse of each other. Native fish abundance decreased significantly downstream, while centrarchids followed the opposite tendency (Figure 3). Patterns of native species richness were similar to those observed for abundance. The number of native species declined in an upstream-downstream manner.

**Table 1.** Fish species captured in South West Iberian basins (Guadiana and Guadalquivir) with their mean abundance, frequency of occurrence, and mean and maximum size. (E) Exotic species. [1]Barbus spp.: B. microcephalus, B. comizo and B. sclateri. [2]Species restricted to Guadiana basin and not included in the correlation analysis.

| Species | Abundance (mean CPUE ± sd) | Biomass (mean BPUE in gr. ± sd) | Frequency of occurrence (n=50) | Mean size (mm) | Maximum size (mm) |
|---|---|---|---|---|---|
| **F. CYPRINIDAE** | | | | | |
| *Barbus* spp.[1] | 298.6 ± 490.8 | 4893.8 ± 8102.2 | 0.96 | 150.9 | 564.1 |
| *Cyprinus carpio* (E,) | 1.3 ± 5.0 | 523.9 ± 1981.8 | 0.16 | 249.0 | 480.0 |
| *Carassius auratus* (E, 2) | 0.1 ± 0.4 | 0.8 ± 5.9 | 0.02 | 100.5 | 103.0 |
| *Chondrostoma lemmingii* | 46.5 ± 127.2 | 208.4 ± 625.0 | 0.26 | 45.2 | 135.0 |
| *Chondrostoma willkommii* | 68.3 ± 243.5 | 152.4 ± 424.9 | 0.52 | 102.6 | 279.0 |
| *Squalius pyrenaicus* | 40.6 ± 105.6 | 140.7 ± 305.2 | 0.44 | 75.2 | 168.0 |
| *Squalius alburnoides* | 157.5 ± 304.6 | 392.9 ± 776.3 | 0.58 | 67.9 | 140.0 |
| *Anaecypris hispanica*[2] | 11.9 ± 77.7 | 8.2 ± 53.9 | 0.08 | 45.8 | 57.0 |
| **F. COBITIDAE** | | | | | |
| *Cobitis paludica* | 16.6 ± 53.8 | 45.1 ± 163.5 | 0.54 | 66.2 | 107.0 |
| **F. BLENNIIDAE** | | | | | |
| *Salaria fluviatilis*[2] | 0.9 ± 2.8 | 4.8 ± 15.2 | 0.18 | 76.7 | 125.0 |
| **F. POECILIIDAE** | | | | | |
| *Gambusia holbrooki* (E) | 4.9 ± 26.8 | 3.2 ± 16.8 | 0.18 | 42.2 | 52.0 |
| **F. CICHLIDAE** | | | | | |
| *Herichthys facetum*[2] | 0.2 ± 0.9 | 0.6 ± 3.4 | 0.04 | 91.5 | 100.0 |
| **F. CENTRARCHIDAE** | | | | | |
| *Lepomis gibbosus* (E) | 16.2 ± 31.8 | 126.4 ± 228.7 | 0.52 | 80.6 | 140.0 |
| *Micropterus salmoides* (E) | 10.2 ± 50.5 | 95.7 ± 290.1 | 0.24 | 104.8 | 280.0 |

These results show that both centrarchid abundance and upstream-downstream gradient (i. e. distance to the main river) were related to native fish abundance and richness. However, only centrarchid abundance was significantly incorporated in a multiple regression analysis when both parameters (centrarchid abundance and river gradient) were considered as predictor variables of native species abundance, richness, biomass and diversity (H') (Table 3).

### The impact of centrachids on native size class distribution

The effect of centrarchids on the population structure of native species was evident. Small size classes were under-represented and large size classes over-represented in sites with centrarchids, in sections >40 km and 20–40 km (Figure 4A and 4B). All size classes accounted for statistical significance (partitioned chi-square analysis, $p<0.05$).

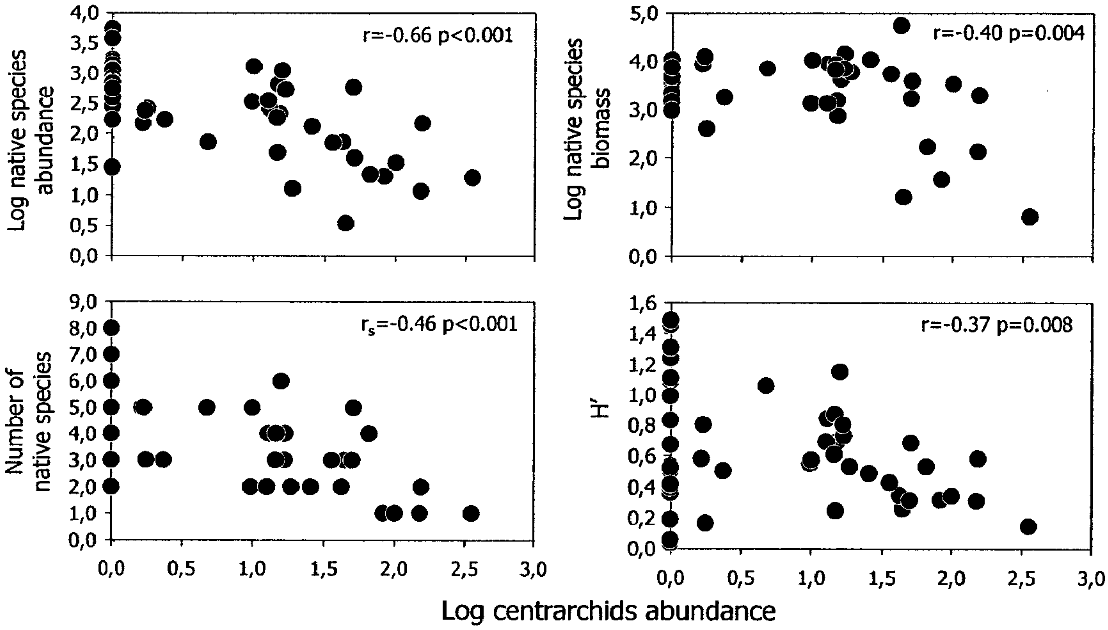

**Figure 2.** Correlations between native fish community attributes and centrarchid abundance (CPUE) in 50 sampling sites in the Guadiana and Guadalquivir river basins (South West Iberian Peninsula). Data on abundance and biomass are log-transformed.

This indicates that the whole native community structure could be heavily affected by centrarchids in these locations. The analysis was not possible in section 0–20 km, because centrarchids were present in all sites in this section.

We also explored the effect of centrarchids on barbel populations. The barbel was the only native taxon that enabled the assessment of the potential influence of centrarchids on population structure, because it occurred in most of the sites. Both groups (sites with and without centrarchids) belonged to the same sector (40–20 km) and the two groups did not differ in distance to the main river channel (t=–0.58, df=5, p=0.59). Barbel tended to be larger in sites with centrarchids (Mann-Whitney U test, Z=19.04, $n_1$=278, $n_2$=2035, p<0.001; Figure 5A); this was largely accounted for by a lack of small size classes and a higher proportion of large individuals (Figure 5B). Furthermore, differences in the proportions of the size classes with and without centrarchids were significant (chi-square value=8897.60, df=4, p<0.001), with all classes contributing to the statistical significance (partitioned chi-square analysis, p<0.05).

## DISCUSSION

### Native fish communities and centrarchids

Despite the low abundance of centrarchids in the study area, they exerted a strong impact on native fish species. Previous results showed a sharp segregation between native fishes and exotic centrarchids (Capítulo 2). Intense interspecific interactions may occur; indeed, centrarchid fishes can reduce the abundance of other fish species. MacRae and Jackson (2001) found that the smallmouth bass (*Micropterus dolomieu*) reduced the abundance of and even extirpated fish species in small lakes in central Ontario. Furthermore, the presence of largemouth bass together with the introduced cichlid *Serranochromis robustus* dramatically reduced the abundance and diversity of *Barbus* species in some streams in Zimbabwe (Gratwicke and Marshall, 2001).

The reduction of species abundance as a consequence of the presence of centrarchids led to the extirpation or exclusion of some species in certain stream reaches, resulting in a net loss of species richness and diversity. This evidence answers $Q_1$. The exception was the Iberian roach. This species seemed to be insensitive to the presence of centrarchids. Two mechanisms could account for this: (1) the Iberian roach effectively escapes the effects of centrarchids and/or (2) this species is so productive that the impact of centrarchids is unnoticed. In general, the composition of native communities becomes impoverished when they are invaded by alien species, due to simplification of the community structure (Godinho and Ferreira, 2000; Latini and Petrere, 2004; Yonukura et al., 2004).

**Table 2.** Pearson correlation between abundance of each native species (CPUE, log-transformed) and centrarchid abundance (CPUE, log-**transformed**). *$p<0.05$, **$p<0.01$ and ***$p<0.001$. (E) Exotic species.

| **Species** | **r** |
|---|---|
| *Barbus sp.* | -0.57*** |
| *Cyprinus carpio* (E) | 0.39** |
| *Chondrostoma lemmingii* | -0.49*** |
| *Chondrostoma willkommii* | -0.32* |
| *Squalius pyrenaicus* | -0.49*** |
| *Squalius alburnoides* | -0.12 |
| *Cobitis paludica* | -0.39** |
| *Gambusia holbrooki* (E) | 0.35* |

### Distribution pattern across the stream gradient

The tendency for fish communities to increase in both abundance and richness from the source to the mouth of rivers is a general rule that is widely observed (Lotrich, 1973; Hawkes, 1975; Horwitz, 1978; Matthews, 1986; Schlosser, 1990). However, following the upper-lower gradient, we observed a progressive decrease in the overall abundance and richness of native species from the headwaters to downstream. Rincón et al. (1990) described a similar reduction in abundance and species richness in an upper-lower manner in the Esla basin (another Iberian river), inverse to the pattern of the abundance of pike (*Esox lucius*), an introduced piscivorous species.

An ideal analysis of the impact of introduced species on the native fish fauna should include a comparison with data from a pre-invaded situation, but unfortunately this information is not available. Indeed, it is almost impossible to obtain data from pristine Iberian rivers, since all large Iberian basins are now invaded by centrarchids (Doadrio, 2001). One plausible alternative is to compare our results with those from small basins poorly invaded by exotic species, such as those studied by Magalhães et al. (2002a). In these basins, located very close to those studied here, the authors detected a downstream increase in the abundance of all native species. In our study area, the abundance of centrarchid fish increased downstream, following the pattern observed for native species in rivers scarcely invaded by exotic fish species. From these results, we argue that centrarchid distribution and abundance is strongly related to the inverse of the expected pattern of fish community characteristics in streams and rivers. Hence, with respect to $Q_2$ we can answer that centrarchids clearly alter the natural distribution pattern of native fish in rivers.

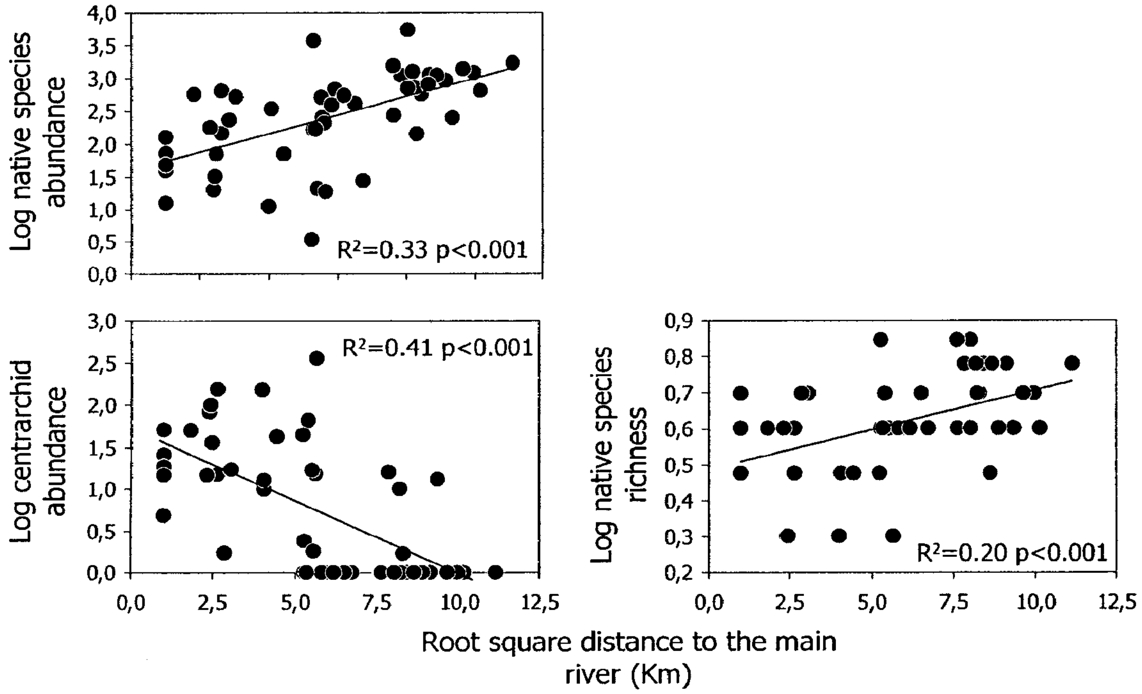

Figure 3. Relationships between the upstream-downstream gradient, measured as distance to the main river channels, native species abundance and richness, and centrarchid abundance in 50 sampling sites in the Guadiana and Guadalquivir river basins (South West Iberian Peninsula).

**Table 3.** Saturated multiple regression analysis showing the effect of centrarchid abundance and river gradient (measured as distance to the main river channel) on native species abundance, richness, biomass and diversity. Data on species abundance, richness and biomass are log-transformed. Distance to main river data (km) is root square-transformed.

| Dependent variable | | d.f. | F-value | p | $R^2$ | Independent variable | d.f. | p | $\beta$ |
|---|---|---|---|---|---|---|---|---|---|
| Native | Model | 2 | | | | Intercept | 1 | <0.001 | |
| species | Error | 47 | 21.5 | <0.001 | 0.48 | Centrarch. abundance | 1 | <0.001 | -0.51 |
| abundance | Total | 49 | | | | Dist. to river | 1 | 0.08 | 0.24 |
| Native | Model | 2 | | | | Intercept | 1 | <0.001 | |
| species | Error | 47 | 15.1 | <0.001 | 0.39 | Centrarch. abundance | 1 | <0.001 | -0.58 |
| richness | Total | 49 | | | | Dist. to river | 1 | 0.637 | 0.07 |
| Native | Model | 2 | | | | Intercept | 1 | <0.001 | |
| species | Error | 47 | 4.3 | 0.018 | 0.15 | Centrarch. abundance | 1 | 0.010 | -0.47 |
| biomass | Total | 49 | | | | Dist. to river | 1 | 0.430 | -0.14 |
| Native | Model | 2 | | | | Intercept | 1 | <0.001 | |
| species | Error | 47 | 3.9 | 0.028 | 0.14 | Centrarch. abundance | 1 | 0.059 | -0.34 |
| diversity (H') | Total | 49 | | | | Dist. to river | 1 | 0.774 | 0.05 |

### Centrarchids and native fish size structure

The impact of centrarchids on the community size structure was clear. The lack of small size classes in sites invaded by centrarchids must be related to their higher vulnerability. Piscivorous fishes such as the largemouth bass are size-selective (Tonn and Pazskowski, 1986), and small fish (or small size classes) are generally more vulnerable to predation than larger ones (Tonn et al., 1992). Furthermore, small fish are probably more sensitive to other types of negative interaction, such as diet overlapping and agonistic behaviour, than larger fish. The coexistence of large barbels with centrarchids is probably related to the fact that their size (>100 mm; Figure 4C) makes them large enough to escape from negative interactions with the bass (a gape-limited fish predator) and/or sunfish (Tonn et al., 1992). Probably, changes in population structure are more detectable in species with a wide spectrum of size classes, such as barbels. The effects of centrarchids on the population structure of small-bodied species are less evident since all size classes may be similarly affected.

With respect to $Q_3$, it can be stated that centrarchids clearly alter the size structure of native fish populations, reducing the proportion of small size classes and consequently increasing the percentage of large/old individuals.

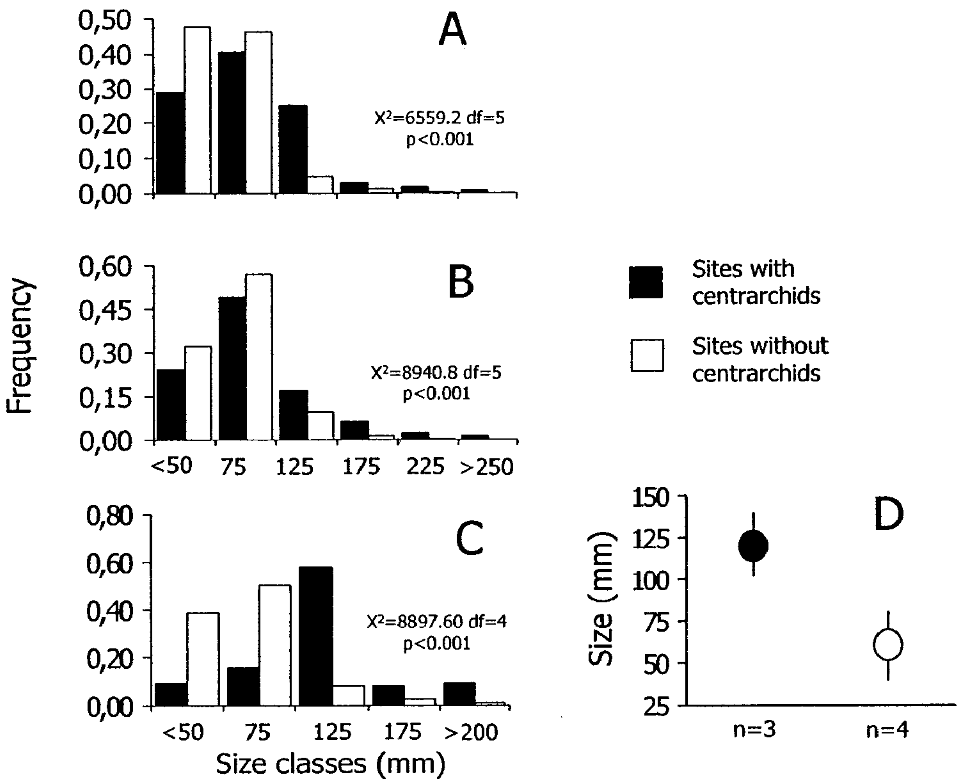

**Figure 4.** Distribution of size classes of native species (A, B and C), comparing sites with and without centrarchids. (A) refers to all species together in sites in the sector 40–20 km (see methods for a detailed description of sectors); (B) refers to all species together in sites in the sector >40 km; (C) represents the frequency distribution of size classes of barbels (three species of genus *Barbus* grouped, see methods). Differences are analysed using chi-square test. (D) shows differences in size (total length) of barbels comparing sites with and without centrarchids in the sector 40–20 km. Circles are the median and whiskers represent the quartiles 25–75%.

## On the mechanisms involved

We do not have direct evidence for the mechanisms involved in the interaction between centrarchids and native species, but these effects are typically caused by predation. The Iberian freshwater fish fauna is especially vulnerable to predation because of the lack of experience with specialist piscivorous fish in their recent evolutionary history (Brown and Moyle, 1991; Godinho and Ferreira, 2000).

Trophic competition in the form of diet overlapping may be another important factor explaining the interaction between native species and centrarchids. The native fish usually feed on detritus, plant material and invertebrates, and diet overlapping with juvenile basses and sunfishes must be occasionally important (Zapata and Granado-Lorencio, 1993). Aggressive behaviour has been cited as an important cause of exclusion between fishes (Ortubay et al., 2002). Agonistic behaviour between centrarchids and the Iberian native fish fauna has not yet been described. However,

this type of aggressive behaviour may easily occur, since both centrarchids (pumpkinseed sunfish and largemouth bass) display active parental care and nest defence (Colgan et al., 1981; Popiel et al., 1996; Cooke et al., 2002). The predatory and agonistic behaviour of centrarchids may increase the competitiveness of these species against native species.

Summer drought is a key factor in the structure of freshwater communities in Mediterranean streams (Prenda and Gallardo, 1996; Pires et al., 1999; Magalhães et al., 2002b) and may increase the intensity of biotic interactions such as competition and predation (Cowx et al., 1984; Matthews, 1988; Magoulick and Kobza, 2003). The food supply becomes scarce in pools through the season (Magalhães, 1993a,b), increasing the competition for food resources (Blanco-Garrido et al., 2003). Moreover, limited movement in isolated pools makes fish more vulnerable to predation. Native predators (e.g. the otter, *Lutra lutra*; Magalhães et al., 2002b) and exotic species (e.g. piscivorous centrarchids) may be involved, but adaptations developed against native predators may not be effective against introduced predators (Gamrad et al., 1997). The virtual absence of piscivory and aggressive behaviour in native species (Table 1) might reflect insufficient anti-predatory mechanisms (Latini and Petrere, 2004). If a native fish population does not possess efficient anti-predatory mechanisms, its persistence in invaded communities, such as those of the study area, will be endangered.

## Conclusions

We have shown that centrarchids impoverish community structure, change the natural distribution patterns of stream fish, and alter the population structure of large-bodied species such as barbels. Apart from the direct effects induced by centrarchids, the impact on other community levels may be important (e. g. Kruuk and Goudswaard, 1990; Maezono and Miyashita, 2003; Townsend, 2003). These types of effects have not yet been sufficiently evaluated in the Iberian Peninsula, though they are known to occur (e.g. Martinez-Solano et al., 2003).

It is worth mentioning that the observed impact of centrarchids on native fish communities occurred in a scenario with a low abundance of the former. What can be expected if the abundance and distribution of centrarchids increase? There is insufficient knowledge about the real impact of exotic species on Iberian biodiversity, yet new exotic species continue to be introduced. Once an exotic species is established, eradication and restoration seem to be impossible (Baltz and Moyle, 1993). The main mechanism of introduction and dispersal is via anglers, and this should be the first line of alien dispersal control. Preventing the establishment of exotic species and understanding their impact are fundamental tools for the conservation of native species.

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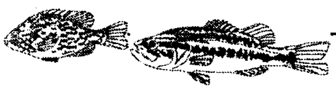

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# CAPÍTULO 4

# POTENTIAL INFLUENCE OF HABITAT AND EXOTIC FISH SPECIES ON THE DISTRIBUTION AND CONSERVATION OF TWO IBERIAN ENDANGERED FISHES IN THE MIDDLE GUADIANA BASIN: JARABUGO (*Anaecypris hispanica*) AND FRESHWATER BLENNY (*Salaria fluviatilis*)

*Influencia del hábitat y los peces exóticos en la distribución y conservación de dos especies ibéricas amenazadas: jarabugo* (Anaecypris hispanica) *y el pez fraile* (Salaria fluviatilis)

Enviado a Aquatic Conservation

# POTENTIAL INFLUENCE OF HABITAT AND EXOTIC FISH SPECIES ON THE DISTRIBUTION AND CONSERVATION OF TWO IBERIAN ENDANGERED FISHES IN THE MIDDLE GUADIANA BASIN: JARABUGO (*Anaecypris hispanica*) AND FRESHWATER BLENNY (*Salaria fluviatilis*)

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## ABSTRACT

The jarabugo (*Anaecypris hispanica*), an Iberian endemism exclusive of Guadiana basin, and the freshwater blenny (*Salaria fluviatilis*) are among the most endangered fishes in Iberian freshwaters. In this work we analysed their distribution patterns and the fish-habitat associations in the middle Guadiana basin, and examined their spatial relationships with the exotic centrachids present in the area. An analysis of habitat use-availability showed that each species was not randomly distributed. Jarabugos showed a very restricted range of distribution and were present in only four locations (out of 28) at very low densities. Jarabugos seemed to prefer small streams with aquatic submerged vegetation and avoided downstream stretches characterized by high flow, large channel width and high current velocity. Blennies was captured in nine locations at low densities as well. They seemed to select sites with opposite characteristics to jarabugos': downstream stretches with the highest flow and water availability. A Monte Carlo test showed that although jarabugos and centrarchids never coexisted, the probability of co-occurrence expected by chance was very high. However, blennies distributed randomly with respect to exotic species, since it coexisted with them at all sites. Large dams in the study area, which transform and fragment the original stream habitat and generate suitable conditions for the proliferation of exotic fish, must be considered as the main threat for the conservation of both species, specially for the most endangered one: the jarabugo.

Key words: Iberian fish endemisms, biotic interactions, exotic centrarchids, large dams, Mediterranean streams, habitat use, fish conservation

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## INTRODUCTION

Freshwater ecosystems are currently among the most threatened by human activities around the world (Mason, 1991; Dynesius and Nilsson, 1994; Saunders et al., 2002), suffering a high biodiversity loss rate (Richter et al., 1997; Harding et al., 1998; Ricciardi and Rasmussen, 1999; Aparicio et al., 2000). In this context, freshwater fishes, the most diverse group of vertebrates, present the highest proportion of endangered species as well (Bruton, 1995; Leidy and Moyle, 1998; Duncan and Lockwood, 2001).

Iberian freshwater fish fauna follows the same scheme, with many native species being currently rare (low distribution extent) and scarce (low abundance) (e.g. Elvira, 1995; Doadrio, 2001). The jarabugo (*Anaecypris hispanica*), an Iberian endemism of the Guadiana basin, and the freshwater blenny (*Salaria fluviatilis*, simply blenny hereafter) are among the most threatened fishes in Iberian freshwaters (SNPRCN, 1991; Collares-Pereira et al., 1999; Doadrio, 2001). The jarabugo is considered "Endangered" in Spain and "Critically Endangered" in Portugal, while blennies are considered "Endangered" both in Spain and in Portugal (Doadrio, 2001; Portuguese Institute of Nature Conservation, 2005). Both species present highly fragmented and localized populations (Changeux and Pont, 1995; Elvira, 1995; Corbacho and Sánchez, 2001; Salgueiro et al., 2003), and little is known about their specific ecological requirements (see Ribeiro et al., 2000 and Filipe et al., 2002).

Habitat disturbance, as well as river regulation and water pollution, have been proposed as the main threats both for jarabugos and blennies (Collares-Pereira et al., 2000; Doadrio, 2001). The exotic fish species, whose expansion is frequently favoured by river damming (e.g. south basins of the Iberian Peninsula, Clavero et al., 2004), are also influencing the decline of native fishes worldwide (Bruton, 1995; Moyle, 1997). Many exotic species are currently widespread in Iberian rivers and expanding their ranges (Aparicio et al., 2000; Doadrio, 2001). Two of these species are the North American centrarchids, pumpkinseed sunfish (*Lepomis gibbosus*) and largemouth bass (*Micropterus salmoides*). Their abundance (mainly that of sunfish) has increased in the Guadiana basin during the last decades (Bernardo et al., 2003). In this basin, centrarchids have become a potential threat for native species (Doadrio, 2001), most of them endemics (as jarabugos) and highly endangered (as both jarabugos and blennies).

In the current situation, understanding the factors regulating the distribution of endangered species, such as jarabugo and blenny, is important in regard to identifying problems and creating strategies for their conservation. This paper addresses the three following objectives: (i) to determine distribution trends of jarabugos and blennies and to provide abundance estimates for both species, (ii) to describe the features of the habitats used by jarabugos and blennies and (iii) to examine the spatial relationship between these endangered species and exotic fishes.

## METHODS

### Study area

The Guadiana river basin, with an extension over 66.960 Km$^2$ (17% of the overall basin area in Portugal and 83% in Spain), is the fourth largest Iberian basin. The climate is typically Mediterranean with a highly variable but predictable rainfall during autumn-winter and a summer drought. The mean annual precipitation is about 600 mm, falling mainly from October to March. There is also a certain irregularity in the weather between years. These climatic features strongly affect the hydrological network, causing high intra- and inter-annual variability of flow regime. In summer, with average climate conditions, most tributaries become intermittent with a succession of pools of variable dimensions with little or no water flow. More permanent and stable hydrological conditions exist in downstream stretches or in the main river.

The study area is located between the tributaries Olivenza and Chanza streams (Figure 1), in the left margin of the basin. The Mediterranean climate regime produces a high pressure for exploitation of water resources (Collares-Pereira et al., 2000). In fact, three of the five tributaries investigated (Táliga, Friegamuñoz and Alcarrache streams) are affected by the recent Alqueva Reservoir (dam closed in February 2002), the largest European artificial lake. The Chanza river is flooded by two reservoirs, Chanza and the recent Andévalo dam (closed in January 2004), located just upstream of the Guadiana River (Figure 1).

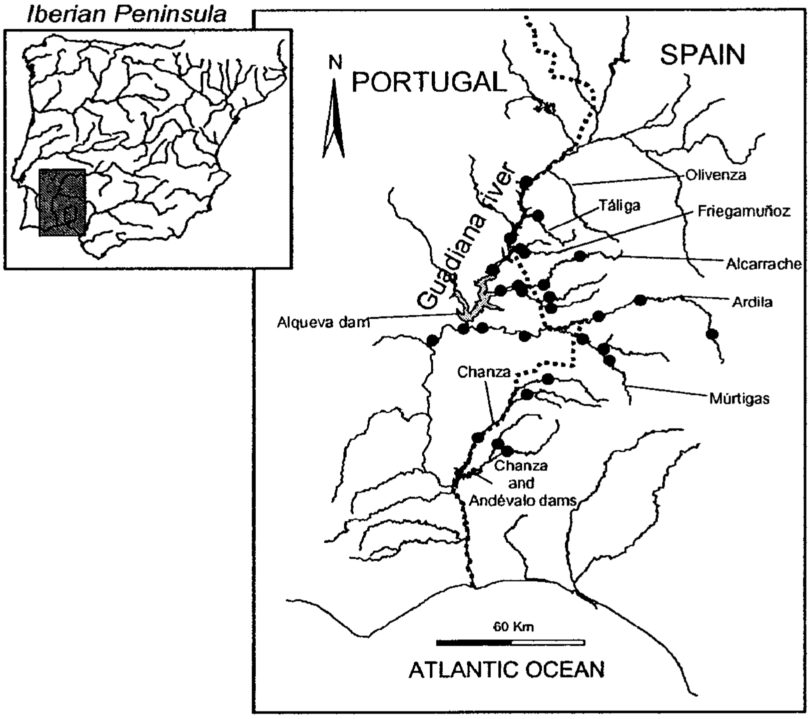

**Figure 1.** Map of the middle-lower Guadiana river basin and its location in the Iberian Peninsula, showing the location of fish sampling points. Alqueva and Andévalo dams were under construction during the sampling period.

### Fish sampling

The field work was carried out from April to June 2001, when Alqueva and Andévalo dams were under construction. A total of 28 locations were sampled (Figure 1). The fishes were captured by electrofishing. Sampling was conducted once in each site, along a stretch of approximately 100 m and during a one hour period. Captures per unit effort (CPUE) was the number of captures*100 $\text{m}^{-1}$*1 $\text{h}^{-1}$ fishing. Fish were identified to species level except for some individuals of the genus *Barbus*, which showed intermediate characteristics between *Barbus comizo* and *Barbus microcephalus* (the two *Barbus* species present in the study area), so they were included in the category *Barbus* sp. (simply barbel hereafter).

## Fish distribution analysis

To assess major changes in the distribution of jarabugos and blennies in the Spanish sector of the Guadiana basin, we compared the distribution maps of these species obtained in the study area with those published by Doadrio (2001) in UTM 10x10 Km squares.

## Habitat characterisation

Two different approaches were used to describe fish habitat for both species. First, we measured or estimated 15 habitat variables (Table 1) in each of the sampled locations (mesohabitat scale). Water temperature, dissolved oxygen, pH, conductivity and turbidity were measured with portable probes. Substrate coarseness was characterised using a modified Wentworth scale. Current velocity was assigned to one of four categories (1= no velocity; 2= scarce; 3= moderate; 4= high velocity). Depth was measured with a rigid metre, while width of the water course was measured with a flexible one. Current velocity, depth and width were used to estimate the flow in each site. Availability of effective fish shelter was estimated using a qualitative scale (1= no shelter; 2= scarce; 3= moderate; 4= high availability of shelter). The vegetation cover (%) was estimated separately for submerged, emergent and riparian bank vegetation (grass, bushes and trees). Secondly, we calculated a set of macrohabitat variables extracted from topographical and hydrological maps (1:100.000) (Table 1). Both sets of variables (mesohabitat and macrohabitat) were considered separately for statistical analysis. Presence/absence of exotic fish species (all species together and centrarchids alone) were used as variables for a probability analysis described below.

## Fish-habitat analysis

A Principal Component Analysis (PCA) was applied to mesohabitat variables to describe the main habitat gradients. The distribution of jarabugos and blennies along sampling sites were related to habitat gradients to explore distribution patterns of both species. A second PCA was run using macrohabitat variables. Before PCA, percentages were arcsine transformed, distance to the main channel was square-root transformed and the remaining variables, except temperature, conductivity and pH, were log transformed [log(x+1)]. Principal Components extracted from both PCA are hereafter referred as $PC_{mes}$ (mesohabitat variables) and $PC_{mac}$ (macrohabitat variables). The habitat gradients represented by $PC_{mes}$ and $PC_{mac}$ were equally divided into three classes with the same amplitude based on PC scores. We calculated the frequency distribution of each principal component score (sampling sites) into these classes to provide the relative availability of specific habitat configurations. To test the preferences of jarabugos and blennies for any particular habitat class, the previously described frequency distribution was compared to the frequency distribution of the sites where each species was present (Prenda et al., 1997). A Chi-square analysis was conducted to determine whether significant differences existed between both frequency distributions. If fish species occupied sampling sites randomly, then distributions for habitat availability and expected frequency should not be significantly different. When a significant result was obtained, a partitioned Chi-square analysis was conducted to determine which classes contributed to the statistical significance, i.e. in which habitat class the species was over or under represented (Prenda et al., 1997).

**Table 1.** Environmental variables used to characterise the 28 locations where fish sampling was performed in the middle Guadiana basin.

| MACROHABITAT | MESOHABITAT | |
|---|---|---|
| **Variables** | **Variables** | **Method** |
| Drainage area in each sampling point (Km$^2$) | Temperature (°C) | Portable probes |
| Stream order (Strahler, 1964) | Conductivity (µs/cm) | Portable probes |
| Distance to the main channel (Km) | Turbidity (FTU) | Portable probes |
| Altitude (m) | Dissolved oxygen (mg/l and %) | Portable probes |
| | pH | Portable probes |
| | Current velocity | Floating object, 3 replicates |
| | Depth (cm) | Rigid meter |
| | Width of water course (m) | Flexible meter |
| | Substrate coarseness | Wentworth scale |
| | Submerged vegetation cover (%) | Visual estimate, 2-3 observers |
| | Emergent vegetation cover (%) | Visual estimate, 2-3 observers |
| | Herbaceous cover (%) | Visual estimate, 2-3 observers |
| | Shrub cover (%) | Visual estimate, 2-3 observers |
| | Tree cover | Visual estimate, 2-3 observers |
| | Fish shelter (stones, submerged branches, cavities between rocks, ...) | Visual estimate, 2-3 observers |

## Relationships between the presence of jarabugos and blennies and the occurrence of exotic fish species

A probability analysis was performed to assess the relationships between exotic species' presence and jarabugo and blenny's distribution patterns. A Monte Carlo simulation test was applied to test the null hypothesis of random community assembly. Four matrices of 28 columns (sample sites) and 1000 rows were generated, where random distributions of jarabugos, blennies, all exotic species and centrarchids were assigned (1-presences, 0-absences), maintaining in all cases the probability of occurrence (i.e. the frequency of occurrence) observed in the field in each cell (number of occurrences/ total number of sample sites) (Manly, 2001). These matrices were used to calculate random coincidences between jarabugo/blenny and exotic fishes. The probability of coincidence of jarabugo/blenny and exotics expected by chance was calculated as follows:

P(i)= C(i)/ total number of randomised coincidences

where P(i) is the probability of coincidence in i sites, C(i) is the number of coincidences in i sites and i=0,1,2,...,28 sites.

The significance level was assigned to $P<0.05$ (Manly, 2001). If jarabugos or blennies had a probability of no co-occurrence with exotic species or centrarchids expected by chance $P<0.05$, but these species did not coexist with them, then the observed distribution is not random and the species tend to occur at distinct locations. If a species was distributed randomly in relation to exotic species or to centrarchids, no significant results should be expected (i.e. probability of coincidence must be $P>0.05$).

## RESULTS

### Summary of captures

A total of 15 fish species were collected, six of them being exotics (37.5%) and nine natives (Table 2). The barbel group was the most widespread followed by Iberian roach (*Squalius alburnoides*) and the pumpkinseed sunfish (*Lepomis gibbosus*) (Table 2). Iberian roach was also the most abundant species. Infrequent species included the exotics goldfish (*Carassius auratus*), mosquito fish (*Gambusia holbrooki*), common carp (*Cyprinus carpio*) and the chameleon cichlid *Herichthys facetum* (Table 2). Largemouth bass (*Micropterus salmoides*) was especially abundant in one site located in a Chanza tributary.

**Table 2.** List of native and exotic species captured in the study area, including their frequency of occurrence at 28 locations and mean density (± standard deviation)

| Species | Family | Frequency of occurrence (%) | Mean density ± SD (Number of captures 100 m$^{-2}$) |
|---|---|---|---|
| Native | | | |
| *Anaecypris hispanica* | Cyprinidae | 14.3 | 6.5±31.1 |
| *Salaria fluviatilis* | Blenniidae | 32.1 | 0.4±0.8 |
| *Barbus sp.* | Cyprinidae | 92.9 | 97.8±265.6 |
| *Barbus comizo* | Cyprinidae | 28.6 | 0.28±0.5 |
| *Barbus microcephalus* | Cyprinidae | 71.4 | 22.3±61.5 |
| *Chondrostoma willkommii* | Cyprinidae | 42.9 | 35.9±118.7 |
| *Chondrostoma lemmingii* | Cyprinidae | 25.0 | 25.7±94.8 |
| *Squalius pyrenaicus* | Cyprinidae | 39.3 | 13.3±39.3 |
| *Squalius alburnoides* | Cyprinidae | 85.7 | 325.4±1354.6 |
| *Cobitis paludica* | Cobitidae | 64.3 | 23.8±89.9 |
| Exotic | | | |
| *Cyprinus carpio* | Cyprinidae | 7.1 | 0.2±1.14 |
| *Carassius auratus* | Cyprinidae | 3.6 | 0.02±0.1 |
| *Lepomis gibbosus* | Centrarchidae | 75.0 | 6.2±11.8 |
| *Micropterus salmoides* | Centrarchidae | 25.0 | 4.4±16.1 |
| *Herichthys facetum* | Cichlidae | 7.1 | 0.1±0.4 |
| *Gambusia holbrookii* | Poeciliidae | 7.1 | 0.2±0.9 |

### Distribution and abundance of jarabugos and blennies

Jarabugos had the most restricted distribution of all native species and was only collected in four locations (14.3%, n=28) (Figure 2). Important changes have been detected in jarabugos' distribution in the study area, comparing our data with those published by Doadrio (2001) (Figure 2). Of the seven UTM 10x10 km squares prospected in this study where it had been previously cited by Doadrio (2001), it was not detected in six (86% reduction of its original area). Abundance was very low(Table 2), excepting the location of Alcalaboza. In this location

On the other hand, blennies were recorded in nine sampling stations (32.1%, n=28). This species had previously been cited in just one UTM 10x10 km square (Doadrio, 2001), where it was not found in this study. However, it was found in seven new UTM 10x10 km squares. Abundance estimates were very low (Table 2) and varied from 0.3 ind. 100 $m^{-2}$ (G2 and G3 stations) to 3.0 ind. 100 $m^{-2}$ (M2 station) (Figure 2). The percentage of contribution of the freshwater blenny to the fish community ranged from 0.3% (C3 station at lower Chanza river) to 2.8% (G1 station at Guadiana river).

### Fish-habitat relationships

The mesohabitat variables were summarized in two main gradients (Table 3) related (1) to stream size ($PC1_{mes}$), running from narrow stretches and low flows to larger ones and high flows and (2) to water physico-chemical parameters ($PC2_{mes}$). At the macrohabitat scale, an upstream-downstream gradient could be defined ($PC1_{mac}$) (Table 3). There was a high concordance between this gradient and the $PC1_{mes}$ gradient ($r=0.80$ $p<0.001$).

Jarabugos occurred preferentially in small streams with high submerged vegetation cover (class 1 $PC1_{mes}$, partitioned $\chi^2$-analysis, $p=0.032$) and was absent from the largest river stretches (high flow, channel width and current velocity) (class 3 $PC1_{mes}$, partitioned $\chi^2$-analysis, $p<0.001$) (Figure 3). However, this species was distributed randomly in relation to the water physico-chemical gradient defined by $PC2_{mes}$ ($\chi^2$-analysis, $p=0.127$) and to the upstream-downstream gradient ($PC1_{mac}$; $\chi^2$-analysis, $p=0.127$).

Blennies used sites with opposite habitat features to those of jarabugos (Figure 3). It was over-represented in the largest rivers (class 3, $PC1_{mes}$, partitioned $\chi^2$-analysis, $p<0.05$), while they rejected class 1 zones, which demonstrated opposite features and were characterized by high submerged vegetation cover (partitioned $\chi^2$-analysis, $p<0.001$). Also, the proportion of blennies in the community increased throughout the $PC1_{mes}$ gradient ($r=0.93$, $p=0.002$), which reinforce the apparent preference for the largest streams. These are the sites with more stable water availability. This result concurred with that obtained for the habitat gradient described by $PC1_{mac}$. The species was over-represented in stretches located downstream or in the Guadiana river channel (class 3, partitioned $\chi^2$-analysis, $p<0.05$).

Finally, the physico-chemical gradient defined by $PC2_{mes}$ had a significant effect on the distribution of freshwater blenny (Figure 3), since the species occurred more frequently in stretches with higher dissolved oxygen, pH and conductivity (class 3, partitioned $\chi^2$-analysis, $p=0.035$).

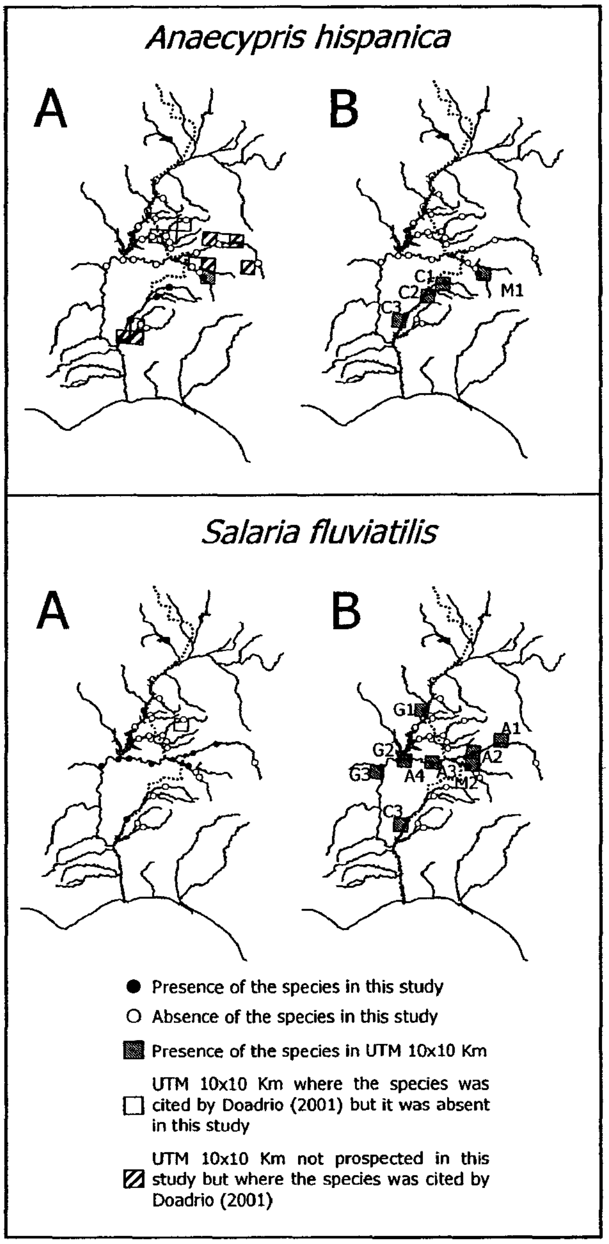

**Figure 2.** Distribution of jarabugos (*Anaecypris hispanica*) and blennies (*Salaria fluviatilis*) in the middle Guadiana. A) Distribution published by Doadrio (2001) in UTM 10x10 km squares. B) Distribution in UTM 10x10 km squares obtained in this study. Location codes of the sites where the species were collected begin with the first letter of the name of each river.

### Relationships between the presence of jarabugos and blennies and the occurrence of exotic fish species

Jarabugos and centrarchids never appeared together, although the probability of co-occurrence expected by chance was very high (Monte Carlo test P=0.965, prob. no co-occurrence P=0.035, Figure 4). For the entire Spanish sector of the Guadiana basin, jarabugos were cited in 54 UTM 10x10 Km squares and centrarchids (pumpkinseed sunfish and largemouth bass) in other 78 UTM 10x10 Km squares (Doadrio, 2001). For this sector, jarabugos and centrarchids coincided in only six UTM squares and according to the previous result, the probability of that occurring by chance was extremely low (Monte Carlo test, P<0.0001). These results reveal a strong spatial segregation between these species. No significant result was obtained considering the exotic species altogether. There was one site with jarabugos and exotics (*H. facetum*). The probability of this co-occurrence expected by chance was P=0.114 (Monte Carlo test, Figure 4), indicating a random distribution pattern.

Blennies coexisted with exotic species in all locations, with a probability expected by chance of P=0.127 (Monte Carlo test, Figure 4). Similar results were obtained considering centrarchids; they appeared together in eight locations with a probability expected by chance of P=0.151 (Figure 4). These results indicate that the distribution of blennies was random in relation to that of exotic species.

## DISCUSSION

In this work, we only found four occurrences of jarabugos and nine of blennies. The low density of both species can limit their detection in the field, mainly in the case of blennies. Their cryptic morphology and their preference for bottom habitats and current velocity (Côté et al., 1999;García-Berthou and Moreno-Amich, 2000) can further hinder their detection. Despite this, new locations for blennies were found in the Spanish sector of the Guadiana basin during this study.

Despite its limitations, this work provides significant progress on the knowledge of the ecology of both species. In fact, this is the first work that demonstrates the spatial segregation between the endangered jarabugos and the exotic centrarchids. These findings are important and provide interesting inputs towards developing conservation strategies of both species.

### Fish-habitat relationships

Despite of the limited number of occurrences of jarabugos, the results are in agreement with the observations on habitat use published in previous works (e.g. Collares-Pereira et al., 1999; Ribeiro et al., 2000). Jarabugos inhabited small streams with low current and flow and with presence of submerged vegetation.

Though blennies are tolerant to a wide range of environmental conditions (Freeman et al., 1990; Hernández et al., 2000), they were generally found in downstream stretches or in the main river channel, characterized by high values of flow, channel width and current velocity. This species has specific requirements for heterogeneous substrates made of large stones (more than 180 $cm^2$ on average), that provide adequate refuge for nest building (Côté et al., 1999). This type of substrate was abundant in downstream stretches or in the main river, with the highest current in the study area. On the other hand, the breeding period of blennies, that extends from the end of May to the beginning of August (the driest period in the study area) (Côté, 1993; Vila-Gispert and Moreno-Amich, 1998), may force this species to select those zones with more stable and permanent water availability, usually found downstream. Freeman et al. (1990) observed that prolonged drought conditions apparently prevented successful reproduction and also caused high juvenile and adult mortality. Downstream stretches, with larger drainage area and stream order, have a greater probability of experiencing continuous flow, even during the summer, compared to stretches located up or middle stream, with smaller basin and lower order (Filipe et al., 2002).

**Table 3.** Pearson correlations (r) between the habitat variables included in both PCA and the extracted principal components. The percentage of the original variance explained by each component is indicated. * $p<0.05$; ** $p<0.01$; ***$p<0.001$

| Mesohabitat variables | $PC1_{mes.}$ 25.37% | $PC2_{mes.}$ 17.63% |
|---|---|---|
| Temperature | -0.42* | 0.28 |
| Conductivity | 0.56* | 0.62*** |
| Turbidity | 0.34 | 0.42* |
| Dissolved $O_2$ | 0.07 | 0.78*** |
| pH | 0.13 | 0.64*** |
| Current velocity | 0.74*** | 0.25 |
| Flow | 0.90*** | 0.03 |
| Width of water course | 0.85*** | -0.005 |
| Substrate coarseness | 0.02 | 0.20 |
| Submerged vegetation cover | -0.75*** | 0.24 |
| Emergent vegetation cover | -0.47* | 0.46* |
| Herbaceous cover | -0.33 | 0.38* |
| Shrub cover | -0.24 | 0.56** |
| Tree cover | 0.28 | 0.12 |
| Fish shelter | -0.24 | 0.36 |
| **Macrohabitat variables** | $PC1_{mac.}$ 73.57% | |
| Stream order | 0.91 | |
| Drainage area | 0.88 | |
| Distance to the main channel | -0.84 | |
| Altitude | -0.80 | |

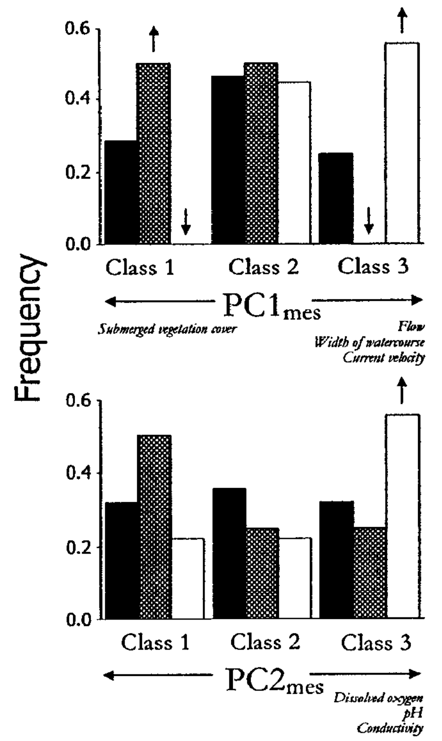

**Figure 3.** Habitat preferences of jarabugos (*Anaecypris hispanica*) and blennies (*Salaria fluviatilis*). Black bars are habitat availability, pointed bars are habitat use by jarabugos and white bars are habitat use by blennies. PC1mes and PC2mes represent habitat gradients. The meaning of each gradient is under the arrows. Habitat types in which the species was over-represented or under-represented are indicated by vertical arrows (partitioned $\chi^2$-analysis, $p<0.05$). Jarabugo n=4; freshwater blenny n=9.

## Relationships between the presence of jarabugos and blennies and the occurrence of exotic fish species

Introduction and dispersal of exotic fishes has been supposed as one of the main threats to the conservation of jarabugos (Collares-Pereira et al., 1999; 2000; Ribeiro et al., 2000; Doadrio, 2001). However, no analytical effort had been made to demonstrate objectively the relationship between exotic fishes and the distribution of this endangered species. In this work, we show a strong spatial segregation between these species. Jarabugos appeared only in those sampling stations where centrarchids were absent, even though the probability of co-occurrence expected just by chance was very high. Differences in habitat requirements may be the reason for this type of segregation. However, there is evidence that point to the existence of a negative impact of centrarchids on jarabugos. Many works have shown that the introduction of exotic species negatively affects native species at a local scale, frequently causing extirpation of local populations (e.g. Ross, 1991). Bernardo et al. (2003) reported an increase of centrarchids proportion (mainly pumpkinseed) in the Portuguese sector of the Guadiana basin during the 1980-1995 period. For the same area and years, Collares-Pereira et al. (1999) observed a dramatic decline of jarabugos (a species that three decades ago was relatively abundant in Portugal), both in abundance and distribution range. Our results confirm this negative trend, since jarabugos have apparently disappeared from most of the Guadiana's tributaries studied here and where they had been previously cited (Doadrio, 2001). Five out of the six UTM squares where the species had been previously found and they seem to have disappeared, are currently invaded by centrarchids (which they were not previously cited by Doadrio 2001). This finding together with the strong spatial segregation observed, allows us to propose centrarchids as the main factor responsible for the decline of jarabugos in the study area. Other possible factors, such as habitat disturbance or water pollution, were not detected.

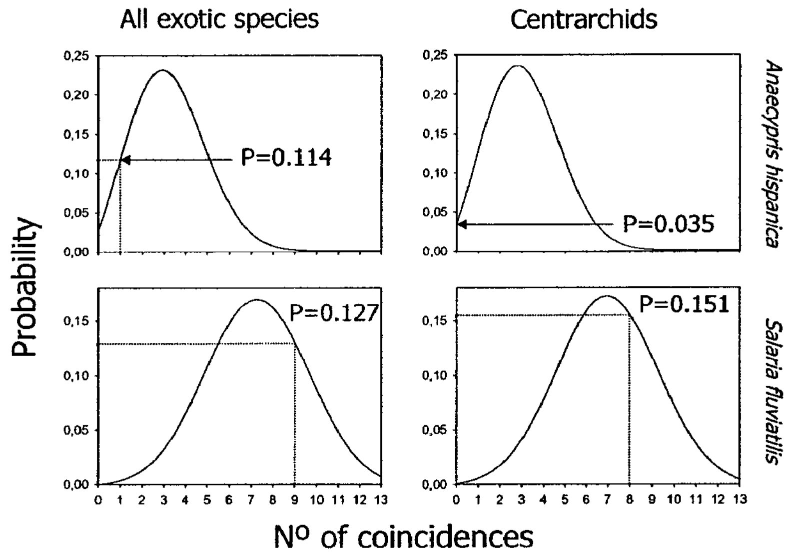

**Figure 4.** Probability coincidence models between jarabugo/ blenny and exotic species derived from Monte Carlo simulation test. P values are the probability of coincidence expected by chance for the number of locations where each species pair coexists in the field. See methods for a detailed description of the analysis.

The mechanisms explaining the interactions between jarabugos and centrarchids that result in the observed exclusion pattern and the restricted distribution of this endangered species remain unknown. However, some of the following explanations could be proposed. For example, adults and juveniles of jarabugos could be seen as potential preys for basses or pumpkinseeds because of the small size of this cyprinid (maximum total length 7 cm, Collares-Pereira and Cowx, 2001). Also, jarabugo's eggs consumption by sunfish may be significant, though there is no evidence of high predation on fish eggs (Garcia-Berthou and Moreno-Amich, 2000a). However, as Bernardo et al. (2003) pointed out, egg consumption is very probably underestimated because of rapid digestion and short duration of the incubation period, easily missed by sampling. Fish eggs predation might strongly affect species recruitment. Finally, aggressive behaviour has been cited as an important cause of exclusion between fishes (Ortubay et al., 2002). Agonistic behaviour between centrarchids and Iberian native fish fauna has not been described yet. Nevertheless, this type of aggressive behaviour is easy to occur, since centrarchids display territoriality, active parental care and nest defence (Colgan et al., 1981; Popiel et al., 1996; Cooke et al., 2002). Agonistic behaviour might also account for the absence of jarabugos in the presence of centrarchids.

On the contrary, the presence of exotic species did not have any evident influence on the distribution of blennies, since they occurred even in sites where exotic species were highly abundant. Previous works showed that blennies seem to be tolerant to exotic fish presence (e.g. Prenda and Mellado, 1993; García-Berthou and Moreno-Amich, 2000). Blenny was the only common native species after the introduction of more than 12 exotic fish species in Lake Banyoles (García-Berthou and Moreno-Amich, 2000). These authors suggested that the persistence of blennies seems to be related to its particular ecological features such as its cryptic morphology, small size and benthic preferences.

## Conservation status of jarabugos and blennies in the Guadiana basin

The distribution range and abundance of jarabugos have been drastically reduced in the last 30 years. Certainly, if no efficient conservation measures are put into practice, their disappearance seems unavoidable (Collares-Pereira et al., 1999, 2000). Two main factors are identified as potential threats for the conservation of jarabugos in the study area, the invasion of centrarchids and the increasing number of large dams. Three large dams are located in the study area, Alqueva, Chanza and Andévalo, the former being the largest reservoir in Europe. Dams, which drastically modify freshwater habitats and generate suitable conditions for the proliferation of exotic species (centrarchids and others) (Taylor et al., 2001; Clavero et al., 2004), act as a barrier for fish movements and recolonization events (Clavero et al., 2004). Molecular studies have demonstrated the strong isolation of the few populations of jarabugo (Alves et al., 2001). Alqueva dam, with a capacity of 3150 hm3, could accentuate this isolation and increase the probability of local extirpations at medium-short term and, eventually, species extinction in the long term. Further research focused on the potential negative influence of centrarchids on jarabugos is needed. The correct selection of priority conservation areas for the species, as those recently proposed by Filipe et al. (2004), demands the clarification of the real effect of centrarchids on jarabugos.

The conservation status of blennies in the study area does not seem to be as critical as that of jarabugo's. We found blennies in nine locations distributed along the Ardila, Chanza and in the main river (Guadiana) where the species had not been previously cited. The impact of the large reservoirs on blennies' distribution and conservation seem less strong than in the case of jarabugos (Rodríguez-Jiménez, 2001). However, substrate is generally more homogeneous in reservoirs than in stream or river beds, due to sediment deposition. The specific requirements of blennies for substrate heterogeneity (Côté et al., 1999) make this species vulnerable to changes in benthic habitat structure, as it has been observed in other species with similar habitat preferences (e. g. Knaepkens et al. 2004). Hydraulic actions proposed in the Guadiana basin, as impoundment of rivers or water interbasin transfers, are diminishing the water flow. This represents a great threat to the conservation of blennie's populations in the Guadiana basin, a basin in which still persists in having the richest, best

preserved and most diverse freshwater fish fauna of the Iberian Peninsula (Filipe et al., 2002).

## ACKNOWLEDGEMENTS

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# CAPÍTULO 5

## EURASIAN OTTER (*Lutra lutra*) DIET AND PREY SELECTION IN MEDITERRANEAN STREAMS INVADED BY EXOTIC CENTRARCHIDS

*Dieta de la nutria* (Lutra lutra) *y selección de presa en ríos mediterráneos invadidos por centrárquidos*

# EURASIAN OTTER (*Lutra lutra*) DIET AND PREY SELECTION IN MEDITERRANEAN STREAMS INVADED BY EXOTIC CENTRARCHIDS

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## ABSTRACT

In this study, the diet of the otter (*Lutra lutra*) was determined by analysing 1633 spraints collected in 71 sites distributed along the Guadiana, Guadalquivir and Tinto river basins (south-west Iberian Peninsula). At the same time, fish availability was quantified by electrofishing techniques. Fish was the otters' main prey category, representing more than 60% of total individuals and more than 80% of total consumed biomass. To search for possible preference for or avoidance of centrarchids (*Lepomis gibbosus* and *Micropterus salmoides*) by the otter, we studied prey selection of fish in sites from the Guadiana basin (this area was the most by centrarchid invasion). Fish availability was determined by electrofishing. Otters preyed on most of the fish species captured in the field, however the consumption of exotic centrarchid fishes was low. Although the otters preyed on all available centrarchid size classes, application of Jacobs' index of preference showed a clear rejection of both centrarchid species by the otter. Consumption of native fish genera (*Squalius*, *Barbus* and even *Chondrostoma*) by the otters increased as a response to an increase of these prey in the environment. In contrast, increasing numbers of pumpkinseed in the field was not reflected in otter consumption, as these fish were practically ignored by the otters. The general decline of native freshwater fish fauna in Iberian rivers, traditionally the preferred prey of otters, together with the spread of exotic fish species (centrarchids and others) may force otters to consume centrarchids in a higher proportion than they do at present. However, the maintenance of healthy populations of Iberian otters depends not only on the prey consumed, but on the amount of energy provided.

Key words: Iberian freshwater fishes, predation, invasive species, Mediterranean streams, Guadiana basin, conservation

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## INTRODUCTION

The otter (*Lutra lutra*) is a top predator in freshwater ecosystems. Fish are the otter's main prey, but its opportunistic feeding behaviour allows it to feed on whatever prey is available (Erlinge, 1968; Mason and Macdonald, 1986). Exotic centrarchids (pumpkinseed sunfish, *Lepomis gibbosus*, and largemouth bass, *Micropterus salmoides*) are increasing their range in Iberian freshwater habitats (Doadrio, 2001) and specially in the Guadiana river basin (Bernardo et al., 2003). These species are known to impact severely on native fish fauna, becoming the dominant fish species in many areas (Capítulo 3). The question arises as to whether this new potential trophic resource is being consumed by native predators that largely depend on fish for their food. If Iberian otters consume centrarchids in sufficient quantities, they could benefit from the introduction of these exotic species. This is what happened with the introduction and expansion of the American crayfish (*Procambarus clarkii*). Crayfish was introduced to Spain in 1973/74, spread rapidly in most Iberian rivers (Hasburgo-Lorena, 1983), and became important prey for the otter and other predators (Adrián and Delibes, 1987; Delibes and Adrián, 1987; Correia, 2001).

In this work, we analyse the importance of exotic centrarchids in the otter diet in a large area from the lower Guadiana basin (south-west Iberia), and the selectivity displayed by the mustelid for both pumpkinseed sunfish and largemouth bass. Finally, we discuss the role of the otter as a potential natural controller of the expansion of these exotic centrarchids.

## METHODS

### Study area

The field work was carried out in 71 localities distributed along the Guadiana, Guadalquivir and Tinto river basins. The area is characterised by a Mediterranean climate with seasonal flooding (autumn, winter and early spring) and severe droughts (summer) that vary markedly between years.

The unpredictable climate produces a high pressure for exploitation of water resources (Collares-Pereira et al., 2000), and eight large dams (more than 100 hm$^3$) are situated in the area. The Alqueva dam (3150 hm$^3$), which impounds the middle reaches of the Guadiana basin, is considered the largest artificial lake in Europe.

The Tinto is a very special river. Its basin drains part of the Iberian Pyrite Belt, a region containing massive sulphide deposits. The mining activity, together with the natural alteration of these mineral masses, have long been a source of pollution to this river. The extreme water acidity (pH~2-3) and the high concentration of dissolved heavy metals (Amaral-Zettler et al., 2002) do not allow the existence of macroscopic life. Under this situation fishes and other potential prey for otters remain isolated into unpolluted tributaries or sub-basins, without the possibility of interconnection between populations (Clavero et al., 2004). In spite of this, the otter inhabits the entire basin, even using the main river as a corridor between the unpolluted tributaries (personal observation).

### Analysis of otter diet

The diet of the otter was determined by the analyses of its faeces (referred to as 'spraints' hereafter). Spraints contain those parts of the food intake that cannot be digested (bones, scales, hair and feathers). We collected and analysed a total of 1633 spraints (Table 1), collected in the 71 sampling sites (Guadiana 28 sites, 547 spraints; Guadalquivir 28 sites, 493 spraints and Tinto 15 sites, 593 spraints). On average, 23 spraints were collected per site, and diet analyses followed standard methodology (Beja, 1997). Prey remains were identified using published works (Webb, 1980; Roselló, 1987; Prenda et al., 2002) and our own collection for comparison. Each identified prey class was considered an 'occurrence'. The relative frequency of occurrence (RFO) was the percentage of the total number of occurrences corresponding to a certain prey class (Mason and Macdonald, 1986).

The minimum number of individuals of each prey class present in a spraint was estimated by the number and position (left-right) of diagnostic hard parts (mainly mouth bones for fish and endopodites/exopodites for the crayfish, *Procambarus clarkii*), which were measured with a calliper to the nearest 0.1 mm. In cases where these diagnostic pieces were not found, the remains of a certain prey item were considered to belong to a single individual.

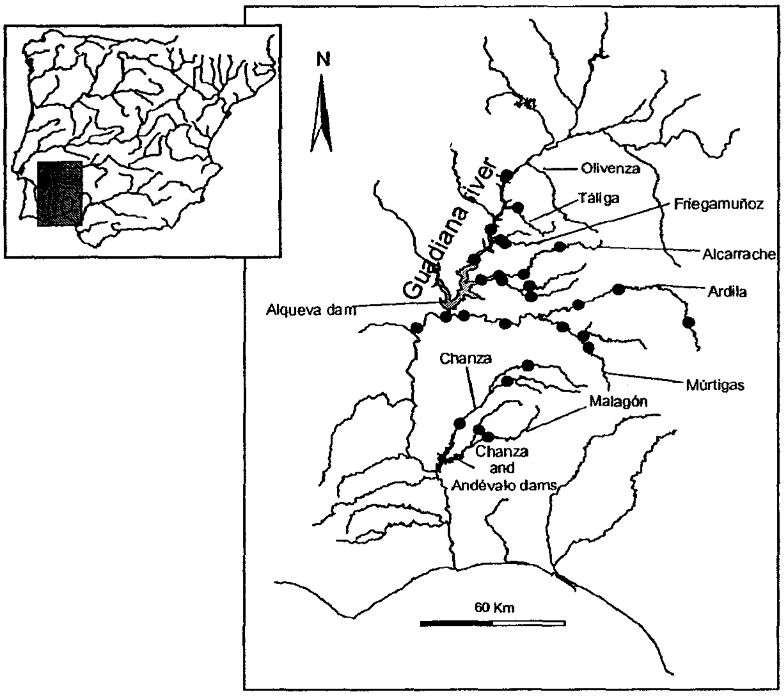

**Figure 1.** Map of the study area showing the localization of sampling sites in the Guadiana river basin, were otter prey selection was studied. This area correspond to the most affected zone by centrarchid invasion.

We applied regression equations to estimate the original size and weight of the prey consumed by the otters. In the case of fish, regressions between the size of key bones and original length (Prenda et al., 2002), and between length and weight (Prenda et al., 2002; own unpublished data) were computed. The original size of ingested crayfish was estimated using a regression between the size of the endopodite/exopodite and the total length, measured from the rostrum to the uropodo, computed from a crayfish sample (own unpublished data). The weight of crayfish was estimated by regression between total length and crayfish weight without claws (they were rarely found in otter spraints).

The original lengths and weights of fish and crayfish individuals without measured key hard parts were distributed following the frequencies of lengths and weights in the estimated samples of each prey class. Constant weights were assigned to the remaining prey classes: insects, 1 g; *Rana perezi*, 10 g; *Bufo bufo*, 50 g; unidentified amphibians, 10 g; *Natrix* sp., 50 g; *Mauremys leprosa*, 100 g; birds, 100 g; small mammals, 100 g (Beja, 1996).

## Fish prey selection

To search for possible preference for or avoidance of centrarchids (pumpkinseed sunfish and largemouth bass) by the otter, we studied prey selection of fish in sites from the Guadiana basin (Figure 1), since streams and rivers of this area were the most affected by centrarchid invasion. The relative availability of fish prey was estimated by electrofishing. All captured fish were identified and measured for total length, and then returned to the water. We applied regression equations between fish length and weight (Prenda et al., 2002; own unpublished data) to estimate the available fish biomass. Fish species that appeared rarely in the otter diet or in the environment were removed from the analysis of prey selection (Table 2). In many cases, it was difficult to assign fish remains to a certain species within congeneric species (*Squalius*, *Barbus* and *Chondrostoma*), due to the similarity of their bones. To solve this problem, congeneric fish species were pooled into a single prey item, so six fish prey categories were used in the analysis: *Squalius*, *Barbus*, *Chondrostoma*, *Cobitis paludica*, *Lepomis gibbosus* and *Micropterus salmoides*.

Chi-square analysis was used to test for differences between individuals and the biomass of the items consumed by otter, and those present in the environment. To obtain an absolute preference or avoidance value for each fish prey, we also used Jacobs' (1974) selection index, calculated as:

$D= (r-p)/\ [(r+p)-2rp]$;

where $r$ is the proportion of the prey category in the diet and $p$ is the proportion in the environment. The index ranges from -1 to +1, -1 being total avoidance, 0 no preference and +1 full preference. $D$ was calculated for each prey category in each site. To interpret the index objectively, we compared alternative hypotheses, in which the null hypothesis corresponded with a Jacobs' index equal to zero (the prey is used as available in the environment). The obtained value was compared using Student's t-test (Revilla et al., 2000).

## RESULTS

Spraint analysis showed that fish was the otter's main prey category, representing more than 60% of total individuals and more than 80% of total consumed biomass (Table 1 and 2). Otter diet was very similar considering as the entire study area (Guadiana, Guadalquivir and Tinto river basins) as the Guadiana basin alone (Table 1 and 2). In the Guadiana basin otters consumed most of the fish species captured in the field (Table 2). However, *Anaecypris hispanica*, *Gambusia holbrooki* and *Herichthys facetum* were captured during the field work but were absent from otter spraints. In contrast, no eels (*Anguilla anguilla*) were captured, but one individual was detected in an otter spraint. Within fish, *Barbus* spp. were the main prey item, representing 22.2% of total individuals and 48.6% of total consumed biomass in the entire study area (Table 1).

**Table 1.** Otter diet in the study area (Guadiana, Guadalquivir and Tinto river basins) expressed as relative frequency of occurrence (RFO), percentage of individuals (% Ind), biomass (% Bio). Total number of analysed spraints n=1633. * *Barbus* sp. include at least three species (*B. comizo, B. microcephalus* and *B. sclateri*).

| | Diet | | |
|---|---|---|---|
| | RFO n=3046 | % Ind n=4707 | % Bio (g) n=140022.3 |
| ***Fish*** | *57.8* | *61.7* | *85.9* |
| Family Cyprinidae | | | |
| *Barbus* sp*. | 22.2 | 24.6 | 48.6 |
| *Chondrostoma lemmingii* | 0.6 | 0.5 | 0.03 |
| *Chondrostoma willkommii* | 1.8 | 1.8 | 1.2 |
| *Chondrostoma sp.* | 0.5 | 0.6 | 0.05 |
| *Squalius alburnoides* | 4.6 | 6.4 | 0.6 |
| *Squalius pyrenaicus* | 5.2 | 5.4 | 1.3 |
| Family Cobitidae | | | |
| *Cobitis paludica* | 5.7 | 4.8 | 0.5 |
| Family Blenniidae | | | |
| *Salaria fluviatilis** | 0.1 | 0.05 | 0.01 |
| Family Anguillidae | | | |
| *Anguilla anguilla** | 1.5 | 1.1 | 1.8 |
| Introduced species | | | |
| Family Salmonidae | 0.3 | 0.2 | 1.1 |
| Family Cyprinidae | | | |
| *Carassius auratus* | 5.0 | 6.1 | 6.8 |
| *Cyprinus carpio* | 1.6 | 1.4 | 17.1 |
| Family Centrarchidae | | | |
| *Micropterus salmoides* | 2.0 | 2.5 | 2.3 |
| *Lepomis gibbosus* | 1.2 | 1.2 | 0.3 |
| Family Poeciliidae | | | |
| *Gambusia holbrooki* | 1.2 | 2.2 | 0.1 |
| *Undetermined fish* | 4.2 | 3.0 | 4.1 |
| ***Other prey*** | ***42.2*** | ***38.3*** | ***14.1*** |
| *Procambarus clarkii* | 22.1 | 21.8 | 6.0 |
| *Insects* | 2.8 | 2.2 | 0.1 |
| *Rana perezi* | 13.2 | 11.0 | 3.7 |
| *Bufo bufo* | 0.2 | 0.1 | 0.2 |
| *Undetermined amphibian* | 1.3 | 1.4 | 0.5 |
| *Natrix* sp. | 2.0 | 1.3 | 2.2 |
| *Mauremys leprosa* | 0.2 | 0.1 | 0.4 |
| *Birds* | 0.2 | 0.1 | 0.4 |
| *Mammals* | 0.3 | 0.2 | 0.6 |

**Table 2.** Otter diet in the Guadiana basin expressed as relative frequency of occurrence (RFO), percentage of individuals (% Ind), biomass (% Bio) and the occurrence, the percentage of individuals and biomass of each fish species in the field determined by electrofishing. Total number of analysed spraints n=547. * Fish species excluded for the fish prey selection analysis.

| | **Diet** | | | **Environment** | | |
|---|---|---|---|---|---|---|
| | RFO n=1013 | % Ind n=1480 | % Bio (g) n=36768.4 | Occurr. | % Ind n=31197 | % Bio (g) n=166420.4 |
| ***Fish*** | *57.8* | *62.1* | *81.1* | | | |
| Family Cyprinidae | | | | | | |
| *Barbus* sp. | 29.2 | 29.5 | 67.7 | 92.9 | 23.8 | 69.7 |
| *Chondrostoma lemmingii* | 0.3 | 0.5 | 0.01 | 25.0 | 4.1 | 0.9 |
| *Chondrostoma willkommii* | 1.7 | 1.3 | 2.8 | 42.9 | 7.3 | 1.5 |
| *Chondrostoma sp.* | 1.1 | 1.4 | 0.06 | | | |
| *Squalius alburnoides* | 12.9 | 19.2 | 2.2 | 85.7 | 54.1 | 16.0 |
| *Squalius pyrenaicus* | 0.6 | 0.5 | 0.4 | 39.3 | 2.3 | 1.0 |
| *Anaecypris hispanica** | - | - | - | 14.3 | 1.4 | 0.2 |
| Family Cobitidae | | | | | | |
| *Cobitis paludica* | 5.5 | 4.4 | 0.7 | 64.3 | 4.0 | 1.6 |
| Family Blenniidae | | | | | | |
| *Salaria fluviatilis** | 0.2 | 0.1 | 0.02 | 32.1 | 0.2 | 0.2 |
| Family Anguillidae | | | | | | |
| *Anguilla anguilla** | 0.1 | 0.1 | 1.5 | - | - | |
| Exotic species | | | | | | |
| Family Cyprinidae | | | | | | |
| *Carassius auratus** | 0.7 | 0.9 | 0.4 | 3.6 | 0.02 | 0.02 |
| *Cyprinus carpio** | 0.3 | 0.2 | 0.4 | 7.1 | 0.1 | 4.7 |
| **Family Centrarchidae** | | | | | | |
| ***Micropterus salmoides*** | **0.8** | **0.8** | **0.4** | **25.0** | **1.1** | **1.1** |
| ***Lepomis gibbosus*** | **2.7** | **1.9** | **0.9** | **75.0** | **1.5** | **3.0** |
| Family Poeciliidae | | | | | | |
| *Gambusia holbrooki** | - | - | - | 7.1 | 0.05 | 0.01 |
| Family Cichlidae | | | | | | |
| *Herichthys facetum** | - | - | - | 7.1 | 0.03 | 0.1 |
| *Undetermined fish** | 1.7 | 1.3 | 3.6 | | | |
| ***Other prey*** | *42.2* | *37.9* | *18.9* | | | |
| *Procambarus clarkii* | 24.2 | 21.5 | 8.2 | | | |
| *Insects* | 1.6 | 1.3 | 0.04 | | | |
| *Rana perezi* | 12.4 | 11.9 | 4.6 | | | |
| *Bufo bufo* | 0.3 | 0.2 | 0.2 | | | |
| *Undetermined amphibian* | 0.9 | 1.0 | 0.4 | | | |
| *Natrix* sp. | 2.2 | 1.5 | 0.9 | | | |
| *Mauremys leprosa* | 0.4 | 0.3 | 2.7 | | | |
| *Birds* | 0.1 | 0.1 | 1.1 | | | |
| *Mammals* | 0.1 | 0.1 | 0.8 | | | |

These percentages were even higher considering data from the Guadiana basin (Table 2). Crayfish (*Procambarus clarkii*) was the second main prey item (21.8% of individuals and 6.0% of biomass, Table 1). Other categories can be considered as secondary. Predation on amphibians (mainly *Rana perezi*) was frequent, while birds, reptiles and mammals were rather rare in the otters' diet (Table 1 and 2).

Overall, prey consumption was significantly different from availability, considering both individuals and the biomass of each fish prey consumed by the otter (individuals: $\chi^2=3179.9$ d.f.=5 $p<0.001$; biomass: $\chi^2=13246.9$ d.f.=5 $p<0.001$). Using Jacob's preference index for individuals, *Barbus* was the only preferred fish prey (mean D=0.42), while centrarchids were avoided (pumpkinseed sunfish: mean D=-0.40; largemouth bass: mean D=-0.66) and the remainder were used as available (Figure 2A). Similar results were obtained for biomass; *Barbus* was again the only preferred prey (mean D=0.34). Both centrarchids (pumpkinseed: mean D=-0.61; largemouth bass: mean *D*=-0.84) and *Squalius* (mean *D*=-0.43) were avoided fish prey (Figure 2B). Despite the clear rejection of pumpkinseed sunfish and largemouth bass, the otter preyed on all available centrarchid size classes (Figure 3).

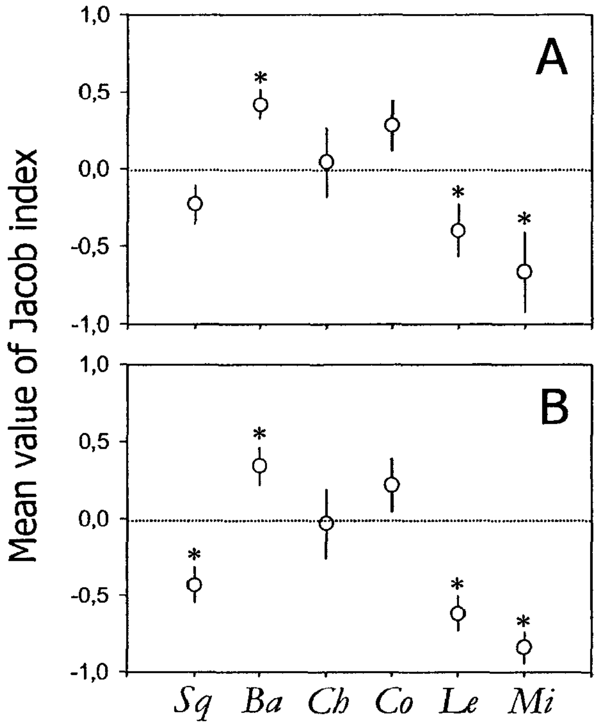

**Figure 2.** Jacobs' index for each fish prey consumed by the otter (*Lutra lutra*). Positive and negative values indicate preference and avoidance, respectively. A) prey selection on individuals, B) Idem for biomass. Sq-*Squalius* sp., Ba-*Barbus* sp., Ch-*Chondrostoma* sp., Co-*Cobitis paludica*, Le-*Lepomis gibbosus* and Mi-*Micropterus salmoides*. Bars are the standard error. * mean is significantly different from zero (t-Student, p<0.05).

Otters incorporated more *Squalius* and *Barbus* into their diet in response to an increase of these prey in the environment (Figure 4). This pattern was partially followed by *Chondrostoma* (Pearson correlation, r=0.34, p=0.08). However, there was no such change in the consumption of pumpkinseed sunfish; that is, increasing numbers of this species in the field were not reflected in consumption, as this fish was practically ignored by the otter (Figure 4). The response of the otter to the increase of largemouth bass in the field could not be adequately addressed, since the otter consumed this prey in only two locations.

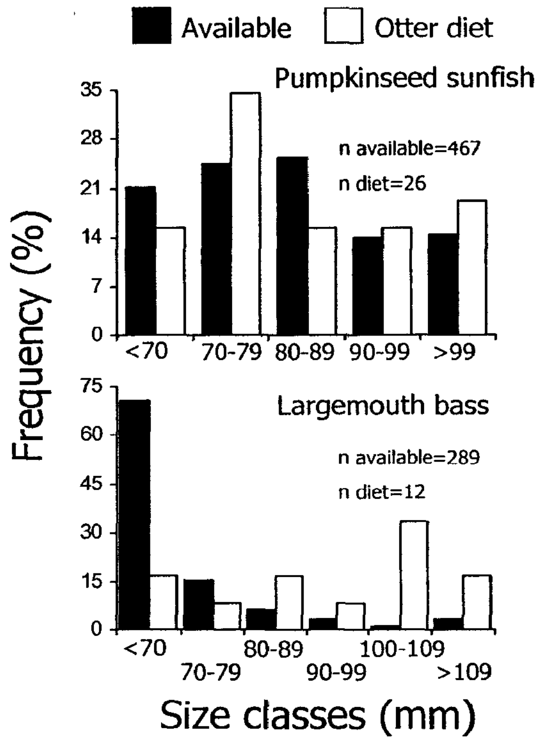

**Figure 3.** Size classes of centrarchids in the environment and consumed by the otter.

## DISCUSSION

Despite the recognized opportunistic trophic behaviour of otters, they showed preference for or avoidance of certain fish prey in the study area. Barbels (*Barbus* spp.) were preferred, accounting for the largest portion of individuals and biomass consumed by otters. Both pumpkinseed sunfish and largemouth bass were clearly rejected, appearing in a significantly lower proportion in the diet than in the environment. Other fish predators, such as the grey heron (*Ardea cinerea*), have incorporated pumpkinseed in their diet (Peris et al., 1995). However, the relative importance, in terms of biomass, of this prey is minimal compared with other fish species.

The mechanisms involved in this avoidance pattern may be related to the anti-predator body structures of the two centrarchids. It has been demonstrated that fish species with anti-predator morphological structures are usually consumed less than those that lack such structures (Beyerle, 1978; Hoyle and Keast, 1987; Eklöv and Hamrin, 1989). The pumpkinseed sunfish possesses spiny rays in both the dorsal and the anal fin. Similarly, the largemouth bass has hard rays in the dorsal fin and a strong, spiny opercle. These morphological features might impede their capture and increase the possibility of damage to the predator during ingestion. Heggberget and Moised (1994) showed similar results in a study of coastal otter prey selection. They found that, relative to their trapping frequency, prey species with hard, spiny exoskeletons and integuments were much less frequently found in otter spraints than fish with soft integuments. Moreover, otters prefer cyprinids to spiny-rayed percids (Mason ad MacDonald, 1986; Taastrøm and Jacobsen, 1999; Jacobsen, 2005). Cyprinids are also preferred over percids by other fish predators, such as pike (*Esox lucius*) (Eklöv and Hamrin, 1989). Not only fish with hard structures may be rejected by otters, but also other prey with such features. Adrian and Delibes (1987) suggested that the Spanish ribbed newt (*Pleurodeles waltl*) was not consumed by otters because of its skin glands and external sharp-pointed ribs.

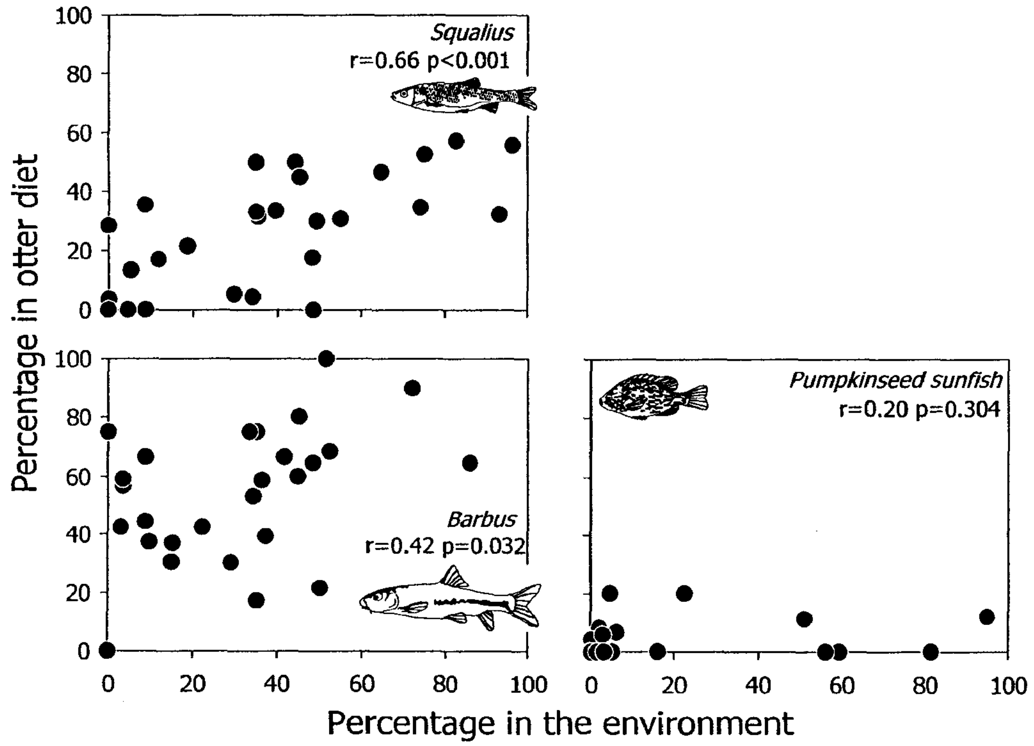

**Figure 4.** Relationship between the percentage of individuals of *Squalius* sp., *Barbus* sp., and pumpkinseed sunfish (*Lepomis gibbosus*) present in the environment and in otter diet.

Lack of acclimation of the otter to this new trophic resource may lead to its partial rejection. Indeed, though otters fed on all available centrarchid size classes, an increase of pumpkinseed in the environment was not followed by a similar increase of this centrarchid in their diet, and it was practically ignored by the otters. As in other cases, otters can show negative preferences for centrarchids if alternative prey resources are available (Erlinge, 1968). Otters showed a wide trophic spectrum in the study area, consuming at least 14 fish species, aquatic invertebrates, amphibians, reptiles, birds and small mammals (Tables 1 and 2). This great variety of alternative prey might allow otters to reject centrarchids. However, the spreading of centrarchids in the study area (Doadrio, 2001; Bernardo et al., 2003) together with the negative effect that these exotic species have on native fish fauna (Capítulo 2 y 3) might result in a noticeable reduction of alternative prey, mainly native fish species. Under this situation, otters may be forced to consume centrarchids in a higher proportion than they do at the present. Thus, what effect will the large reservoirs located in the study area, particularly the Alqueva dam, have on the otters' diet? These new lenthic habitats will drastically transform the original flowing ecosystems. This modification of the aquatic habitat can favour even further the colonization and proliferation of exotic species (Taylor et al., 2001; Clavero et al., 2004) such as centrarchids. If otters acclimate to this new possible trophic resource, their diet could vary enormously. At present, otters are not consuming centrarchids as they are available in the environment; on the contrary, otters usually reject them. From such evidence, it can be seen that otters are not acting as native controllers of exotic centrarchids.

In the Iberian Peninsula, there is a general decline of native fish fauna (Aparicio et al., 2000; Doadrio, 2001), traditionally the preferred prey of otters (Prenda, 1996), together with an increase of exotic fish species (Clavero et al., 2004). However, no attention has been paid to the potential effect that this change in fish populations could have on Iberian otters. The acclimation of Iberian otters to centrarchids does not necessarily imply the persistence of otter populations. Indeed, Kruuk and Goudswaard (1990) found that, though the related spotted-necked otter (*Lutra maculicollis*) changed its diet following the introduction of exotic fish (Nile perch, *Lates niloticus* and others) in Lake Victoria, its population declined in most of the lake's coasts. If centrarchids and other potential exotic fish prey become dominant, the maintenance of healthy populations of Iberian otters not only depends on whether these prey are consumed by the otters, but whether they remain at a sufficient density and provide similar amounts of energy as do native fish fauna.

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# CAPÍTULO 6

## SPATIAL SEGREGATION ALONG ENVIRONMENTAL GRADIENT WITHIN ENDEMIC CONGENERIC MEDITERRANEAN STREAM FISHES

*Segregación espacial entre especies congenéricas a lo largo de un gradiente ambiental en ríos mediterráneos*

# SPATIAL SEGREGATION ALONG ENVIRONMENTAL GRADIENT WITHIN ENDEMIC CONGENERIC MEDITERRANEAN STREAM FISHES

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## ABSTRACT

This study provides strong evidences on spatial segregation between the morphologically similar endemic congeneric species of *Chondrostoma* and *Squalius* genus (Cyprinidae) inhabiting the southwest of Iberian Peninsula. *Ch. lemmingii* and *S. pyrenaicus* occupied preferentially the highly variable habitats located in the headwaters, being progressively replaced by *Ch. willkommii* and *S. alburnoides* towards the relatively more stable downstream stretches. Moreover, the two *Chondrostoma* species never coexisted in completely isolated small basins; *Ch. lemmingii* appeared in the smallest catchments whereas *Ch. willkommii* inhabited the largest ones. Also, the largest *Ch. lemmingii* and *S. pyrenaicus* specimens tend to inhabit upstream, just the opposite to *Ch. willkommii* and *S. alburnoides*. It reinforces the observed preferences for upstream or downstream reaches of each pair of species, respectively. Two mechanisms acting simultaneously could explain this pattern: 1) different habitat requirements and 2) biotic interactions. The conservation of the described distribution pattern for *Chondrostoma* and *Squalius* species, and ultimately to preserve them, demands the maintenance of precise environmental conditions related to habitat gradients and absence of exotic predators, between other factors.

Key words: Mediterranean rivers; Cyprinid ecology; habitat partitioning; biotic interactions; fish distribution

## INTRODUCTION

Spatial relationships between species can be determined by biotic, abiotic or even random factors (Jackson *et al.*, 2001). It is well known that the distribution of fishes changes along stream habitat gradients (Schlosser, 1982). This variation is suggested as an adaptation to changes in habitat conditions (Schlosser, 1982; Pires et al., 1999) and frequently generates complementary distributions of fish species producing a river zonation based on fish composition along the longitudinal gradient (Gilliam et al., 1993; Winston, 1995; Taylor, 1996; Vila-Gispert et al., 2002).

The knowledge of the spatial relationships between species is of great importance from a conservation perspective, specially in those endangered or inhabiting fragile ecosystems. Mediterranean rivers are highly threatened habitats due to an intense use of water and the introduction of exotic fish species (Collares-Pereira et al., 2000; Clavero et al., 2004; Prenda et al., 2006). So a large part of their aquatic fauna and flora are consequently deeply affected by these human activities (e. g. Doadrio, 2001). Information about the distribution patterns and habitat preferences of most freshwater

species in Iberian flowing waters is almost lacking. Although this information is basic to the adequate design and implementation of management strategies to preserve species and habitats.

In the Iberian Peninsula the genus *Squalius* and *Chondrostoma* (Cyprinidae) are widespread in freshwater habitats and possess a high degree of diversification between different basins, with two species per genus within the largest basins (Zardoya and Doadrio, 1998; Doadrio, 2001; Clavero et al., 2004). Despite of the strong decline that both genus are suffering in Iberian freshwaters, essential ecological aspects useful in conservation practices are poorly addressed in the scientific literature (but see Filipe et al., 2002; Magalhães et al. 2002 and Filipe et al., 2004). The distribution patterns of these genus remain poorly unknown and most of studies made are focused on life history aspects such as growth patterns, reproductive strategies or diet (e.g. Rodriguez-Jiménez, 1987; Fernández-Delgado and Herrera, 1995; Blanco-Garrido et al., 2003; Ribeiro et al., 2003).

This work analyses the spatial relationships of two pairs of congeneric stream fish species: the genus *Chondrostoma* (the Pardilla nase *Chondrostoma lemmingii* and the Iberian nase *Ch. willkommii*), and the genus *Squalius* (the Iberian chub *Squalius pyrenaicus* and Iberian roach *S. alburnoides*) in several Mediterranean basins in SW Iberian Peninsula.

## METHODS

### Study area

The study area was located in streams and rivers belonging to Guadiana, Guadalquivir, Tinto and Odiel river basins, on the southwest of Iberian Peninsula (Figure 1). All studied basins posses similar characteristics regarding topography, as well as similar climatic and hydrologic regimes. Altitude above sea level varied between 5 and 570 m (mean= 227.8 m, S.D.= 143.9). The whole area is characterized by a typical Mediterranean climate. The mean annual rainfall is about 700 mm, falling mainly between October and March. There is a 3 to 4 months long summer drought (June-September).

The Tinto and Odiel are very special rivers. Their basins drain the Iberian Pyrite Belt, a region containing massive sulphide deposits. The mining activity, together with the natural alteration of these mineral masses, have long been a source of pollution to these rivers. The extreme water acidity (pH~2-3) and the high concentration of dissolved heavy metals (Amaral-Zettler et al., 2002) do not allow the existence of macroscopic life. Under this situation fishes remain isolated into unpolluted tributaries or sub-basins, without the possibility of interconnection between populations (Figure 1) (Clavero et al., 2004).

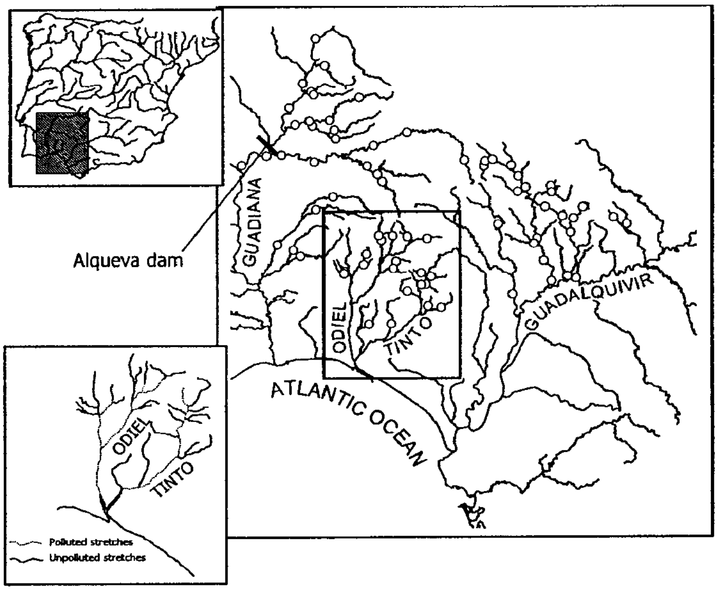


**Figure 1.** Map of the study area with indication of the location of sampling sites. The Tinto and Odiel basins are highly polluted by acid mine drainage (pH~2-3), containing a high concentration of dissolved heavy metals. Under this circumstance, fishes only survive in a few unpolluted and isolated small sub-basins.

### Fish sampling

A total of 73 stream stretches were sampled in the study area between April and July 2001 (Guadiana and Tinto basin), 2002 (Guadalquivir basin) and 2003 (Odiel basin), after the rainy season but before the summer drought, when river flows were stabilized. Although the fish sampling was done in three different years, the climatic conditions were very similar during this period (the average rainfall for the three years in five stations randomly distributed over the study area was 677, 640 and 732 mm, Kruskal-Wallis, $\chi^2$=3.75 df=2, p=0.15; and the mean temperature in the same sites for 2001, 2002 and 2003 was 17.3, 17.1 and 17.4 C, K-W, $\chi^2$=0.53 df=2, p=0.77). Fish sampling was carried out before the filling of the Alqueva dam, that impound the middle reaches of Guadiana river and it is considered the largest artificial lake in Europe (3150 Hm$^3$). Fishes were captured by electrofishing techniques, using portable or fix gears depending on the accessibility of sampling sites. Sampling was conducted once in each location running a length between 100 to 200 m. Fishing time (minutes) was noted to calculated captures per unit effort (CPUE) as nº of captures*100 m$^{-1}$*1 h$^{-1}$ fishing. CPUE was used as an index of species abundance. In 20 sites fish sampling was completed using fyke nets, which were set for at least five hours and always in pairs with different mesh sizes (15 mm and 7 mm). Between 5 and 8 pairs of nets were placed in these sites and always fishing during day time. In this case CPUE was calculated as nº of captures*1 h$^{-1}$ fishing, but it was not used in the statistical analysis.

All fishes were identified to species level, measured for total length (TL) (to the nearest mm) and then returned to the water.

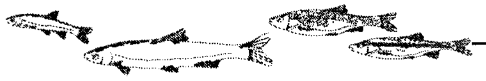
### Habitat characterisation and statistical analysis

After collecting the fishes 14 habitat variables were measured *in situ* in each of the surveyed stretches (Table 1). These variables were recorded at 5-10 points within each stretch and included physico-chemical parameters (water temperature, conductivity, dissolved oxygen and turbidity) channel structure and flow descriptors (substratum coarseness, width of water course, depth and current velocity) and riparian and aquatic vegetation cover, including fish shelter. The availability of shelter was visually estimated in each of the 5-10 points as the area (in m$^2$) occupied by the structures within the wetted channel that could serve as an effective refuge for fish (submerged roots and branches, cavities between bedrocks, ...). This estimation was made in a 4m length channel section comprising the whole channel width (Prenda *et al.*, 1997). The average values of each variable were used to summarize each site's characteristics. Visual estimations were averaged after two or three independent observers.

A physiographical and climatic description of the studied basins was made using a GIS approach (ArcView 3.2 software, ESRI, 1996). Two climatic variables, annual rainfall and evapotranspiration were obtained from Elena (1997) and Instituto do Ambiente (2005). Physiographical parameters were stream order, altitude, slope, catchment area (drainage surface of the catchment in each site) and relative position of each site within the basin (the ratio between the distance to the highest site of the stream and its total length). A site with a high relative position will be located downstream.

Exotic centrarchid species has been considered as main determinants of native fish distribution and dynamics in the study area (Collares-Pereira et al., 1999; Capítulos 2 y 3), as in other Iberian rivers. So its abundance was included as a descriptor variable in the environmental matrix.

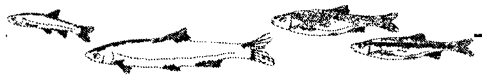
A PCA was applied to environmental variables to summarise the main sources of variation. Pearson correlation analysis between the first two PCA axes and the original variables was employed to detect significant habitat gradients. Four sets of sites (1-4) were made describing the presence/absence of congeneric species at a particular site (1: both congeneric species coexisting; 2: both congeneric species absent; 3: presence of one species and absence of its congener; 4: sites complementary to 3). To analyse possible differences in the position of the different sets (1-4) along environmental gradients, ANOVAs were then performed on PC scores. Post hoc comparison (Tukey HSD test) was applied whenever significant differences were observed. If one pair of congeneric species tend to be dissociated in the environment (i.e. they occupy different habitats), the sets 3 and 4 must distribute separately on the basis of the habitat gradient defined by PC's. On the contrary, sets 3 and 4 must show no statistical differences if the two species tend to occupy similar habitats. In contrast with other techniques that maximize differences among groups (e.g. Discriminant Analysis) the



use of PCA allows a "blind" habitat characterisation, since groups are not defined when the analysis is performed. Combinations of no congeneric species (*Chondrostoma* spp. vs. *Squalius* spp.) were also submitted to the same analysis described above to assess concordance between results. Those environmental gradients (PC's) that maximized the differences between the defined groups (1-4) were divided into four classes based on PC scores. The frequency distribution of each PC scores (sampling sites) into these

**Table 1.** Environmental gradients observed at several river basins in south-western Iberia after a PCA. r: Pearson correlation between the variables included in the PCA (for further details on methods see Clavero et al. 2005) and the two principal components. In brackets the percentage of the original variance explained by each component. * $p<0.05$; ** $p<0.01$; ***$p<0.001$.

| | r (n=73) | |
|---|---|---|
| | PC1 (30.4%) | PC2 (10.0%) |
| **Physico-chemical parameters** | | |
| Water temperature (°C; portable probe) | 0.30** | 0.39*** |
| Conductivity (µs $cm^{-1}$; portable probe) | 0.41*** | 0.04 |
| Dissolved oxygen (mg $l^{-1}$; portable probe) | 0.13 | 0.09 |
| Turbidity (FTU; portable probe) | 0.58*** | 0.15 |
| **Channel structure and flow descriptors** | | |
| Current velocity ($ms^{-1}$; floating object, 3 replicates) | 0.65*** | -0.05 |
| Depth (cm; rigid meter) | 0.68*** | -0.08 |
| Channel width (m; flexible meter) | 0.78*** | -0.38*** |
| Substratum coarseness (modified Wentworth scale) | -0.03 | -0.19 |
| Fish shelter ($m^2$ in four meters channel lenght; visual estimate, 2-3 independent observers) | 0.34** | 0.47*** |
| Submerged vegetation cover (%; visual estimate, 2-3 independent observers) | -0.22 | 0.60*** |
| Emergent vegetation cover (%; visual estimate, 2-3 independent observers) | 0.22 | 0.74*** |
| Herbaceous cover (%; visual estimate, 2-3 independent observers) | -0.04 | 0.48*** |
| Shrub cover (%; visual estimate, 2-3 independent observers) | -0.30** | 0.04 |
| Tree cover (%; visual estimate, 2-3 independent observers) | -0.22 | -0.30** |
| **Physiographical and climatic parameters** | | |
| Stream order (Strahler, 1964) | 0.84*** | -0.28* |
| Catchment area ($Km^2$) | 0.93*** | -0.15 |
| Relative position | 0.87*** | -0.10 |
| Altitude (m) | -0.42*** | -0.11 |
| Slope (‰) | -0.77*** | 0.13 |
| Evapotranspiration (mm) | -0.66*** | -0.07 |
| Annual rainfall (mm) | -0.60*** | -0.26* |
| **Biotic parameter** | | |
| Centrarchid abundance (CPUE) | 0.60*** | 0.40*** |



classes (habitat available) was compared to the frequency distribution of sites where each species was present (habitat used) using Chi-square test. This analysis allows to detect the possible preference of the species for any particular habitat class (Prenda et al., 1997; Prenda et al., 2001). Partitioned Chi-square was applied whenever significant results were obtained to identify the class or classes that account for statistical significance.

Prior to analysis, continuous variables that did not meet the normality conditions were logarithmically (base 10) transformed and variables involving proportions were arcsine transformed.

## RESULTS

Both PCs obtained after a PCA of environmental variables ordered the sampling sites along an upstream-downstream gradient (Table 1). Upstream sites were characterized by high altitude , slope, evapotranspiration, annual rainfall and shrub cover in the riparian zone. Downstream sites had opposite characteristics and in addition had larger catchment areas, higher stream order, higher relative position within the basin, larger channels with abundant fish shelter and turbid, relatively saline and warm waters inhabited by exotic invasive centrarchids (Table 1). The upstream sites, with high evapotranspiration and rainfall that determines high flow fluctuations, are considered more unstable zones compared to downstream ones (Taylor and Warren, 2001).

The distribution pattern of both components of each congeneric species pair was quite different (Figure 2). The sites exclusively used by each congeneric species (sets 3 and 4) were clearly separated along this environmental gradient (*Chondrostoma*: ANOVA for PC1 scores, $p=0.005$, Tukey HSD test, $p=0.003$, Figure 2A and 2C; *Squalius*: ANOVA for PC1 scores, $p<0.001$, Tukey HSD test $p<0.001$, Figure 2B and 2D). *Ch. lemmingii* and *S. pyrenaicus* inhabited upstream areas rejecting the lower stretches (partitioned $\chi^2$-analysis: 2 d.f. $p<0.05$, for *Ch. lemmingii* and 3 d.f. $p<0.01$, for *S. pyrenaicus*; Figure 3). Both species are longer towards upstream than downstream (Pearson correlation Total length (TL)-PC1, $r<-0.43$, $p<0.04$, $n=23$, for *Ch. lemmingii* maximum and mean TL; $r=-0.28$, $p<0.098$, $n=36$, for *S. pyrenaicus* maximum TL).

On the contrary *Ch. willkommii* and *S. alburnoides* avoided the headwaters and used mostly the middle-lower stream zones (partitioned $\chi^2$-analysis: 3 d.f. $p<0.01$, for *Ch. willkommii* and 3 d.f. $p<0.001$, for *S. alburnoides*: Figure 3), where reached a significant longer size (pearson correlation TL-PC1, $r>0.47$, $p<0.007$, $n= 32$, for *Ch. willkommii* maximum and mean TL; $r>0.47$, $p<0.006$, $n=33$, for *S. alburnoides* maximum and mean TL).

The habitat where the congeneric species coexisted (set 1) or were absent (set 2) did not differ statistically for both genus (Tukey HSD test $p>0.05$) and in average occupied the middle portion of the environmental gradient (Figure 2C and 2D). When coexisted, *S. alburnoides* was more abundant than its congener (t-test, 18 d.f., p<0.001, n=10 sites), but the abundance of the *Chondrostoma* species did not differ (t-test, 16 d.f., p=0.215; n=9).

The environmental gradient defined by PC2, mostly related to aquatic vegetation cover and fish shelter (Table 1), did not separate any group spatially (ANOVA p>0.05 for both genus).

All the possible combinations *Chondrostoma* vs. *Squalius* were performed to test concordance with the patterns obtained above for congeneric species (Table 2). The PC1 scores of the sets of sites 1-4 were compared for each species combination with an ANOVA and the results agreed with the segregation observed above within *Chondrostoma* and *Squalius* genus (Table 2). As before, in none of the cases did the PC2 separate any group spatially (ANOVA p>0.05).

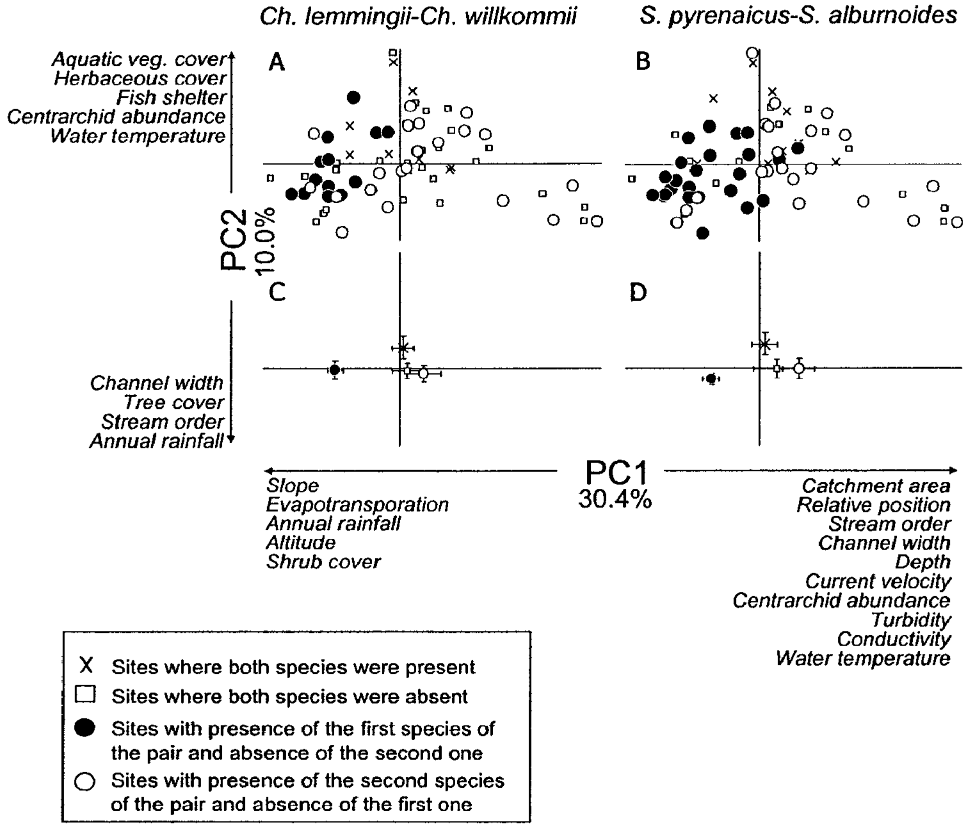


**Figure 2.** Distribution of sampled sites in the space defined by the two first principal components of a PCA of environmental variables. A) Spatial relationship between Iberian nase and Pardilla nase. B) The same for Iberian chub and Iberian roach. C) and D) represent the mean of the scores for each set of sites (see Methods for a full description) and whiskers are the standard error. The interpretation of each habitat gradient (PCs) is under arrows (see Table 1).

If the observed spatial segregation was mostly a consequence of a competitive interaction within each congeneric pair, there should be statistical differences in mean fish size for each species when in sympatry or when in allopatry. To test it, the mean total length of both *Chondrostoma* and both *Squalius* species were compared, after removing the effect of the environmental gradient (PC1) on fish size (TL). The residuals of the regression line between PC1-fish mean TL did not vary between both *Chondrostoma* species when in allopatry or when in sympatry (Table 3). Similarly *S. alburnoides* size did not vary when coexisting with its congener or when in allopatry (Table 3). In all these cases size was independent of the presence or absence of its respective congenerics and there not seemed to be a negative interaction of one congeneric over the other. On the contrary *S. pyrenaicus* were longer in sympatry with *S. alburnoides* than in allopatry (Table 3). Here, the effect was positive pointing out to another possible interaction, different from competition.

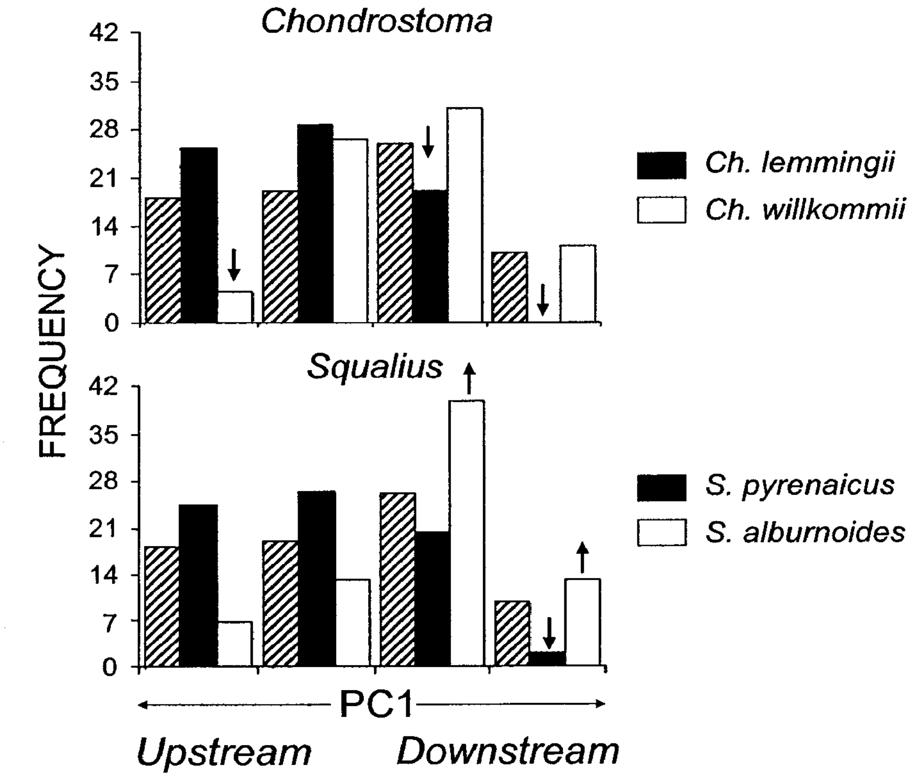


**Figure 3.** Habitat preference of *Chondrostoma* and *Squalius* along the environmental gradient defined by PC1. Stripped bars are the available habitat. The meaning of the gradient is under the arrow. Available and used habitat is compared with a $\chi^2$-analysis. The portions of the gradient preferred (up) or avoided (down) are represented by vertical arrows (partitioned $\chi^2$-analysis $p<0.05$). See methods for a full description of the analysis.

**Table 2.** Habitat segregation between all the possible combinations of *Chondrostoma* and *Squalius* species. Set 1, sites where both species coexisted; set 2, sites where both species were absent; set 3, sites where the first species of the pair was present and the second one was absent; set 4, sites where the first species of the pair was absent and the second one was present. See methods for a full description of the analysis.

| Combination | ANOVA | Greatest differences (Tukey HSD test p<0.05) | Comments |
|---|---|---|---|
| *Ch. lemmingii-S. pyrenaicus* | PC1: F=7.09 p=0.0003<br>PC2: F=1.90 p=0.138 | Sets 1 and 2 | No differences between their exclusive habitat type (sets 3 and 4). A pair of species with similar distribution patterns. |
| *Ch. willkommii-S. alburnoides* | PC1: F=4.82 p=0.004<br>PC2: F=1.38 p=0.254 | Sets 1 and 4 | No differences between their exclusive habitat type (sets 2 and 3). A pair of species with similar distribution patterns. |
| *Ch. lemmingi-S. alburnoides* | PC1: F=5.54 p=0.002<br>PC2: F=2.10 p=0.108 | Sets 3 and 4 | Their exclusive habitat types were different. Similar to the pattern displayed between *Ch. lemmingi-Ch. willkommii* or *S. pyrenaicus- S. alburnoides* |
| *Ch. willkommii-S. pyrenaicus* | PC1: F=6.04 p=0.001<br>PC2: F=0.14 p=0.931 | | |

**Table 3.** Comparison of the mean fish size of species when they appeared in sympatry or allopatry with their congeners. To remove the effect of the habitat gradient (see Table 1) on fish length, the t-test was applied to the residuals of the regression line PC1-Fish length.

| Species | Sympatry (mean±stand. deviation) | Allopatry (mean±stand. deviation) | t-test |
|---|---|---|---|
| *Ch. lemmingii* | 16.91±8.48 | 12.89±8.50 | t=1.12 df=21 p=0.27 |
| *Ch. willkommii* | 39.45±18.39 | 32.89±26.58 | t=0.70 df=30 p=0.49 |
| *S. alburnoides* | 11.07±13.05 | 9.34±7.54 | t=0.49 df=31 p=0.63 |
| ***S. pyrenaicus*** | **30.93±26.10** | **16.83±12.46** | **t=2.21 df=34 p=0.03** |

Though the probability of coexistence between *Ch. willkommi* (n=4 sites) and *Ch. lemmingii* (n=11 sites) was very high in the isolated tributaries of Tinto and Odiel rivers (Figure 1) (Monte Carlo test with 10.000 repetitions, Manly, 2001) (93%, marginal significance p=0.07), they never occurred together and displayed a typical checkerboard pattern (Diamond, 1975). *Ch. lemmingii* inhabited exclusively the smallest sub-basins while *Ch. willkommii* lived only in the largest ones.

## DISCUSSION

The results of this study demonstrate the spatial segregation between the morphologically similar congeneric species of *Chondrostoma* and *Squalius* genus inhabiting the southwest of Iberian Peninsula. No other study has been focused to test the complementary distribution within congenerics in Iberian freshwaters, yet being this phenomena a common distribution pattern among cyprinid species (e.g. Winston, 1995). The habitat configuration of sites exclusively used by each congeneric species was statistically different. *Ch. lemmingii* and *S. pyrenaicus* selected upstream sites in contrast to *Ch. willkommii* and *S. alburnoides* which inhabited middle-lower stream zones. The few sites where congeneric species coexisted presented average environmental characteristics.

These results fit well with those reported by Filipe et al. (2002), who found that both *Ch. lemmingii* and *S. pyrenaicus* occurred frequently in small streams in Guadiana basin far from the main river channel (i.e. upstream), while *Ch. willkommii* was more frequent at high order streams. Moreover, Prenda (1993) reported in another Mediterranean stream that *S. pyrenaicus* was dominant in upper stretches while *Ch. willkommii* increased downstream. These different distribution patterns can be explained by the individual requirements or preferences of each species and/or by interespecific biotic interactions (competition for food and space, agonistic behavior, reproductive parasitism and predation).

In Mediterranean streams not only a gradient in habitat conditions can be defined but also in environmental stability, both affecting fish populations at several levels. Upper zones of Mediterranean streams are subjected to a high variability in flow conditions, fluctuating between catastrophic flash floods during the wet season and almost a complete lack of flow during summer (Prenda et al., 1993; Taylor and Warren, 2001; Clavero et al., 2004). Here, as the dry season progress habitat suitability in the few extant pools deteriorates with a general decrease in water volume and quality, (an increase of hypoxia, hyperthermia and eutrophia) (Gasith and Resh, 1999). It subsequently determines an increase in fish density (Pires *et al.*, 1999; Bravo et al., 2001) which can drive a rise in competitive interactions for food and space (Cowx et al., 1984; Matthews, 1988; Blanco-Garrido et al., 2004). Also, the reduction in habitat volume makes fish more vulnerable against predators, still increasing their stress levels (Magalhães et al., 2002). These adverse environmental conditions tend to ameliorate downstream, as habitat stability increase due to a high and more stable water supply (Filipe *et al.*, 2002).

So, only fish with effective adaptations can survive under the harsh circumstances found upstream, as seem to be the case of *Ch. lemmingii* and *S. pyrenaicus*. Both species are characterized by fast growth, early maturity, high level of reproductive effort and a short life-span (Fernández and Herrera, 1995; Pires et al., 2000; Soriguer et al., 2000). Also, *Ch. lemmingii* is considered one of the most resistant Iberian fishes to low concentration of dissolved oxygen (Gómez-Caruana and Díaz-Luna, 1991). Both *Ch. lemmingii* and *S. pyrenaicus* are small-bodied species, a requirement to inhabit environments subjected to a high variability (Poff and Ward, 1989; Schlosser, 1990), and display a wide trophic spectrum (Rodríguez-Jiménez, 1987; Blanco-Garrido et al., 2004). All these life-history traits are typical of species living in unstable environments, where adult mortality is high, variable or unpredictable.

On the other hand, *Ch. willkommii* inhabiting mostly middle or downstream stretches, is a long lived species that reaches large maximum sizes, up to 350 mm total length (Prenda, 1993), displays a narrower trophic niche than its congeneric (Rodríguez-Jiménez, 1987), and spawns over large stones in high water current stream reaches. All these requirements may justify the habitat preference of this species and its almost absence from the more unstable upstreams zones.

At large basins both *Chondrostoma* are spatially segregated although can coexist in some sites (Figure 2). However a complete species segregation occur in small isolated catchments, *Ch. lemmingii* living in the smallest ones and *Ch. willkommii* in the largest, which provide more stable environmental conditions and/or enough refuge to survive during harsh periods. Two mechanisms acting simultaneously could explain this pattern: 1) different habitat requirements (*Ch. lemmingii* preferring unstable/smallest isolated basins and *Ch. willkommii* more stable water supply/largest isolated basins) and 2) biotic interactions (*Ch. willkommii* outcompete *Ch. lemmingii* via agonistic behaviour and diet overlap) (Rodriguez-Jiménez, 1987; Robalo et al., 2003; pers. obs.). It produces a typical checkboard distribution pattern within *Chondrostoma*.

The size distribution of *Chondrostoma* along the environmental gradient reinforces its observed preference for upstream reaches and/or unstable basins. The largest *Ch. lemmingii* specimens tend to inhabit upstream. It is just the contrary for *Ch. willkommii*. But the mean fish size observed in allopatry or sympatry do not support the biotic interaction hypothesis. Although coexisting congenerics can interact negatively, it do not seem to affect the fish length.

*S. alburnoides*, although being also a typical r-selected species as *S. pyrenaicus* (small-bodied species, multiple spawner, displaying early maturity and possessing a sex-ratio strongly deviated to females) (Ribeiro et al., 2003), inhabits preferentially the middle-lower stretches where coexists with *Ch. Willkommii*. The size of the Iberian roach also increases dowstream. This habitat partitioning between both *Squalius* -apart from historical, ecological and adaptative reasons (Gorman, 1992; Winston, 1995)- could also be explained by a differential sensibility to exotic centrarchids by each congeneric. *S. alburnoides* seemed to tolerate exotic centrarchids but *S. pyrenaicus* did not. Centrarchids abundance increased in an upstream-downstream sense (see Table 1 and Figure 2), coinciding with the distribution of *S. alburnoides*, while *S. pyrenaicus* inhabited sites lacking centrarchids or sites where they were very scarce.

The higher abundance of *S. alburnoides* than *S. pyrenaicus* when coexisting could be a consequence of the highly peculiar reproductive mode displayed by *S. alburnoides*. This species uses hybridogenesis with *S. pyrenaicus* as the main reproductive mode (Alves et al., 1997; Carmona et al., 1997). Most individuals of *S. alburnoides* have an hybrid origin being *S. pyrenaicus* one of its parental species (see Alves et al., 1998 for a major description). This may produce a "reproductive parasitism" of *S. alburnoides* females over *S. pyrenaicus* males, whose main effect can be the dilution of the latter species population on the former one and subsequently the dominance in numerical terms (CPUE) of *S. alburnoides* over *S. pyrenaicus*. In agreement with this hypothesis *S. pyrenaicus* tend to be larger when in sympatry with its congener than when in allopatry. The consequences of this reproductive parasitism on fish mean size should be as follows: *S. pyrenaicus* when alone reproduce exclusively intraspecifically. However, if *S. alburnoides* is present, at least part of the *S. pyrenaicus* male population reproduce with its congeneric and produce new *S. alburnoides*, but not young *S. pyrenaicus* increasing subsequently its average size.

The conservation of the described distribution pattern for *Chondrostoma* and *Squalius* species, and ultimately to preserve them, demands the maintenance of precise environmental conditions related to habitat gradients and absence of exotic predators, between other factors. It implies specifically: 1) to keep the natural flow patterns and stability gradients along the watercourses, which guarantee the long term coexistence of different congeneric species as those studied here. 2) To maintain the natural conectivity within basins. On the contrary, an extensive fragmentation (i.e. due to dam building) can drive to some species to local extinctions, as apparently occurred with the *Chondrostoma* genus in the fragmented Tinto and Odiel basins. 3) To minimize reservoir construction to avoid the establishment of exotic fish, specially centrarchids, which cause the extinction or depletion of the native fish fauna and modify its natural distribution patterns.

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Universidad de Huelva

Reunido el Tribunal integrado por los abajo firmantes en el día de la fecha, para Juzgar la Tesis Doctoral de D. / Dª. Francisco Blanco Garrido

Titulada Ecología, distribución y conservación de peces continentales en el cuadrante suroccidental ibérico

acordó otorgarle la calificación de Sobresaliente cum laude

Huelva, 27 de octubre de 2006

El Vocal

El Vocal

El Vocal

El Presidente

El Secretario

El Doctorando

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