Universidad de Huelva

Departamento de Biología Ambiental y Salud Pública

Ríos mediterráneos, aproximación a la evaluación del estado ecológico y planificación para la conservación a través de los peces

Memoria para optar al grado de doctor
presentada por:

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RÍOS MEDITERRÁNEOS, APROXIMACIÓN A LA EVALUACIÓN DEL ESTADO ECOLÓGICO Y PLANIFICACIÓN PARA LA CONSERVACIÓN A TRAVÉS DE LOS PECES

Memoria presentada por el Licenciado Virgilio Hermoso López para optar al grado de doctor por la Universidad de Huelva

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LICENCIADO

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Paco, has supuesto un pilar fundamental durante todo este tiempo. He estado empapándome de tu conocimiento e interés por los ríos y sus habitantes, y el resto del mundo natural en general. Hemos compartido muchas horas de campo y, más días y meses aun, de sufrimiento en nuestro "zulo". Dificilmente se pueden olvidar todos esos buenos ratos y desde luego es con lo que me quedo.

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(...) I heard the sound of a thunder, it roared out a warning,
Heard the roar of a wave that could drown the whole world,
Heard one hundred drummers whose hands were blazing,
Heard ten thousand whispering and nobody listening,
Heard one person starve, I heard many people laughing (...)
(...) I'll walk to the depths of the deepest black forest,
Where the people are many and their hands are all empty,
Where the pellets of poison are flooding their waters,
Where the home in the valley meets the damp dirty prison.

(*A hard rain´s a-Gonna fall*, Bob Dylan, 1963)

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INTRODUCCIÓN Y SÍNTESIS GENERAL

INTRODUCCIÓN

El gran problema

Los ecosistemas acuáticos continentales se encuentran entre los medios más ricos en especies y diversos de todo el planeta (Revenga y Mock, 2000). La biodiversidad que atesoran es enorme si tenemos en cuenta el escaso espacio en el que se desarrollan. A los ecosistemas acuáticos les corresponde apenas el 0,8% de la superficie del planeta y contienen el 2,4% de todas las especies conocidas. Mientras que para los ecosistemas terrestres o los marinos estas cifras son del 28,4% de superficie y 77,5% de especies para los continentes y del 70,8% de superficie y el 14,7% de especies para los mares y océanos (McAllister et al. 1997). Como ejemplo tan solo los peces de agua dulce suponen un cuarto de la cuota total de biodiversidad de vertebrados del planeta (Abell, 2002). Sin embargo también son uno de los ecosistemas más gravemente amenazados como consecuencia del uso intensivo que el hombre hace del imprescindible recurso agua (Malmqvist y Rundle, 2002). Entre otras causas de perturbación han sido destacadas 1) la degradación y fragmentación del hábitat, 2) el deterioro de la calidad del agua (contaminación y eutrofización), 3) la sobreexplotación del recurso mediante embalsado y extracción y 4) la introducción de especies exóticas (Allan y Flecker, 1993; Collares-Pereira y Cowx, 2004; Prenda et al., 2006).

Esta grave situación se ve reflejada en el preocupante estado de conservación de las comunidades biológicas, puesto que un 30% de las especies de peces de agua dulce está incluido en alguna de las categorías de amenaza de la UICN (World Conservation Union, 2000).

En particular el problema se ve agravado en medios áridos y semiáridos, como pueden ser los Mediterráneos, por la mayor demanda de agua que se requiere para el consumo, la agricultura, la industria y la obtención de energía (Collares-Pereira & Cowx, 2004). En el contexto Mediterráneo, el 56% de las especies de peces dulceacuícolas de distribución exclusivamente mediterránea, se encuentra amenazado (18% En Peligro Crítico, 18% En Peligro y el 20% Vulnerables), mientras que tan sólo el 21% están consideradas como de Menor Interés por parte de la UICN (Smith and Darwall, 2006). Este hecho es especialmente remarcable dado el elevado grado de endemicidad de estas cuencas.

A pesar del gran valor que se les supone por todo lo comentado hasta el momento, los ecosistemas acuáticos continentales no han recibido la atención que se merecen. En una revisión realizada por Abell (2002) sobre el total de artículos científicos publicados en *Conservation Biology*, una de las revistas internacionales más reconocidas en el ámbito conservacionista, durante el periodo 1997-2001, tan sólo el 4% estaba directamente enfocado a especies y hábitats de aguas continentales. Sin duda, existen otras revistas específicamente dedicadas al estudio de estos medios, pero quizás el dato sí pueda ser indicador de la insuficiente atención dedicada a ellos en un contexto global.

Camino a una solución

Existe por tanto una urgente necesidad de atención hacia los ecosistemas acuáticos continentales para evaluar su estado de conservación, y en qué medida y magnitud se están viendo afectados por las perturbaciones (Revenga and Kura, 2003). Como respuesta a esta necesidad han surgido diversas normativas internacionales como *The Clean Water Act* en Estados Unidos o la Directiva Marco del Agua (DMA) en Europa (European Council, 2000). Ambas tratan de afrontar el problema del mal estado de conservación de estos medios, ampliando las exigencias relativas a los estándares de calidad en el ámbito biológico. La DMA garantiza la aplicación de principios de protección y mejora de la calidad de los ecosistemas acuáticos mediante el desarrollo de programas de seguimiento en los que deben ser tenidos en cuenta especialmente sus componentes biológicos. En definitiva, se alienta el uso de herramientas basadas en bioindicadores como medio para evaluar el estado de conservación y vía fundamental para la mejora de su estado. Este enfoque supone un importante avance en materia de evaluación de la calidad de los ecosistemas acuáticos continentales, puesto que hasta el momento ésta estaba siendo caracterizada casi en exclusiva a través del uso de indicadores de calidad química.

Cinco grandes grupos taxonómicos son propuestos por la DMA como elementos de calidad biológica. Entre ellos se encuentran los peces, que deben ser utilizados tanto para evaluar el estado de conservación del medio, como para diagnosticar al agente o los agentes causales del posible deterioro encontrado. Además, deben servir de guía para la puesta en marcha de medidas correctoras eficientes. De esta forma todos los ríos europeos deberían estar en Buen Estado Ecológico antes de 2015, garantizando la mejora en general de estos medios.

Sin embargo, además de todo lo anterior, parece necesario otro tipo de actuaciones que afianzen la conservación de la biodiversidad acuática. Por ejemplo, la identificación de áreas prioritarias donde centrar los esfuerzos conservacionistas o el diseño de reservas que garanticen el objetivo final de la protección del conjunto de la biodiversidad. En este sentido destaca el desarrollo en las últimas décadas de la planificación sistemática para la conservación (Margules y Pressey, 2000). Una vez que se ha establecido una reserva, ésta debe garantizar a largo plazo la biodiversidad que contiene a través de 1) el mantenimiento de sus procesos naturales, y 2) la mitigación de los efectos de las principales amenazas sobre ella (Margules et al., 2002; Wilson et al., 2005). El diseño de espacios protegidos ha conllevado tradicionalmente, bien el uso de criterios de expertos sobre el valor de la biodiversidad de cada zona y su importancia para la conservación, o el uso de criterios totalmente ajenos a la conservación. Entre estos últimos podemos citar algunos comúnmente utilizados como pueden ser valores paisajísticos, grado de naturalidad, inaccesibilidad, bajo potencial para producción económica o simple disponibilidad (Margules et al., 1988; Pressey et al., 1996; Sarkar, 1999). Este tipo de aproximaciones conduce a estrategias de conservación *ad hoc* centradas en las áreas más fáciles de proteger y con menos necesidades a corto plazo (Pressey, 1994; Knight, 1999; Pressey et al., 2000). Para encarar de forma más eficiente la protección de la biodiversidad y lograr una puesta en práctica efectiva de los escasos recursos destinados a estas tareas (Knight et al., 2007) existe lo que se denomina *planificación sistemática para la conservación*. Mediante ella se pretende diseñar redes de espacios protegidos atendiendo a criterios básicos que garanticen la persistencia de todas las especies y resuelvan los problemas comentados de limitada disponibilidad de recursos.

En el ámbito de los ecosistemas fluviales, son pocas las reservas declaradas explícitamente para la conservación de la biodiversidad acuática (Saunders et al., 2002). Los ríos han sido tratados tradicionalmente de forma lateral en cuestiones de conservación, a menos que supusieran una oportunidad importante para alcanzar objetivos conservacionistas en medios terrestres (Nel et al., 2007). De hecho, la mayoría de los tramos de fluviales incluidos en alguna reserva son usados como meros límites administrativos, y de ninguna forma son suficientes para garantizar de forma efectiva la conservación de la biodiversidad acuática. No obstante, en los últimos años han surgido estudios que encaran específicamente cuestiones sobre la planificación sistemática para la conservación en estos ecosistemas de aguas corrientes (Nel et al., 2007; Linke et al., 2007). En ellos se aplican los principios desarrollados para los ecosistemas terrestres, pero haciendo especial hincapié en la necesidad de tener en cuenta sus características singulares respecto a los terrestres (Dunn, 2003). Los trabajos sobre ríos deben tener en consideración, sobre todo, la conectividad natural de estos medios, factor clave definitorio de su estructura y funcionamiento. Además, ello supone un reto importante para solucionar problemas tradicionalmente relacionados con los efectos acumulados de las perturbaciones que amenazan gravemente la conservación de su biodiversidad localizadas, no en el tramo protegido, sino aguas arriba o abajo, (Pringle, 2001). Sin embargo, la conectividad y continuidad no han recibido la atención que requieren en los trabajos previos relativos a la conservación de medios fluviales (Pringle, 2001).

El uso de los peces como bioindicadores

Al igual que en el resto de grupos taxonómicos, la estructura de las comunidades de peces está controlada por diversos factores bióticos y abióticos, como el régimen de caudales, la calidad del agua, la estructura del hábitat o las interacciones bióticas (Hynes, 1970). Sin embargo, los peces poseen una serie de características que los hacen especialmente interesantes para su uso como indicadores de calidad. En primer lugar ocupan posiciones altas en las redes tróficas, lo que los hace buenos indicadores de los procesos ecológicos globales (Hynes, 1970). Debido a sus amplios dominios vitales, desde el punto de vista del espacio (la escala espacial a la que desarrollan sus ciclos vitales es considerablemente más amplia que la del resto de componentes de la biocenosis acuática) y el tiempo (los ciclos vitales también son amplios) son muy buenos integradores de la calidad a ambas escalas (Harris, 1995). Además, las especies de peces son más fáciles de identificar en el campo que las de macroinvertebrados o microalgas bentónicas, por ejemplo.

Sin embargo las comunidades de peces de ríos mediterráneos poseen características diferenciales de las de ambientes más templados que podrían dificultar su uso como bioindicadoras. Los peces continentales mediterráneos han evolucionado en ambientes altamente variables, en los que han de responder a eventos dramáticos como pueden ser sequías extremas o avenidas periódicas. Para ello han desarrollado estrategias de vida caracterizadas por ciclos de vida relativamente cortos, hábitos ecológicos generalistas y oportunistas y madurez sexual temprana (e. g. Velasco et al., 1990; Vila-Gispert y Moreno-Amich, 2002). El efecto de la historia evolutiva de estas especies sobre su sensibilidad o capacidad de respuesta ante perturbaciones no ha sido evaluado hasta el momento en peces mediterráneos por lo que poco o nada es sabido al respecto. En su defecto se está utilizando el criterio de experto, con las limitaciones e incorrecciones que ello de hecho implica.

Evolución del concepto de Índice de Integridad Biótica (IIB)

El uso de los peces como indicadores de calidad se remonta hasta hace más de un siglo (Simon, 1999), aunque fue James Karr quien en 1981 por primera vez ideó un índice de calidad basado en los peces para medir el estado ecológico de los ecosistemas fluviales de ríos templados de Estados Unidos (Karr, 1981). Se trataba de un índice que incluía un total de doce métricas o variables elaboradas a partir de inventarios piscícolas no redundantes y sensibles a la perturbación. Estas métricas recogían diferentes aspectos sobre la estructura y funcionamiento de las comunidades estudiadas incluidas en tres grandes bloques: (i) Riqueza y composición de especies, (ii) Composición trófica y (iii) Abundancia y condición de los ejemplares. Con ellas se pretendía evaluar diferentes aspectos definitorios de una comunidad de peces en buen estado ecológico o con buena Integridad Biótica. Es por ello que este índice se denominó Índice de Integridad Biótica (IIB). La Integridad Biótica se define como "*la capacidad de un ecosistema para mantener una comunidad de organismos equilibrada, integrada y adaptativa teniendo una composición de especies, diversidad y organización funcional similar a aquella que pudiéramos encontrar en una zona natural de características similares*" (Karr and Dudley, 1981). Se trata en definitiva de la capacidad de un ecosistema para mantener una comunidad no perturbada por actividades antrópicas. En esta definición se vislumbraba además el concepto de *Condición de Referencia* (Hughes et al., 1986; Reynoldson, 1997, Bailey et al., 1998) ampliamente utilizado hoy en día en el campo de la evaluación del estado ecológico. Según este concepto, para evaluar objetivamente el estado de ecológico de un sistema se debe comparar la situación actual observada en él con la existente en un sistema de características similares, libre de cualquier tipo de perturbación, que sería la situación de referencia. En el caso del IIB esto se hacía a través de una línea de máxima riqueza, que representa el máximo valor de cada métrica a alcanzar por un sistema en ausencia de perturbaciones y teniendo en cuenta la variación natural de las mismas. Finalmente el área bajo esta línea se dividía en tres porciones equivalentes para puntuar cada una de las métricas (5, 3 y 1 en función del grado de desviación respecto a la línea de máxima riqueza) y sumar en un único valor las evaluaciones parciales.

El IIB de Karr fue adaptado a zonas muy diferentes de todo el planeta (Roset et al., 2007) e incluso se aplicó a ecosistemas terrestres (O´Connell et al., 1998), lo que demuestra la flexibilidad del método. En Europa existen numerosas aplicaciones siguiendo estrictamente las pautas marcadas en el original (Didier y Kestemont, 1996; Belliard et al., 1999; Chovanec et al., 2000; Belpaire et al., 2000; Kesminas y Virbickas, 2000), aunque en los últimos años han surgido nuevas aproximaciones que introducen algunas modificaciones.

En el contexto europeo, actualmente se pueden distinguir dos grandes tendencias, diferenciadas básicamente por la forma utilizada para identificar las condiciones de referencia propuestas por la DMA. Schmutz et al. (2007) siguen una aproximación tipológica para el establecimiento de estas condiciones de referencia, en la que se parte de una clasificación previa, basada en la composición taxonómica. Según ella, se identifican grupos biológicamente homogéneos a los que posteriormente se asigna sentido ambiental, para los que se escogen aquellas variables ambientales que mejor diferencian entre grupos. Posteriormente las condiciones de referencia son especificadas para cada uno de los tipos definidos, seleccionando aquellos lugares dentro de cada tipo que muestran una total ausencia de perturbaciones antrópicas o un mínimo impacto humano. Un grave problema que presenta esta aproximación es la falta absoluta de condiciones de referencia para alguno de los tipos, por ejemplo el de los tramos bajos fluviales.

En contraposición, Oberdorff et al. (2002) y más recientemente Pont et al. (2007) evitan el uso de clasificaciones y aplican modelos predictivos para el establecimiento de las condiciones de referencia específicas para cada nuevo sitio a evaluar. Basados en la relación existente entre las características del hábitat y las comunidades de peces en sitios mínimamente perturbados se elaboran modelos capaces de predecir el valor de cada una de las métricas (Pont et al., 2007), o la presencia-ausencia y abundancia de cada especie (en Oberdorff et al., 2002).

Esta última alternativa fue la finalmente elegida durante el desarrollo del proyecto europeo FAME (FAME, 2004) para la elaboración del Índice de Peces Europeo (EFI) (Pont et al., 2007).

En ambos casos, tal y como establece la DMA, la desviación entre el valor de las métricas observadas para un determinado sitio y las esperadas en condiciones de referencia es utilizada como indicador del grado de perturbación.

Existen otras muchas aproximaciones no basadas en métodos multimétricos para la evaluación del estado ecológico de los ríos. Entre ellas destaca por su amplia aplicación a escala mundial el método RIVPACS (también denominado AUSRIVAS por Simpson y Norris -2000- en Australia o MEDPACS por Poquet et al. -2006- en el ámbito mediterráneo). Se trata de un método basado en tipos que utiliza directamente comparaciones entre los valores de riqueza taxonómica observados y esperados como medida del grado de perturbación.

La situación ibérica

En el caso particular de los ríos mediterráneos, la adaptación de este tipo de herramientas se ve dificultada además por las especiales características ecológicas de sus comunidades de peces (Moyle and Randall, 1998; Moyle and Marchetti, 1999), como ya se ha comentado. Al igual que en otros ambientes similares, las comunidades de peces mediterráneos poseen una baja riqueza específica por sitio, un elevado número de endemismos por cuenca y una gran variabilidad especio-temporal. Puesto que el EFI no es aplicable a los ríos mediterráneos, ya que no pudo ser validado para estos medios (Pont et al., 2007), son necesarios esfuerzos adicionales para el desarrollo de índices de calidad adaptados a las condiciones de estos ríos, y útiles para la DMA.

Existen trabajos previos para evaluar el estado ecológico de ríos ibéricos por medio de índices basados en peces, aunque todos centrados en tipologías (Ferreira et al., 1996; Sostoa et al., 2004; Ferreira et al., 2007). Un problema asociado a este tipo de aproximaciones es que tan sólo pueden ser aplicadas a aquellos tipos de ríos para los que han sido creados, lo que unido a lo reducido del área de estudio en la que han sido desarrollados los hacen poco o nada extrapolables al resto del territorio. Queda, por tanto, aun una ardua tarea por delante hasta desarrollar un índice aplicable, idealmente, a todos los ríos del ámbito mediterráneo. Siguiendo las dos tendencias básicas utilizadas en Europa podrían ser evaluadas dos alternativas diferentes: 1) el desarrollo de un índice basado en tipos para todo el territorio ibérico, que cubra la enorme diversidad ambiental mediterránea, ó 2) desarrollo de un índice basado en medidas especificas para cada sitio como en EFI. En el presente trabajo se han centrado los esfuerzos en la segunda alternativa, dada la incapacidad actual para disponer de una base de datos tan extensa como para cubrir todos los posibles tipos mediterráneos y la excelente respuesta que ha demostrado en el resto de Europa el índice especifico para cada sitio.

El marco territorial: la cuenca del río Guadiana

Dadas la especial naturaleza de los ríos y de las comunidades que albergan, estrechamente asociadas a los límites de los cursos de agua, cada cuenca de drenaje se comporta como una isla independiente de las cuencas vecinas. Este hecho es especialmente remarcable en el caso de los peces que no poseen formas dispersivas aéreas como es el caso de otros grupos taxonómicos. Es por ello que se han escogido los límites naturales de una cuenca como área de estudio, en concreto la del río Guadiana.

La del Guadiana se trata en todos los sentidos de una cuenca muy peculiar. En palabras de Hernández-Pacheco (1928) el Guadiana es "*el río más singular, extraño y anómalo de todos los hispanos. Contrariamente a todos los demás, no se origina entre montañas ni en serranías, sino en la llanura más extensa, plana y sin pendientes que existe en la Península: en la llanura de la Mancha. Los diversos segmentos que lo componen no corresponden por sus características, a los tres normales de la generalidad de los cursos fluviales; presentando cada tramo de los que se reconocen en el Guadiana, morfología en la cuenca y en el cauce, y variación en el caudal, muy diferentes de unos a otros.* Estas singularidades se verán posteriormente reflejadas en los análisis de gradientes espaciales.

La cuenca del río Guadiana destaca en el ámbito circunmediterráneo por la importancia de su ictiofauna, tanto por su elevada riqueza específica, sólo comparable a la de otras dos cuencas (río Po en el Norte de Italia y río Orontes en el Sur de Turquía, según Smith y Darwall, 2006), como por el grado de amenaza al que están sometidas sus especies (el 90% está incluida en alguna categoría de la UICN). Esto convierte a la cuenca del río Guadiana en un centro de especial atención para la conservación de la biodiversidad acuática dentro del contexto mediterráneo.

Las características especiales de la comunidad de peces de la cuenca del Río Guadiana lo convierten en un interesante objeto de estudio, no solo por las oportunidades que ofrece, sino también por el interés aplicado que del presente trabajo se pudieran derivar, especialmente aquellos que puedan servir para mejorar el estado de conservación de estos organismos y del hábitat que los acoge. La comunidad de peces que alberga esta cuenca destaca en

OBJETIVOS

El presente trabajo persiguió dos objetivos principales, cada uno de los cuales con una serie de objetivos secundarios imprescindibles para alcanzar los primeros.

1. DESARROLLO DE UN ÍNDICE DE CALIDAD BASADO EN LOS PECES PARA EVALUAR EL ESTADO ECOLÓGICO DE LOS RÍOS MEDITERRÁNEOS.

Antes de la elaboración final del índice se afrontó una serie de tareas básicas que permitiera obtener toda la información necesaria para decidir qué clase de índice iba a ser desarrollado, si basado en tipos ambientales (*type-specific*) o alternativamente en modelos predictivos para cada sitio (*site-specific*). A continuación se expone la secuencia ordenada de objetivos secundarios necesarios para alcanzar el general del epígrafe:

1.1. Construcción de modelos predictivos, basados en la presencia-ausencia de las especies nativas, que permitieran afrontar el resto de objetivos.

1.2. Posteriormente, y puesto que los trabajos previos realizados en la Península Ibérica siguiendo las recomendaciones de la DMA han hecho uso de índices basados en tipologías, se evaluó específicamente la eficacia y sensibilidad de este tipo de índices.

1.3. Dado el enorme vacío existente en el conocimiento de la sensibilidad de las especies nativas a diferentes tipos de perturbaciones antrópicas, a continuación se afrontó su estudio. Con la obtención de valoraciones objetivas se trató de evitar la utilización del juicio de experto común en trabajos previos.

1.4. Haciendo uso de toda la información obtenida con los objetivos anteriores, finalmente se diseñó, calibró y validó un índice de calidad sensible a diferentes tipos de perturbación antrópica. En todo el proceso se prestó especial atención a cuestiones básicas como la consideración del efecto de los cambios naturales, tanto en la evaluación de las sensibilidades como en el desarrollo del índice.

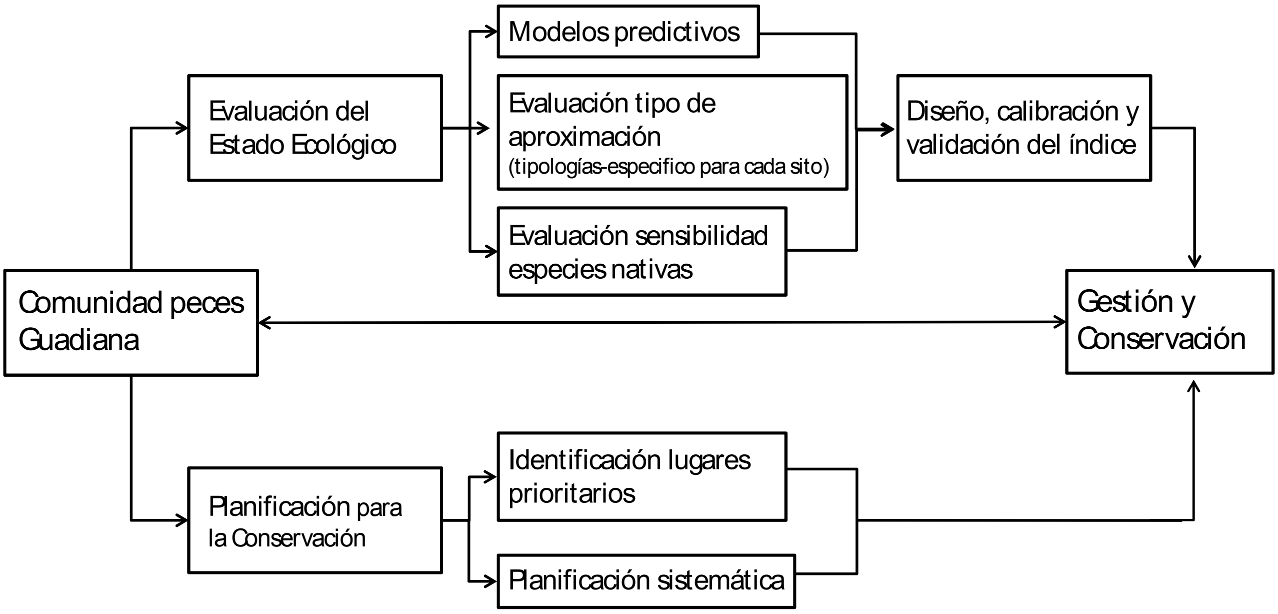

Figura 1. Esquema de los objetivos perseguidos en el presente trabajo.

2. PROPUESTA DE PLANIFICACIÓN PARA LA CONSERVACIÓN DE LA BIODIVERSIDAD DE LAS COMUNIDADES DE PECES DE AGUA DULCE DE LA CUENCA DEL RÍO GUADIANA.

2.1. En una primera aproximación se pretendió identificar aquellas áreas dentro de la cuenca del Guadiana con una biodiversidad más singular y en mejor estado, y seguidamente se planteó el diseño de una red de espacios a proteger utilizando los principios básicos de la planificación sistemática.

2.2. Puesto que este campo ha sido especialmente desarrollado para ecosistemas terrestres, fue preciso adaptar esta herramienta ampliamente utilizada en estas tareas a las características especiales de los ecosistemas acuáticos continentales.

ÁREA DE ESTUDIO

La cuenca del Río Guadiana, con un área total de drenaje de 67.039 Km^2, se localiza en el Suroeste de la península Ibérica y desemboca en el Océano Atlántico. Se trata de una cuenca compartida entre España (81,8%) y Portugal (17,2%), de características claramente mediterráneas desde el punto de vista climático, con una elevada variación intra e interanual en las precipitaciones (Gasith and Resh, 1999). Estas precipitaciones oscilan entre los 350-1200 mm/año, con una media para toda la cuenca de 450 mm/año. La temperatura media anual del aire ronda entre 13.0-18.1 ºC con una fuerte oscilación a lo largo del ciclo anual que en algunos casos supera ampliamente los 40ºC.

A pesar de no ser una cuenca demasiado poblada (28 hab/km^2, cuando en 2006 la media española era de 88,6 hab/km^2) si ha sufrido una profunda transformación durante el último siglo especialmente debido a actividades agrícolas. Casi la mitad de la cuenca (49,1%) se encuentra actualmente ocupada por algún tipo de cultivo, bien intensivo, bien extensivo (30,6% y 18,5% respectivamente). Esta actividad es altamente demandante de agua y por ello existen en la cuenca un total de 86 embalses grandes-medianos (>1 hm^3) y más de 200 pequeños embalses (<1 hm^3) que acumulan más de 13.000 hm^3. Esta capacidad supera con creces la aportación media anual estimada en algo más de 6.000 hm^3, según el Ministerio de Medio Ambiente. Además existen censados casi 61.000 pozos, existiendo un número indeterminado de puntos de extracción ilegal. Otras alteraciones comunes relacionadas con la intensa actividad del hombre son las modificaciones y canalizaciones de los tramos naturales, especialmente en el tramo alto de la cuenca, deterioro o eliminación del bosque de ribera y contaminación por vertidos difusos y puntuales.

En la porción española del Guadiana hay declarados dos Parques Nacionales, tres Parques Naturales y ocho Reservas Naturales, además de 53 Zonas de Especial Protección para las Aves (ZEPAS). En total suponen alrededor de 3.150 km^2 (5,2% de la cuenca), aunque está previsto aumentar la protección legal hasta el 14,7% a través de los numerosos Lugares de Importancia Comunitaria (LICs) propuestos dentro de la red de espacios naturales protegidos Natura 2000.

La ictiofauna de la cuenca estuvo compuesta por un total de 28 especies de las que 16 (57%) fueron nativas y 12 (43%) exóticas. Dentro del grupo de éstas últimas se ha incluido a *Salmo trutta*, que aunque existen referencias históricas de su presencia en la cuenca, en la actualidad se trata de poblaciones reintroducidas en cotos de pesca. El listado completo de las especies y sus áreas de distribución se encuentran en los Anexos I y II. Estos datos pertenecen al inventario realizado en 241 localidades en ríos y 62 localidades en 37 embalses y lagunas (Fig. 2).

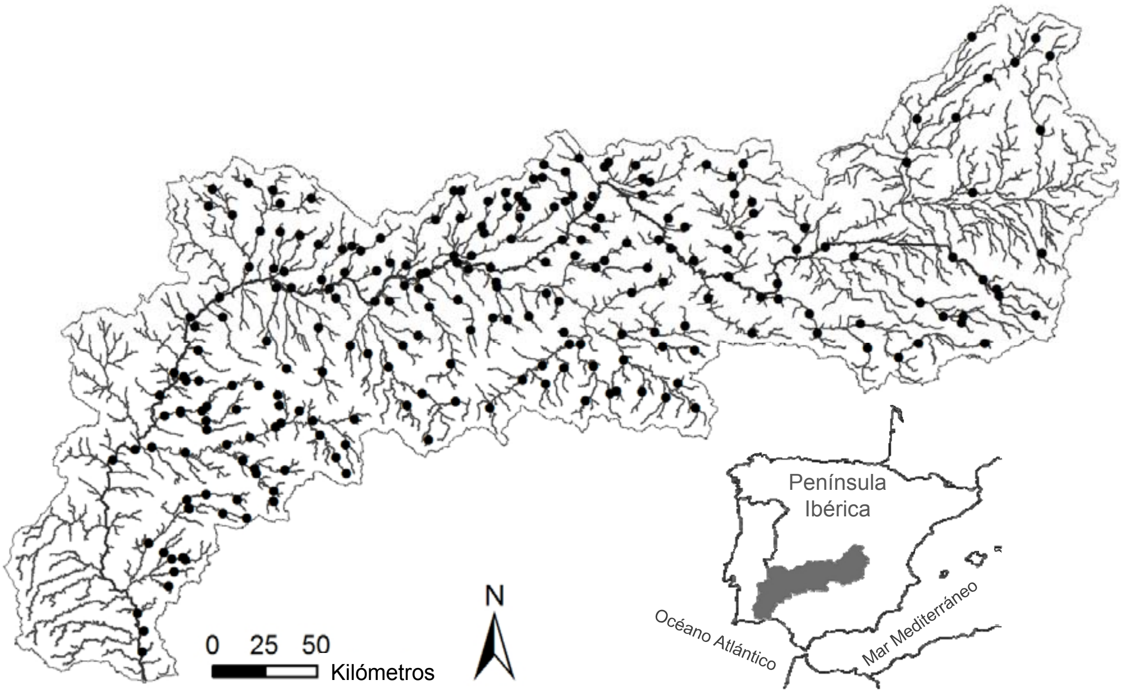

Figura 2. Área de estudio con indicación de las 241 localidades de ríos muestreadas. Puesto que los inventarios en embalses sólo han sido utilizados para completar la distribución de las especies en la cuenca (Anexos I y II) no son mostradas.

Caracterización del hábitat

El hábitat fue caracterizado en cada una de las localidades a través de 40 variables medidas *in situ* o a través de un Sistema de Información Geográfica (SIG) desde cartografía digital. Con ellas se caracterizó el hábitat a tres escalas diferentes: sitio, tramo y cuenca. Para el tramo se utilizó un buffer de 500 m alrededor del punto de muestreo (Tabla 1).

Identificación de localidades de referencia

Para el desarrollo de los trabajos posteriores fueron definidos dos grupos de localidades: de referencia y test. Sólo fueron consideradas localidades de referencia aquellas que mostraron impactos muy bajos en un conjunto de variables independientes utilizadas para caracterizar el estado general de perturbación. Se tomaron 6 de estas variables (distancia a embalses aguas abajo, grado de naturalidad del canal fluvial, alteración de la continuidad del ecosistema fluvial, calidad del bosque de ribera y usos del suelo en la cuenca en un tramo de 500m alrededor de la localidad) y se puntuaron de 1-5 (1=nada perturbado, 5=muy impactado). Los valores de cada

Tabla 1. Conjunto de variables ambientales caracterizadas en cada localidad de muestreo. Se muestran los valores medios y el rango de cada una de ellas. * Indica aquellas variables potencialmente afectadas por la acción del hombre.

Escala	Variable	Método	Código	Media	Rango
Sitio	Profundidad agua (cm)	In situ	PRO	42,8	7,0-200,0
	Disponibilidad de refugio (m^2 de refugio/anchura río)	In situ	REF	5,6	0,0-60,6
	Altura (m)	SIG	ALT	384,1	7,1-974,9
	Posición relativa (distancia a cabecera/longitud total)	SIG	POR	0,47	0,04-1,00
	Orden del río (Strahler)	SIG	ORD	2,1	1,0-6,0
	Distancia a cabecera (km)	SIG	CAB	68,1	3,6-1.036,1
	Distancia al río Guadiana (km)	SIG	GUA	58,2	0,0-196,0
	Anchura (m) *	In situ	ANC	10,8	1,4-123,0
	Tipo de sustrato (Escala Wentworth) *	In situ	SUS	5,3	1,0-9,0
	Calidad bosque ribera (QBR, Munne et al., 2003) *	In situ	QBR	61,8	0-100,0
	NH_4^+ (mg/L) *	In situ	AMO	1,38	0,02-51,60
	NO_2^- (mg/L) *	In situ	NIT	0,10	0,01-2,00
	NO_3^- (mg/L) *	In situ	NIA	4,09	0,50-55,90
	PO_5^{3-} (mg/L) *	In situ	FOS	1,00	0,05-23,20
	SO_4^{2-} (mg/L) *	In situ	SUL	110,1	10,0-2.380,0
	Cl^- (mg/L) *	In situ	CLO	56,1	2,0-834,0
	Temperatura del agua (ºC) *	In situ	TEM	20,5	9,4-32,6
	Conductividad (µS/cm) *	In situ	CON	624,7	38,0-3.230,0
	pH *	In situ	ACD	7,84	2,21-10,63
	Precipitación media anual (mm/m^2)	SIG	PRE	593,1	370,2-1.114,5
	Radiación solar (10 kJ/m^2*día*µm)	SIG	RAD	2.033,9	1.646-2.227
	Temperatura media anual del aire (ºC)	SIG	TMA	15,85	13,0-18,0
	Distancia al embalse más cercano aguas arriba(km) *	SIG	EAA	41,1	0,0-196,0
	Distancia al embalse más cercano aguas abajo (km) *	SIG	EAB	25,9	0,2-115,8
	% Abundancia de exóticas*	In situ	AEX	39,6	0-100
	% Riqueza de exóticas*	In situ	SEX	36,1	0-100
Tramo (500 m)	Pendiente ($^0/_{00}$)	SIG	PEN	5,92	0,00-58,03
	Sinuosidad	SIG	SIN	1,23	1,00-2,79
	Usos suelo	SIG			
	Urbano/Industrial (%) *		TUR	1,0	0,0-36,0
	Agricultura intensiva (%) *	SIG	TAI	29,0	0,0-100,0
	Agricultura extensiva (%) *	SIG	TAE	7,0	0,0-100,0
	Natural (%) *	SIG	TNA	63,0	0,0-100,0
Cuenca	Area drenaje (10^3 km^2)	SIG	ARE	260,1	0,9-5.919,1
	Índice de Gravelius	SIG	GRA	1,68	1,14-2,68
	Usos del suelo	SIG			
	Urbano/Industrial (%)*		CUR	0,4	0,0-6,7
	Agricultura intensiva (%)*	SIG	CAI	22,5	0,0-97,0
	Agricultura extensiva (%)*	SIG	CAE	11,0	0,0-89,1
	Natural (%)*	SIG	CNA	65,8	0,9-100,0
	Embalses (%)*	SIG	CEM	0,32	0,0-21,2
	Densidad de población (Hab/km^2)*	SIG	DEN	21,0	0,0-459,3

una de ellas fueron sumados para obtener un índice general de perturbación. No fue considerada de referencia aquella localidad que presentara de media un valor de magnitud de impacto superior a 2, o lo que es lo mismo, un valor del índice superior a 12 (Pont et al., 2007). De esta forma se seleccionaron aquellas localidades con el hábitat en mejor estado. Para evitar el efecto de las perturbaciones biológicas sobre las comunidades nativas también fueron excluidas todas aquellas localidades con una abundancia de especies exóticas superior al 5% del total (Kennard et al., 2006) (Fig. 3).

Figura 3. Ubicación de las localidades de referencia (en negro).

LOS MODELOS PREDICTIVOS.

Se han seleccionado tres tipos diferentes de metodologías para predecir la probabilidad de presencia de las especies nativas a partir de la composición taxonómica en su conjunto y no especie a especie. Esto permite incluir especies con prevalencias muy bajas (hasta del 5%), cuestión esencial en bases de datos no demasiado extensas como la presente (n=241 localidades, de ellas tan sólo 55 de referencia). A pesar de la capacidad de estos métodos para modelar especies raras, debieron ser eliminadas aquellas con un número demasiado reducido de apariciones (menos de 5 presencias). Con ello se descartó el efecto de asociaciones azarosas entre la presencia y las variables utilizadas como predictoras. Finalmente han sido consideradas diez especies nativas.

RIVPACS vs ANNA.

Estos dos métodos fueron usados para predecir la distribución potencial en ausencia de perturbaciones de las diez especies consideradas. Para ello, del total de variables usadas en la caracterización del hábitat en cada una de las localidades, sólo se emplearon aquellas libres de cualquier influencia humana. Puesto que el número de éstas del que se dispuso en la cuenca del Guadiana no fue suficiente para construir y validar los modelos (n=55 localidades), hubieron de ser usadas un conjunto de localidades de referencia de otras cuencas anexas incluidas en la misma región biogeográfica (Tinto, Odiel y Guadalquivir). Por ello se incluyó a la cuenca como una variable predictora más en los modelos. Finalmente se dispuso de un total de 90 localidades de referencia, de las cuales 70 fueron usadas para construir los modelos y 20 para validarlos.

La principal diferencia entre los modelos RIVPACS y ANNA es la concepción discreta vs continua que, respectivamente, cada uno hace de la relación entre las características del hábitat (variables predictoras) y la comunidad de peces (variables dependientes).

En RIVPACS se parte de una clasificación inicial de las localidades de referencia en base a su composición taxonómica, mediante algún análisis aglomerativo como puede ser un análisis de especies indicadoras de dos vías (TWINSPAN) -en el caso del uso de abundancias (Wright, 1995; Joy and Death, 2002; Clarke et al., 2003)- o un análisis aglomerativo jerárquico (UPGMA) -en el caso de presencias-ausencias (Smith et al., 1999)-. Posteriormente, mediante un Análisis Discriminante Múltiple (ADM) se trata de identificar las variables descriptoras que mejor discriminan entre grupos y que serán usadas para calcular la probabilidad de pertenencia de una nueva localidad a cada grupo biológico. Paralelamente se calcula la probabilidad de aparición de cada taxón dentro de cada grupo (número de localidades dentro del grupo donde aparece/número total de localidades del grupo). Por último, la probabilidad de aparición de cada taxón se obtiene multiplicando la probabilidad de pertenencia de cada localidad a cada grupo biológico por la probabilidad de aparición del taxón en cuestión en cada uno de ellos (Fig. 3). De esta forma la composición taxonómica de los grupos biológicos con un hábitat más similar a la nueva localidad de estudio va a influir más fuertemente en las predicciones.

En el caso de ANNA se evita la clasificación inicial, para considerar de forma más fiel la naturaleza continua de los ríos (Linke et al., 2005), según el principio de *river continuum* descrito por Vannote et al. (1980). Esta metodología encuentra, del conjunto total de localidades de referencia, aquellas ambientalmente más próximas y las utiliza para predecir la comunidad esperada en una nueva localidad dada. Para ello parte de una ordenación espacial tridimiensional de las localidades de referencia en base a su composición taxonómica (presencia-ausencia) mediante un análisis de Coordenadas Principales (*Multidimensional Scaling*) no paramétrico (NMSD). Después a través de regresiones múltiples se dota de sentido ambiental cada uno de los ejes de la distribución (usando como predictoras las variables ambientales y como dependientes las coordenadas de los ejes). De esta forma, conocidas las características ambientales de una nueva localidad, se puede localizar en el espacio tridimesional de ordenación biológica. Por último a través de las medidas de las distancias Euclídeas modificadas desde dicha localidad a las de referencia más cercanas se calcula la probabilidad de aparición de cada taxón (Fig. 3). Si el taxón en cuestión está presente en todas las de referencia escogidas, la probabilidad de presencia en la nueva localidad será muy elevada.

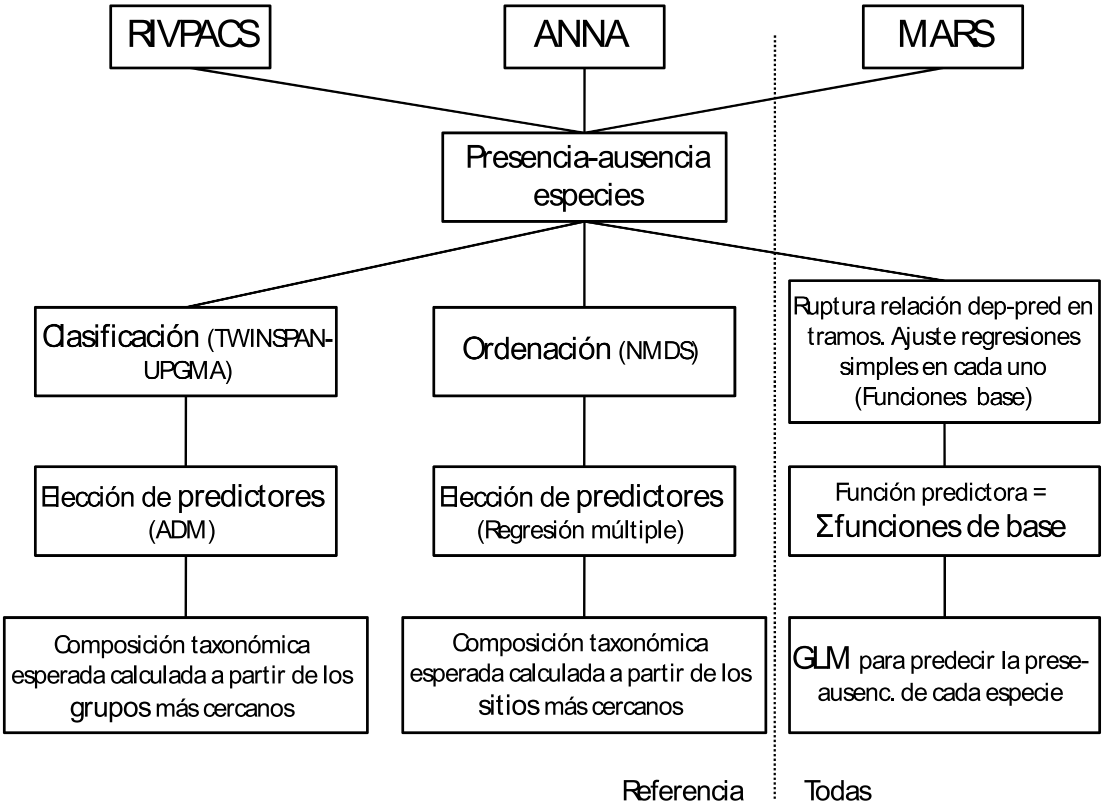

Figura 3. Resumen comparativo de los principales pasos en la construcción de modelos RIVPACS, ANNA y MARS-GLM. La línea de puntos separa los métodos donde sólo han sido utilizadas localidades de referencia para la construcción y validación (distribuciones potenciales) del método basado en toda la información disponible (distribuciones actuales).

Para validar ambos modelos se utilizó la línea de regresión entre la riqueza observada y esperada como indicadora de la fortaleza de la predicción (Oberdorff et al., 2001, Linke et al., 2005). Puesto que las localidades utilizadas con tal fin fueron de referencia, es esperable obtener una línea de pendiente no diferente de 1 y una ordenada en origen no diferente de 0. Además se utilizó como medida de la bondad del ajuste el parámetro R^2 y la Desviación Estándar (DE) de dicha relación. Ésta última fue comparada con respecto a la del mejor modelo que podría obtenerse con los datos usados y con uno nulo, es decir, con el peor posible donde las predicciones no sean mejores que unas obtenidas al azar (Van Sickle et al., 2005). Un buen modelo está caracterizado por una mejora sustancial en la DE respecto a la del modelo nulo y cercana al mejor.

Tabla 2. Variables ambientales caracterizadas a través de un SIG para ser usadas como predictoras en el modelo MARS-GLM. Del total fueron seleccionadas las 9 menos redundantes mediante un Análisis de Componentes Principales. * Indica cada una de las seleccionadas (cada una de las que presentó un factor de carga más elevado para los 9 primeros Componentes Principales).

Escala de cuenca	Escala de subcuenca
% Usos suelo[1]:	% Usos suelo[1]:
Urbano	Urbano
Agricultura intensiva	Agricultura intensiva
Agricultura extensiva	Agricultura extensiva*
Natural	Natural
% Geología[2]:	% Geología[2]:
Depósitos aluviales actuales	Depósitos aluviales actuales
Calcáreo	Calcáreo
Silíceo*	Silíceo*
Volcánico ácido	Volcánico ácido
Volcánico básico	Volcánico básico
Precipitación media anual[3]	Precipitación media anual[3]
Temporalidad precip.[3]	Temporalidad precip.[3]
Temperatura media anual[3]	Precipitación media mes más húmedo[3]
Altura (Media, Máxima y DE)[4]	Precipitación media mes más seco[3]
Pendiente (Media y DE)[4]	Temperatura media anual[3]
Huella ecológica[5]	Rango temperatura anual
Densidad de población[6]	Temperatura media en el mes más cálido[3]*
Área de drenaje*	Temperatura media en el mes más frio [3]*
	Temporalidad temperatura[3]
	Isotermalidad[3]
	Evapotranspiración media anual[3]*
	Altura (Media, Máxima y DE*)[4]
	Pendiente (Media* y DE)[4]
	Índice de calidad del suelo[6]
	Índice de calidad vegetación[6]
	Distancia a cabecera
	Distancia al embalse más cercano aguas abajo
	Distancia a Guadiana
	Huella ecológica
	Densidad de población [6]

Fuentes:

1 CORINE Land-Cover 1:100.000. Confederación Hidrográfica del Guadiana.

2 Mapa geológico de España 1:1.000.000. Instituto Geológico y Minero de España.

3 WORLDCLIM, Version 1.4. La información está descrita en Hijmans et al., 2005.

4 SRTM 90 m Modelo digital de elevaciones obtenido de Jarvis et al., 2006.

5. Huella ecológica. Center for International Earth Science Information Network (CIESIN) at Columbia University (www.ciesin.columbia.edu/wild_areas/). (Agosto 2007)

6. Agencia Medioambiental Europea. (www.eea.europa.eu). (Agosto 2007)

Modelo MARS-GLM.

Las predicciones hechas con los dos modelos anteriores estuvieron restringidas a aquellas localidades muestreadas, en las que fue caracterizado el hábitat, puesto que las variables medidas *in situ* fueron incluidas como predictoras. Esto impidió la propagación de las predicciones a zonas no caracterizadas a dicho nivel de detalle. Para la obtención de las distribuciones reales en toda la cuenca del Guadiana se utilizó una nueva aproximación basada exclusivamente en variables predictoras obtenidas mediante caracterización SIG. Puesto que se pretendió obtener distribuciones reales, se incluyeron variables potencialmente alteradas por el hombre (Tabla 2), así como el total de las localidades muestreadas.

El modelo MARS (*Multivariate Adaptive Regression Splines*) es un método de regresión no paramétrico flexible, útil para modelar relaciones complejas no lineales entre la variable dependiente y las independientes, similar a los Modelos Aditivos Generalizables (GAM, Hastie, 1991).Con MARS se consiguen funciones no lineales para recoger la relación entre la variable dependiente y las predictoras mediante la ruptura del rango global de dicha relación en porciones más pequeñas, donde si pueden ser ajustadas relaciones lineales. Cada una de ellas recibe el nombre de *función de bases*. MARS permite el cambio de la pendiente entre funciones consecutivas, a la vez que asegura que no existen fragmentos de la relación sin ajustar o saltos bruscos de uno a otro (Elith and Leathwick, 2007). La función predictora final está compuesta de una serie de fragmentos lineales conectados, en vez de líneas curvilíneas suavizadas como en GAM. Una característica importante de MARS es que permite explorar interacciones entre predictores pero en vez de sobre el rango total de la dependiente, como es común en otros métodos de regresión, solo sobre determinadas porciones del mismo (Leathwick et al., 2006).

Este tipo de modelos está especialmente diseñado para modelar variables dependientes continuas, mientras que en nuestro caso se trataba de presencias-ausencias. Por ello, siguiendo las recomendaciones de Leathwick et al. (2005), se adaptó el procedimiento general al tipo de información utilizada y así evitar errores en las predicciones. Para ello, tras construir el modelo MARS para todas las especies, se generaron diferentes Modelos Generales Lineales (GLM) para predecir la probabilidad de aparición una a una, utilizando las funciones de bases como predictoras. Las predicciones extraídas de estos últimos sí están restringidas al rango normal de presencias-ausencias (0-1).

RESUMEN DE RESULTADOS

Capítulo 1. ¿Son las clasificaciones derivadas de la DMA adecuadas para evaluar el estado ecológico de los ríos ibéricos de régimen mediterráneo?

Are large-scale landscape classifications derived from the WFD adequate to evaluate the ecological status of Iberian rivers?

La capacidad para definir correctamente las situaciones de referencia en aplicación del concepto de condiciones de referencia limita la precisión para detectar y cuantificar las perturbaciones (Hawkins et al, 2000). Por tanto, este es un paso clave para el desarrollo de un índice eficiente en la evaluación y diagnóstico del estado de conservación y los problemas que aquejan a los ríos.

Puesto que la Directiva Marco del Agua (DMA) propone un par de sistemas de clasificación a usar en la definición de las condiciones de referencia, como primera tarea abordamos la evaluación de la correspondencia entre los grupos de ríos establecidos por el Ministerio de Medio Ambiente (2005) en aplicación del sistema de clasificación B de la DMA y las comunidades de peces de la cuenca del río Guadiana. Este sistema utiliza un conjunto de variables ambientales (altura, pendiente, superficie de cuenca, caudal medio, precipitación media, etc.) para discriminar entre tipos de ríos con características homogéneas. En relación a este sistema de agrupación se definieron para el conjunto de España 29 tipos de ríos diferentes, de los que seis están en la cuenca del Guadiana: (i) *Grandes ejes en ambientes mediterráneos*, (ii) *Ríos mediterráneos continentales mineralizados*, (iii), *Ríos de la baja montaña mediterránea silícea*, (iv), *Ríos silíceos de las llanuras de los Ríos Tajo y Guadiana*, (v) *Ríos de La Mancha* y (vi) *Ríos silíceos del piedemonte de Sierra Morena*. Para que estos grupos fueran útiles en la evaluación del estado ecológico de las masas de agua se precisaría una buena respuesta de las comunidades de peces a los tipos de ríos establecidos. Por el contrario, la falta de sentido biológico de los grupos ambientales –tipos de ríos- se traduciría en errores a la hora de establecer las condiciones de referencia. Por todo ello, en este capítulo se estudió el sentido biológico de la clasificación propuesta por el Ministerio de Medio Ambiente (2005) en la cuenca del Guadiana. Tras evaluar la concordancia entre la clasificación en tipos fluviales y las comunidades de peces se estudió la calidad de las predicciones hechas por diferentes modelos predictivos y, por tanto, sobre los resultados de futuras herramientas para la valoración del estado ecológico.

Para evaluar el sentido biológico de los grupos ambientales, se utilizó un análisis de *escalado multidimensional no paramétrico* (NMDS) sobre una matriz de similaridad de Bray-Curtis basada en las presencias-ausencias de las especies en las localidades de referencia. El tipo *Ríos mediterráneos continentales mineralizados* quedó fuera de los análisis al no contener ninguna localidad de referencia. La ordenación producida no produjo diferencias en la composición taxonómica entre los grupos de ríos testados (Fig. 4). Este resultado se corroboró con dos análisis estadísticos diferentes realizados también sobre la base de datos de referencia. En primer lugar el coeficiente de Fortaleza de la Clasificación (CS), que compara la heterogeneidad dentro de los grupos con la homogeneidad entre los grupos alcanzó un valor muy bajo (CS=0,07). Esto quiere decir que los grupos incluyen localidades con composiciones taxonómicas muy diferentes entre ellas (elevada heterogeneidad dentro de los grupos) y casi indiferenciables uno de otros (elevado homogeneidad entre grupos). Adicionalmente un análisis MRPP demostró que las localidades no estaban mejor agrupadas, respecto a su composición taxonómica, que al azar (A=0,12, P<0,001).

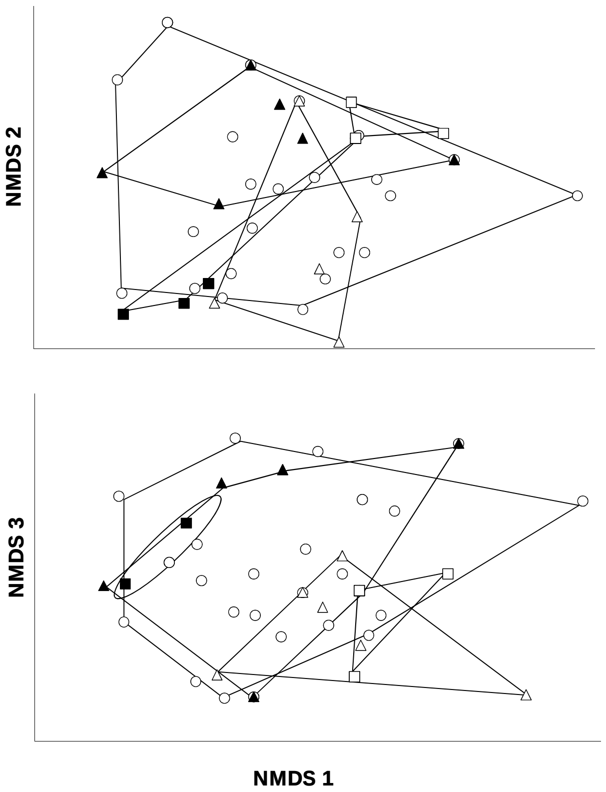

Figura. 4. Ordenación de las localidades de referencia obtenida en el *Multidemensional Scaling* no paramétrico. Los diferentes tipos de ríos están representados como sigue: Ejes principales en ambientes mediterráneos, cuadrados negros; Ríos de la baja montaña mediterránea, círculos blancos; Ríos silíceos de las llanuras del Tajo y Guadiana, triángulos negros; Ríos de La Mancha, cuadrados blancos; Ríos silíceos del piedemonte de Sierra Morena, triángulos blancos.

En contraposición a este resultado, que podría estar originado por una absoluta homogeneidad en la composición taxonómica de las comunidades, fueron encontrados claros gradientes bióticos a lo largo del gradiente natural longitudinal (cabecera-desembocadura). Por lo que la falta de sentido biológico de los grupos evaluados se debe a una agrupación artificial más que a la falta de diferencias biológicas.

Con respecto a la influencia de los resultados anteriores sobre el poder predictivo de los modelos y la idoneidad de seguir manteniendo una aproximación basada en tipos o más centrada en producir medidas específicas para cada sitio, se probaron dos alternativas. En primer lugar se desarrollaron dos modelos RIVPACS para comprobar el efecto de las clasificaciones ineficaces. Un primer modelo RIVPACS fue desarrollado utilizando los grupos ambientales como sistema de agrupación predefinida y sus resultados comparados frente a los de un modelo RIVPCAS estándar. Ninguno de los dos fue completamente válido, como sus pendientes, ordenadas en origen, R^2 y SD demostraron (Tabla 3). Sin embargo, si se apreció una mejoría al evitar el uso de los grupos ambientales.

Estos resultados fueron mejorados por el modelo ANNA (Tabla 3), donde se evitó el uso de cualquier clasificación a priori y se consideró de forma más fiel la naturaleza continua de los ríos.

Tabla 3. Parámetros descriptores de la línea de regresión O/E para el conjunto de localidades de validación (n=20 localidades) en las tres aproximaciones predictivas testadas. Se muestra entre paréntesis la DE (Desviación Estándar) del mejor modelo posible y el modelo nulo según Van Sickle et al., (2005). Sólo aparece un valor que es compartido por todos los modelos.

Método predictivo	Pendiente	Ordenada en origen	R^2	DE (0,38-0,45)
RIVPACS-DMA	0,28	1,37	0,05	0,41
RIVPACS Estándar	1,36	-0,89	0,22	0,40
ANNA	1,06	0,06	0,43	0,39

Queda de este modo probado que las predicciones extraídas de modelos donde se usó algún tipo de clasificación a priori quedaron desbancadas por las del modelo donde se evitó esta aproximación De aquí se deriva la baja eficiencia de las clasificaciones a priori para recoger la variabilidad biológica natural de los cursos de agua.

Como síntesis cabe decir que tal como muestra este estudio las clasificaciones basadas en aspectos medioambientales no siempre se corresponden con diferencias biológicas. Esto tiene inmediatas consecuencias en el establecimiento de condiciones de referencia y en la eficacia de de uno de los métodos predictivos más ampliamente utilizado.

Puesto que la eficiencia de las evaluaciones hechas a través de aproximaciones basadas en tipos depende tan fuertemente de la correspondencia entre las clasificaciones y los cambios bióticos, se recomienda comprobar previamente este hecho. En cualquier caso, como ha quedado demostrado para la comunidad de peces del Guadiana, el uso de aproximaciones que eviten cualquier tipo de clasificación apriorística mejora sustancialmente la calidad de las predicciones y por tanto la fiabilidad de las evaluaciones surgidas de las mismas.

Capítulo 2. Evaluación de la sensibilidad de los peces continentales. Necesidad de tener en cuenta diferentes fuentes de covariación.

Assessing freshwater fish sensitivity. The need to account for different sources of co-variation.

La precisión de las evaluaciones hechas a través de bioindicadores es muy dependiente del conocimiento que se tenga de la sensibilidad de los taxones usados como indicadores. Se han utilizado múltiples formas para su establecimiento: el criterio de expertos (Oberdorff et al., 2002), análisis empíricos (Meador and Carlisle, 2007, Carlisle et al., 2007) o aproximaciones basadas en modelos (Yuan, 2004; Cao and Hawkins, 2005). A pesar de la importancia de este conocimiento, existen muy pocos estudios que evaluen específicamente de la sensibilidad de los peces, y en concreto nada se sabe sobre el caso de peces europeos y mediterráneos. No obstante, estos vertebrados vienen siendo utilizados como indicadores de perturbación en múltiples herramientas de evaluación. Este desconocimiento impide hacer un uso completo de sus capacidades como indicadores en diagnósticos precisos de las causas de perturbación.

Por todo ello, para garantizar un uso adecuado de las especies de peces nativos como herramientas de evaluación del estado ecológico, se afrontó el análisis de la sensibilidad de diez de las especies nativas de la cuenca ante diferentes factores de perturbación. Para ello se utilizó una doble aproximación atendiendo especialmente a la covariación de la presencia-ausencia de las especies a lo largo de diferentes gradientes de perturbación y cambios naturales.

En primer lugar se estudió la sensibilidad de cada especie al grado de perturbación humana en general sin distinguir entre causas concretas. Se comprobó la respuesta de las especies ante esta perturbación eliminando el efecto de la covariación a lo largo del gradiente natural definido por los cambios en el hábitat desde cabecera-tramos bajos. Para ello se realizó un Análisis de Componentes Principales (PCA) sobre la matriz global de hábitat (Tabla 1), del que fueron extraídos dos Componentes Principales (Tabla 4). El primero (PC1) recogió un gradiente claro de perturbación, mientras que el segundo (PC2) estuvo relacionado principalmente con variables descriptoras del gradiente longitudinal cabecera-tramos bajos. Puesto que ambos gradientes fueron completamente ortogonales, se pudo estudiar la respuesta de las especies nativas al gradiente de perturbación global eliminando el efecto de la variación natural a través de un análisis de uso-disponibilidad mediante un test Chi-cuadrado (Prenda et al., 1997). Se analizó la medida en que cada especie utilizó las diferentes porciones del gradiente de perturbación. De esta forma las especies más sensibles al estado de conservación general seleccionarían preferentemente las mejores porciones del gradiente (en mejor estado de conservación) y rechazarían o estarían ausentes de las más degradadas. Las especies tolerantes harían un uso del gradiente no diferenciable de lo disponible o utilizarían preferentemente las porciones más degradadas. En este sentido se encontró que *Luciobarbus sclateri*, *Pseudochondrostoma willkommii* y *Anaecypris hispanica* fueron las especies más sensibles (Fig. 5). Otras especies, como *Luciobarbus microcephalus*, *Luciobarbus comizo* y *Salaria fluviatilis* mostraron valores intermedios de sensibilidad puesto que usaron las mejores porciones del gradiente tal y como estuvieron disponibles, mientras que sólo desaparecieron o evitaron las más degradadas (Fig. 5). Por último las especies menos sensibles se caracterizaron por un uso global del gradiente en la medida en que estuvo disponible (*Iberociprys alburnoides*, *Cobitis palúdica*) o una aparición o uso significativamente superior a lo disponible en ambos extremos del gradiente (*Iberochondrostoma lemmingii* y *Squalius pyrenaicus*) (Fig. 5).

Tabla 4. Análisis de Componentes principales (PCA) llevados a cabo para definir gradientes de perturbación y variación natural. Sólo se muestran las variables con factores de carga |>0,6| siempre que fue posible. Los códigos de las variables ambientales aparecen en la Tabla 1.

Objetivo	Técnica	Variables	Gradiente extraido	% var. exp. (Autovalor)	Extremo negativo	Extremo positivo	Denominación
Extraer un gradiente de perturbación global libre del efecto de la variación natural	PCA	Todas las que aparecen en la Tabla 1	PC1	20,9 (8,14)	**TNA** (-0,77) **CNA** (-0,72) **TEM** (-0,71) **PRE** (-0,62) **QBR** (-0,60)	**CAI** (0,78) **ALT** (0,63) **TAI** (0,62) **SUL** (0,60)	***Gradiente de perturbación global***
			PC2	13,8 (5,39)		**CAB** (0,89) **ARE** (0,87) **ORD** (0,83) **POR** (0,77) **ANC** (0,76) **SUS** (0,67)	***Gradiente longitudinal***
Identificar diferentes gradientes de perturbación humana independientes	PCA	Variables relacionadas con la perturbación humana en la Tabla 1	PC1_Alt	28,9 (5,48)	**CNA** (-0,81) **TNA** (-0,75) **QBR** (-0,61)	**CAI** (0,67) **DEN** (0,64) **CUI** (0,61)	***Naturalidad de la cuenca***
			PC2_Alt	12,0 (2,29)	**FOS** (-0,70)		***Enriquecimiento en P***
			PC3_Alt	8,6 (1,63)	**EAA** (-0,83)	**CEM** (0,81)	***Regulación aguas abajo***
			PC4_Alt	7,2 (1,37)	**NTA** (-0,48)		***Enriquecimiento en N***
			PC5_Alt	6,2 (1,17)	**TAE** (-0,57)		***Agricultura alrededor***
			PC6_Alt	5,7 (1,08)	**EAR** (-0,55)		***Regulación aguas arriba***

Sin embargo a través de este tipo de aproximaciones globales, que han sido utilizadas tradicionalmente, no podemos responder a la pregunta: *¿a qué son sensibles las especies?*, esencial para poder encarar diagnósticos fiables. Además, a través de este tipo de análisis podemos infravalorar la sensibilidad específica de alguna de las especies a una fuente determinada de perturbación, enmascarada en el gradiente global. Por ello en un segundo paso se estudió la respuesta de las presencias-ausencias a fuentes independientes de perturbación.

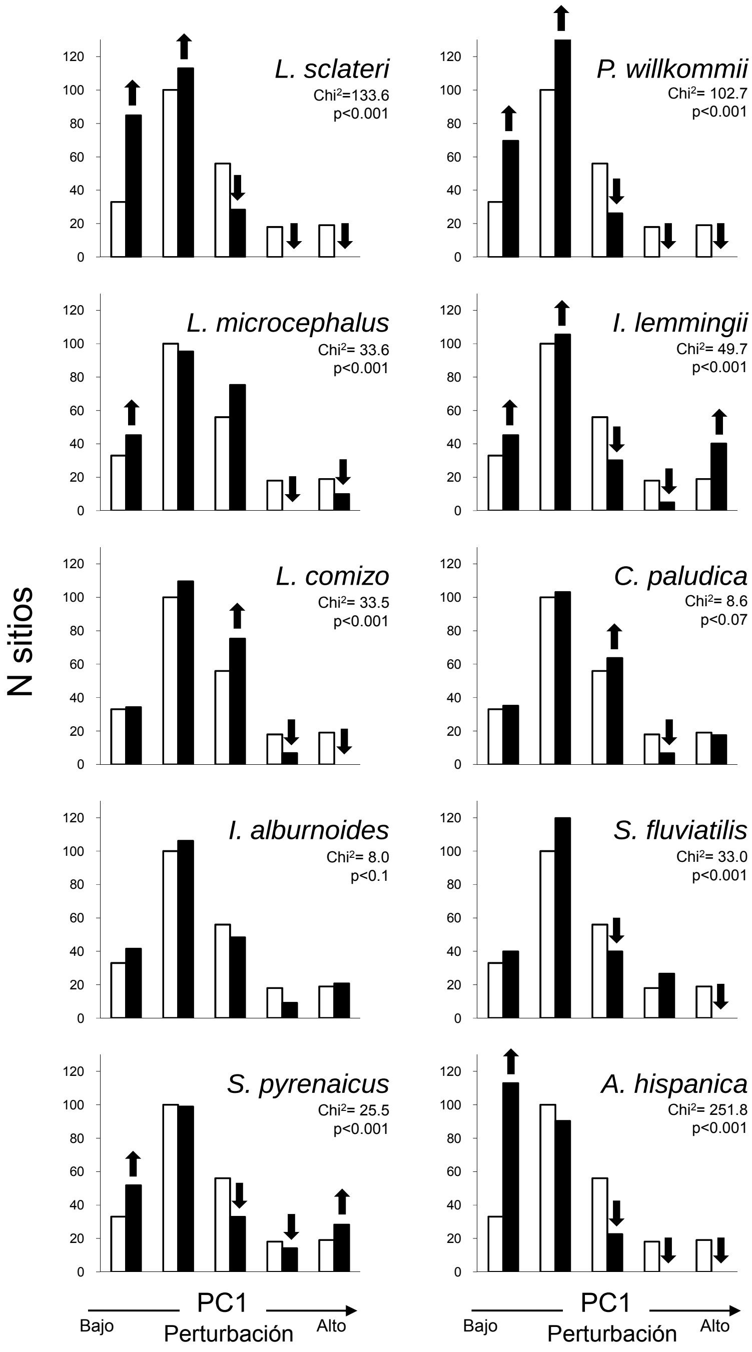

Figura 5. Análisis de uso-disponibilidad realizado sobre las 5 porciones equivalentes en las que fue dividido el gradiente de perturbación humana global. El número de sitios disponible aparece representado en las columnas blancas, mientras que los usados en las negras. Se muestra para cada especie el valor del estadístico Chi-cuadrado y su valor de incertidumbre asociado. Las diferencias significativas para cada porción se encuentran señaladas mediante flechas, las cuales indican además el sentido de la diferencia (infra o sobre-uso con flechas hacia abajo o arriba respectivamente).

Para ello se volvió a repetir un PCA sólo sobre las variables relacionadas con las perturbaciones humanas (Tabla 4), para identificar los gradientes independientes más significativos dentro del área de estudio. En este caso además de los seis primeros Componentes Principales indicadores de perturbaciones humanas, se analizó la respuesta al grado de penetración de las especies exóticas en la comunidad como otro indicador de perturbaciones bióticas. Para ello se utilizó el porcentaje de abundancia y riqueza de las especies exóticas respecto al total de la comunidad. Se usó el porcentaje para evitar el efecto de la variación natural de la abundancia y riqueza local. Para garantizar la independencia de los resultados de sensibilidad del gradiente de variación natural, se comprobó la independencia de cada variable de perturbación (bien gradientes de perturbación humana del PCA o biótica) del mencionado gradiente de variación natural (gradiente longitudinal en Tabla 4). En caso de ser completamente independiente, la sensibilidad de cada especie a una fuente de perturbación dada se estudió mediante la aplicación de un t-test a los valores de la perturbación en los lugares donde dicha especie estuvo presente y en aquellos en que estuvo ausente. En caso contrario (perturbación no independiente del gradiente longitudinal) se utilizó un Análisis de la Covarianza (ANCOVA). En este último, se empleó el gradiente longitudinal como covariable, la presencia-ausencia de cada especie como factor y cada variable de perturbación como variables dependientes del análisis. En ambos casos un efecto significativo de la presencia-ausencia sobre las variables de perturbación significaría que el grupo de localidades donde cada especie apareció fue significativamente diferente de las localidades donde estuvo ausente, independientemente de la posición dentro del gradiente longitudinal donde se encontraran.

Como era esperado aquellas especies que mostraron una elevada sensibilidad al gradiente global también respondieron fuertemente a algunas de las fuentes de perturbación independientes analizadas (*L. sclateri* al enriquecimiento en nutrientes debido a P y N, *P. willkommii* al enriquecimiento en P, los efectos de la agricultura en zonas cercanas al cauce y la regulación del río aguas arriba; *A. hispanica* respondió principalmente al estado de conservación de la cuenca y al enriquecimiento en N). Sin embargo, la escala más detallada de esta segunda aproximación permitió identificar respuestas anteriormente ocultas y, por tanto, definir como sensibles a alguna perturbación a especies que antes fueron incluidas en el grupo de insensibles o tolerantes. En este sentido *S. pyrenaicus* mostró una respuesta clara al enriquecimiento en N y a la regulación aguas arriba; *I. lemmingii* a la regulación aguas arriba; e *I. alburnoides* a los efectos de la agricultura cercana al cauce y la regulación aguas arriba. De la misma forma se encontraron respuestas más fuertes para especies calificadas como medianamente sensibles, como *S. fluviatilis*, *L. comizo* y *L. microcephalus* a la agricultura alrededor del cauce. No se encontró ninguna especie sensible a la regulación aguas abajo tras eliminar el efecto del gradiente longitudinal y sólo *L. comizo* y *S. fluviatilis* no mostraron respuesta ante las perturbaciones bióticas (Fig. 6).

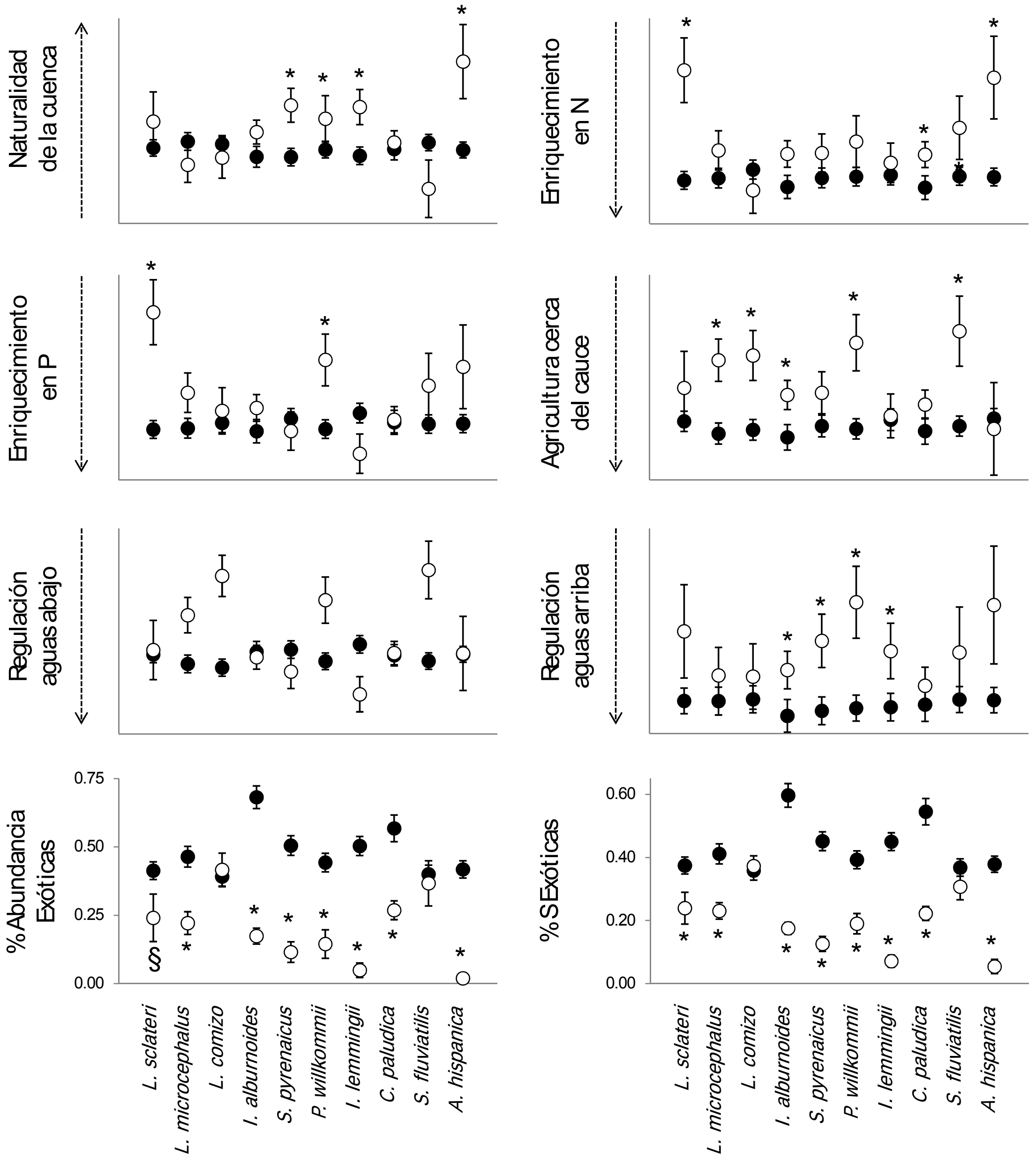

Figura 6. Valores medios ± EE de cada variable de perturbación donde cada especie estuvo presente (puntos blancos) y ausente (puntos negros). * Indica diferencias significativas ($p<0,05$) en el análisis ANCOVA o t-test sin efecto del gradiente longitudinal. § Indica diferencias marginalmente significativas ($p<0,1$).

Por tanto, con esta información sobre la sensibilidad de 10 especies nativas ante diferentes tipos de perturbación humana y biótica disponemos de información objetiva y rigurosa alternativa al uso del siempre subjetivo criterio de experto, a la hora de usar este conjunto de especies en futuras herramientas de evaluación del estado ecológico. Se ha utilizado sólo información de presencia-ausencia para eliminar de dichas futuras herramientas el efecto de las imprecisiones derivadas del uso de la abundancia. La abundancia es altamente variable a lo largo del ciclo anual, con variaciones aun más acusadas en medios tan cambiantes como los mediterráneos (Meffe and Minckley, 1987; Matthews and Marsh-Matthews, 2003; Magalhães et al., 2007), y que además son difíciles de caracterizar adecuadamente. De esta forma adaptamos el uso de las sensibilidades a las condiciones especiales de las cuencas mediterráneas. Finalmente, puesto que todas las especies mostraron algún tipo de sensibilidad ante determinados tipos de perturbación se recomienda la inclusión de todas ellas en futuros índices, ya que en conjunto ofrecen un buen diagnóstico de los agentes de perturbación implicados en el estado ecológico detectado.

Capítulo 3. Evaluación del Estado Ecológico en sistemas pobres en especies a través de un índice basado en peces para los ríos mediterráneos.

Assessing the Ecological Status in species-poor systems: a fish-based index for Mediterranean rivers.

El desarrollo de una herramienta eficaz para evaluar el estado de conservación de los ríos mediterráneos debe estar necesariamente adaptado a las características especiales de estos medios. Como se ha comentado anteriormente todos los esfuerzos realizados en la Península Ibérica han seguido una metodología basada en tipos, mientras que a escala europea está siendo utilizado el EFI basado en medidas específicas para cada localidad. Es por ello que en este trabajo se explora la potencialidad de una aproximación similar a EFI en ríos mediterráneos. Sin embargo, a diferencia de EFI y el resto de adaptaciones de índices en la Península Ibérica, para el desarrollo del aquí presentado se ha seguido una aproximación no basada en métodos multiparamétricos. Los índices multiparamétricos cuantifican el estado ecológico a través de diversos atributos (métricas basadas en gremios y otros aspectos de la estructura y funcionamiento del sistema) descriptores del estado de la comunidad. Puesto que los taxones (en nuestro caso las especies) son los componentes básicos de la comunidad, los cambios originados por perturbaciones podrían verse reflejados más fiel y rápidamente en la composición taxonómica que en niveles estructurales superiores (Norris and Hawkins, 2000; Hawkins et al., 2000). Además, las distintas especies incluidas en cada gremio pueden tener sensibilidades diferentes o incluso respuestas independientes ante una misma perturbación (Thiollay, 1992; Lindermayer et al., 1999; Lindermayer et al., 2000), lo que puede influir negativamente en la propia sensibilidad del índice ante dichas perturbaciones. A lo que se debe añadir que este tipo de aproximaciones son difíciles de seguir en ríos con una riqueza específica por localidad muy baja (Miller et al., 1988) como son los mediterráneos. Para evitar este tipo de problemas, en la propuesta de índice que a continuación se presenta se tendrán en cuenta diferencias en la composición taxonómica entre las comunidades observadas y esperadas como indicador del estado ecológico.

En el desarrollo del índice se utilizaron las predicciones hechas por el método ANNA previamente descrito. La composición taxonómica de las comunidades observadas y esperadas fue comparada especie a especie, derivándose en cada una de ellas una medida parcial del grado de desviación entre lo observado y lo esperado. La diferencia entre la presencia-ausencia observada de cada especie y la probabilidad esperada de aparición fue tomada como indicadora de este grado de desviación. Posteriormente estos valores fueron estandarizados y transformados en valores de probabilidad de pertenecer a sitios de referencia. Por tanto, según la diferencia

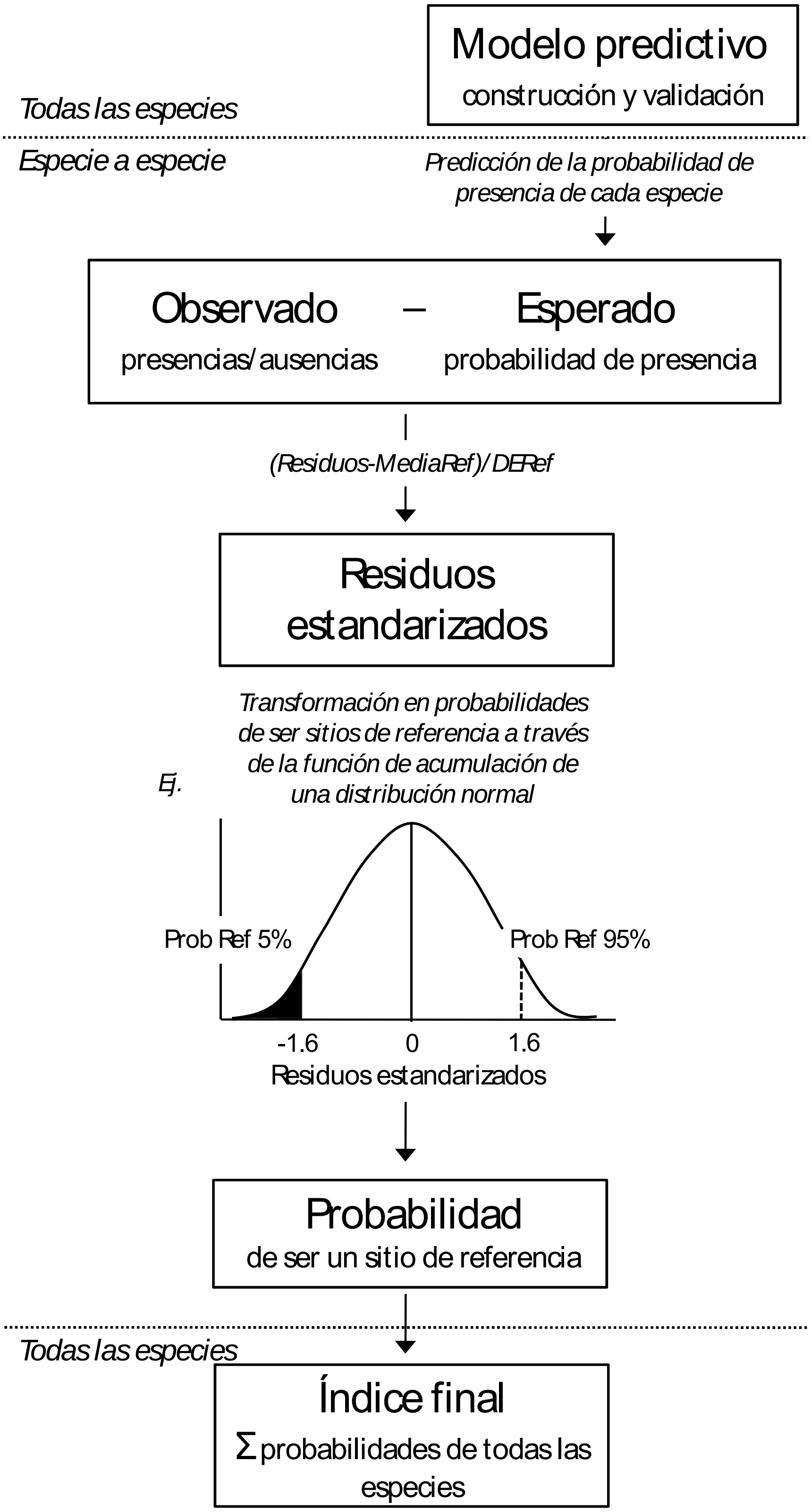

Fig. 7. Esquema seguido para el desarrollo del Índice de calidad. Se muestra un ejemplo de como se transformó la diferencia entre O-E de cada localidad en probabilidades de ser consideradas como de referencia. La probabilidad acumulada para un residuo muy bajo (-1,6) es también muy baja (5%), mientras que la de un residuo alto (1,6) es también alta (95%). Las líneas de puntos separan los pasos en los que se utilizan todas las especies o las evaluaciones parciales especie a especie.

entre la presencia-ausencia de una determinada especie y su probabilidad de aparición tendríamos una evaluación parcial para cada sitio de la probabilidad de que estuviese en condiciones de referencia o, lo que es lo mismo, en buen estado ecológico. Al final todas estas evaluaciones parciales fueron sumadas en un único valor representativo del estado ecológico de cada localidad (Fig. 7). El índice alcanzó valores máximos cuando la composición taxonómica observada fue muy similar a la esperada en ausencia de perturbaciones y mínimos cuando ambas comunidades discreparon significativamente.

Se utilizaron límites entre clases de calidad para maximizar el poder discriminatorio y minimizar el riesgo de cometer errores Tipo I (determinar que hay mal estado ecológico cuando no es así) y Tipo II (no encontrar daño cuando realmente existe) (Tabla 5).

Tabla 5. Límites establecidos entre las cinco clases de calidad y los objetivos perseguidos con cada uno de ellos.

Clase calidad	Punto de corte	Objetivo	Intervalo
MUY BUENO			(6,46-10]
	Percentil 95 sitios test	Menos del 5% de los sitios test son considerados como en muy buen estado (Reducción errores Tipo II)	
BUENO			(5,51-6,46]
	Media sitios referencia	Solo sitios con un valor superior a la media dentro del grupo de referencia serían clasificados como en buen estado	
MODERADO			(4,36-5,51]
	Media sitios test	Sólo sitios con un valor superior a la media de un sitio perturbado son clasificados como Moderados.	
POBRE			(3-4,36]
	Percentil 1 sitios referencia	Menos del 1% de las localidades de referencia serían clasificadas erróneamente como en mala condición (Reducción errores Tipo I)	
MALO			[0-3]

Durante la calibración (análisis de la respuesta ante fuentes de perturbación) el índice no mostró respuesta alguna al gradiente longitudinal (ANOVA, F=0,5, p=0,7), una respuesta moderada a un gradiente general de perturbaciones de hábitat (F=1,8, p=0,1) y una fuerte respuesta a las perturbaciones bióticas (F=22,5, p<0,001) (Fig. 8). Para la definición de estos gradientes se volvió a utilizar un PCA sobre el total de variables ambientales incluidas en la Tabla 1. La buena respuesta del índice fue corroborada durante su validación con los valores de los índices IBMWP y QBR (ANOVA, F=13.5, p<0,001 para IBMWP and F=4.6, p<0,001 para QBR). A través de un análisis previo se despejó la duda de posibles correlaciones entre los gradientes usados para calibrar el índice y los usados en la validación (Correlación Pearson, r <|0,5|), evitando la circularidad en este proceso. Se utilizaron los valores finales de IBMWP y QBR en vez de las respectivas clases de calidad, puesto que sólo se pretendió comprobar respuestas similares y no intercalibrar los diferentes índices. Por supuesto este objetivo debería ser abordado en futuros trabajos, para dotar al presente índice de sentido completo. La buena respuesta del índice ante los valores de los otros dos testados (Fig. 8) muestra no sólo su validez, sino corrobora la sensibilidad ante la perturbación del hábitat (calidad del agua y calidad del hábitat ripario), poco destacada en la calibración.

Puesto que la sensibilidad de cada especie a las diferentes fuentes de perturbación es conocida, se puede hacer un diagnóstico de qué especies son responsables de los valores de estado ecológico observados. Ello permitirá identificar la causa o conjunto de causas más probablemente asociadas a la degradación y, por tanto, facilitará la propuesta de medidas correctoras encaminadas a la consecución de la exigencia de la DMA relativa al alcance del buen estado ecológico de todas las masas de agua en 2015.

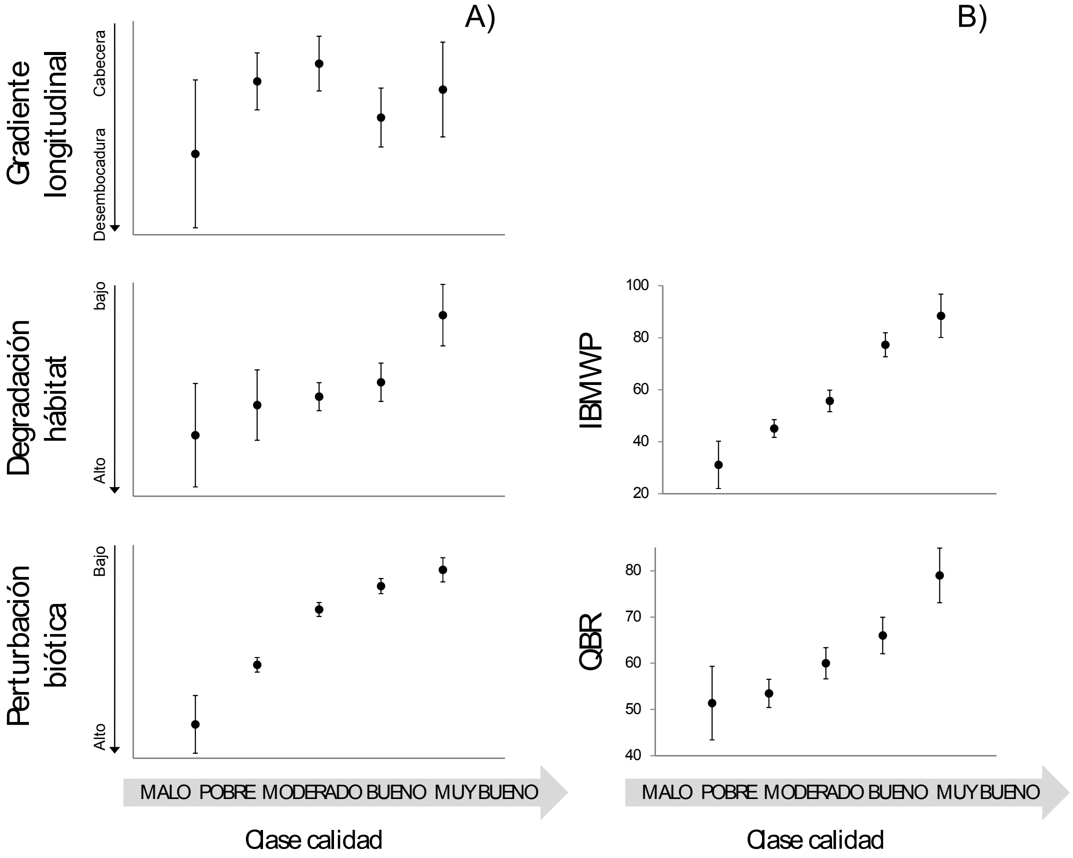

Figura 8. Calibración A) y validación B) del índice. Se muestran valore medios ± EE de las diferentes variables en cada clase de calidad.

Por tanto, el presente índice no sólo es sensible a perturbaciones del hábitat, sino también a alteraciones de tipo biológico. Este es un hecho especialmente remarcable, puesto que se trata de la primera vez que se evalúa la capacidad de un índice de calidad para detectar este tipo de perturbaciones. Las alteraciones bióticas inciden gravemente sobre el estado ecológico de los ecosistemas fluviales y suponen una de las amenazas más graves para la conservación de su biodiversidad (Kaufman, 1992; Godinho and Ferreira, 2000; Clavero et al., 2004). Sin embargo, no son fáciles de detectar a través de otros medios, ya que en la mayoría de los casos no se reflejan en ningún otro cambio a excepción del daño producido sobre las comunidades nativas.

Capítulo 4. Identificación de lugares prioritarios para la conservación de la biodiversidad de peces de agua dulce en una cuenca mediterránea con un elevado grado de endemismos amenazados.

Identifying priority sites for the conservation of freshwater fish biodiversity in a Mediterranean basin with a high degree of threatened endemics.

La elevada biodiversidad de peces continentales que habitan en el río Guadiana y el grado de amenaza al que se hayan sometidos hacen de la ictiofauna de esta cuenca una de las más sobresalientes en el área circunmediterránea (Smith and Darwall, 2006). Por ello es imprescindible acometer estudios que promuevan su conservación. Uno de los principales problemas en la planificación para la conservación es la cuantificación del valor relativo de cada área para la conservación de la biodiversidad local, que evite las tradicionales e ineficientes estrategias de conservación *ad hoc* o a priori.

Para afrontar la identificación de aquellas áreas de especial interés para la conservación de la ictiofauna de la cuenca del río Guadiana adaptamos a las comunidades de peces el procedimiento propuesto por Linke y Norris (2003) para macroinvertebrados en Australia. Puesto que sólo aquellos lugares con 1) una comunidad poco o nada alterada y 2) con elementos singulares, son merecedoras de ser destacadas del total, este método se basa en una evaluación estructurada en dos pasos, que integra medidas tanto de pérdida de biodiversidad potencial, como de la evaluación del valor de conservación. Para ello, en primer lugar se midió la pérdida de biodiversidad potencial que había sufrido cada localidad a través del Índice de Pérdida de biodiversidad (OE50) (Linke y Norris, 2003; Simpson y Norris, 2000). Aquí se utilizó el cociente entre la riqueza observada (O) y esperada (E), calculada a través de las predicciones derivadas del modelo ANNA para aquellas especies con una probabilidad de aparición >0,5. De esta forma sólo se incluyeron en este índice aquellas especies con una mayor probabilidad de aparición, o lo que es lo mismo, aquellas especies que con una mayor fiabilidad deberían estar presentes. Para asegurar una probabilidad de error Tipo I (establecer que el sitio ha sufrido una pérdida de biodiversidad significativa, cuando en realidad no ha sido así) del 10%, se utilizó el percentil 10 de los valores de las localidades de referencia como punto de corte para determinar la pérdida significativa de biodiversidad (Linke y Norris, 2003; Simpson y Norris, 2000) (Fig. 9).

En un segundo paso se calculó un Índice de Valor de Conservación (CV) [también llamado O/E (BIODIV) en Linke y Norris, 2003]. Aquí fueron empleados sólo aquellos sitios que no presentaron una pérdida significativa de biodiversidad. Así se aseguró no invertir esfuerzos en aquellos lugares en los que la comunidad ha perdido una parte importante de su riqueza nativa. El índice CV, de forma similar al caso anterior, surgió del cociente entre la riqueza observada y esperada para aquellas especies que presentaban una probabilidad de aparición <0,5, o especies raras. Si el número observado de especies poco esperadas era superior al predicho (CV>1) entonces la localidad en cuestión podría ser considerada como un lugar interesante para la conservación (Linke y Norris, 2003), puesto que además de no haber sufrido pérdida de biodiversidad cuenta con un elevado número de especies raras.

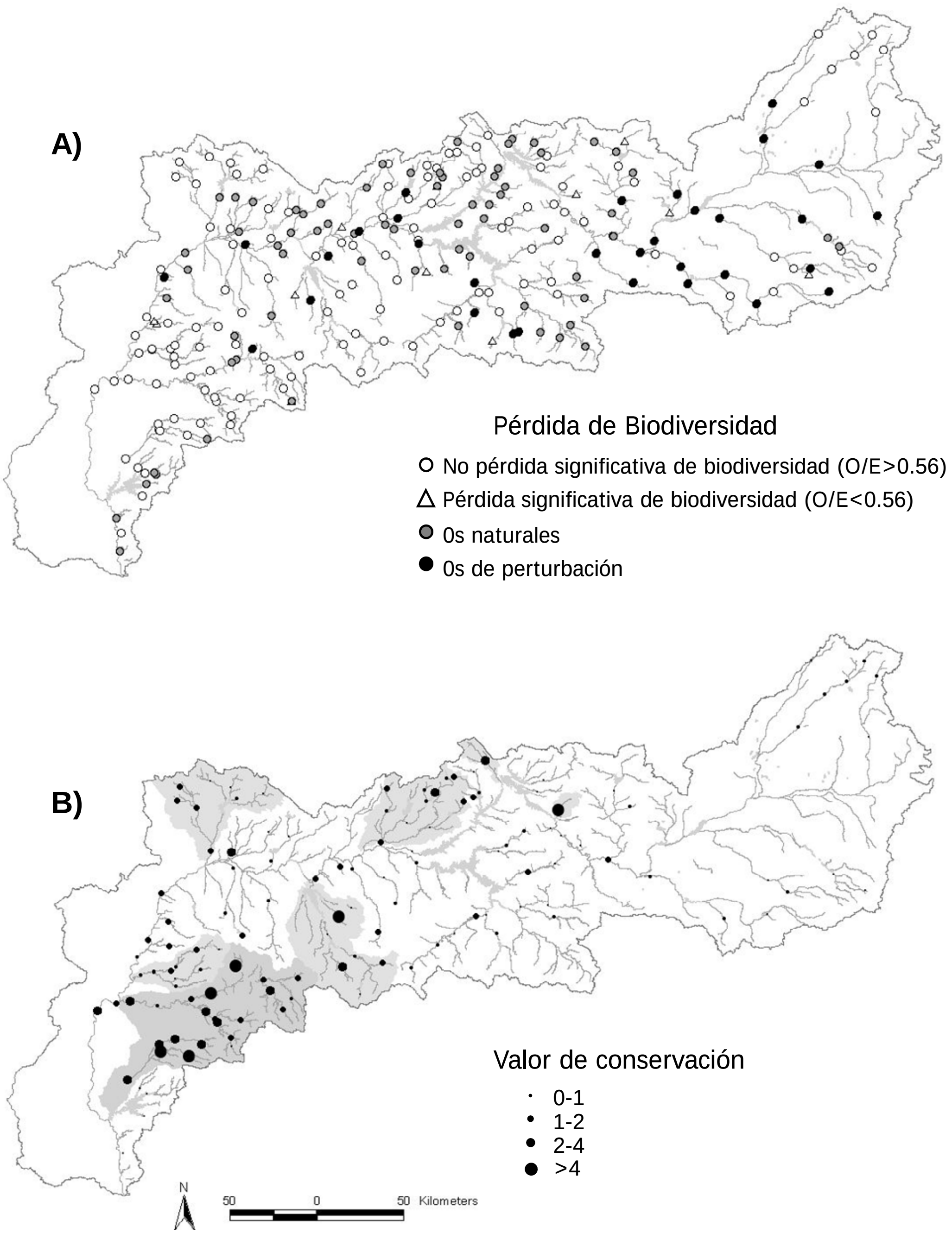

Figura 9. A) Mapa del Índice de pérdida de biodiversidad (n=241). Los puntos blancos indican ninguna pérdida de biodiversidad y por tanto aquellos sitios considerados en el segundo paso. Los triángulos blancos indican aquellos sitios donde si hubo alguna pérdida de biodiversidad, aunque se encontró alguna especie nativa. Los puntos grises y negros representan los 0s naturales y debidos a perturbaciones. B) Puntuación del Índice de valor de conservación. Las subcuencas más importantes han sido remarcadas en gris. Sólo se muestran aquellos ríos incluidos en el estudio.

La inestabilidad hidrológica puede limitar seriamente la colonización o la existencia continua de poblaciones ícticas en ecosistemas acuáticos. Es el caso de los medios temporales o efímeros (Mathews y Styron, 1982; Ross et al., 1985; Schlosser, 1995). Por ello, se trató de discriminar entre aquellos lugares en los que el valor 0 del índice de pérdida de biodiversidad (no se encontró ninguna especie nativa) no estuvo ligado a actividades humanas, sino que estuvo originado de forma natural por la inestabilidad hidrológica; de aquellos otros lugares en los que los valores 0 fueron debidos algún tipo de perturbación antrópica. Así, se realizó un PCA sobre una matriz de variables ambientales del conjunto de localidades con valor 0 para el índice de pérdida de biodiversidad (n=94 localidades). El principal gradiente ambiental obtenido en este PCA (PC1) mostró un cambio desde el extremo negativo, con localidades situadas en tramos medios-bajos muy perturbados, hasta localidades muy cercanas a cabecera poco o nada perturbadas, en el extremo positivo (Tabla 6). Este gradiente estuvo evidentemente muy correlacionado con el Índice de Perturbación usado para discriminar entre localidades de referencia y test (Correlación de Pearson, r=-0,78, p<0,001). El valor medio del índice a lo largo de seis tramos equivalentes del gradiente fue utilizado como indicador para distinguir entre los valores 0 debidos a la inestabilidad hidrológica natural del hábitat y debidos a presiones humanas (tramos con un valor medio inferior a 12, el límite establecido para diferenciar entre

Tabla 6. Factores de carga del Análisis de Componentes Principales realizado sobre las localidades que no presentaron ninguna especie nativa (n=94 localidades). Sólo se muestran las variables con factores >|0,5|. *p<0,05; **p<0,01; ***p<0,001.

Variable	**PC1** (24.4%)	**PC2** (11.6%)
% usos naturales cuenca	0.80 ***	
Precipitación media anual	0.78 ***	
Superficie cuenca	-0.76 ***	
% agricultura intensiva cuenca	-0.74 ***	
Distancia a cabecera	-0.73 ***	
% usos naturales tramo	0.68 ***	
Índice de Gravelius	-0.67 ***	
Cl^- (mg/L) *	-0.67 ***	
Densidad de población	-0.66 ***	
% suelo urbano-industrial cuenca	-0.61 ***	
QBR	0.60 ***	
SO_4^{2-} (mg/L) *	-0.58 ***	
Orden del río	-0.56 ***	-0.58 ***
Pendiente media del tramo	0.53 ***	
% agricultura extensiva cuenca	0.53 ***	
Posición relativa dentro de la cuenca	-0.50 ***	-0.62 ***
Anchura del cauce		-0.71 ***
Temperatura del agua		-0.51 ***
Distancia al cauce del Guadiana		0.56 ***
Altura		0.58 ***

localidades de referencia y test). Aquellas localidades situadas en el extremo más positivo del gradiente, donde la ausencia de peces no pudo ser relacionada con presiones antrópicas se incluyeron en el grupo de valores 0 por causas naturales. El resto fue considerado valor 0 debido a perturbaciones. Como se ha comentado anteriormente las localidades con valores 0 naturales estuvieron principalmente localizadas en tramos de cabecera, donde los cursos de agua fueron excesivamente temporales como para permitir el desarrollo de comunidades de peces.

Por último, se evaluó el efecto sobre la pérdida de biodiversidad observada y el valor de conservación de uno de los principales agentes de perturbación en áreas mediterráneas: los embalses (Clavero et al., 2004). Para ello se calculó el valor medio de ambos índices en localidades ubicadas dentro de cuencas reguladas por el mismo embalse, sólo considerando grandes embalses (>1 hm^3). Posteriormente se analizó el patrón mostrado por estos valores medios a lo largo del tiempo (agrupados por décadas). Se encontró que la pérdida de biodiversidad crecía a medida que lo hacía el tiempo que llevaba regulada la cuenca (Fig. 10), y el valor de conservación fue claramente inferior en cuencas reguladas desde hace 50-60 años, que en las reguladas a partir de las décadas de los 80-90s.

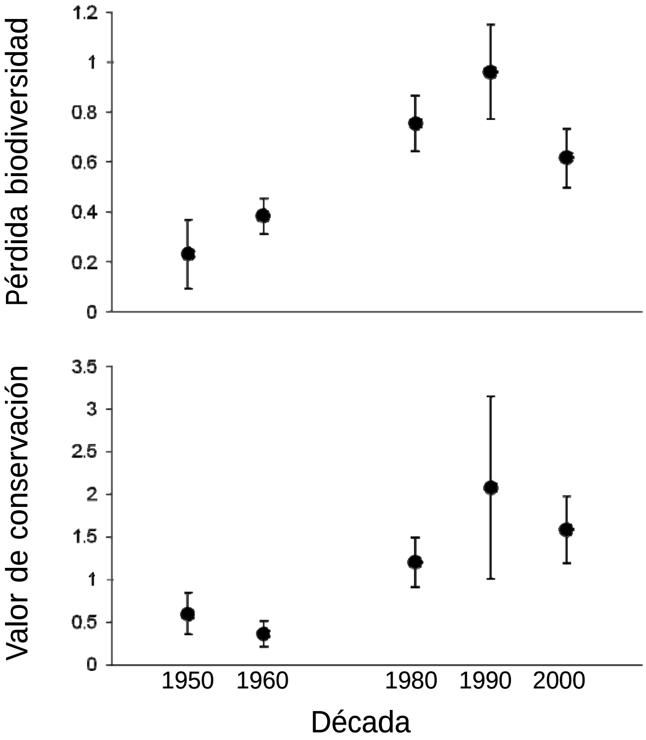

Figura 10. Efectos de la regulación de las cuencas sobre la pérdida de biodiversidad y el valor de conservación de la ictiofauna. Se muestran las puntuaciones (Media ± EE) de los índices agrupados por subcuencas reguladas dentro de la misma década por un gran embalse (>1Hm^3). Para los embases de Alqueva y Pedrogao construidos en el año 2002, tan sólo se han utilizado aquellas localidades muestreadas posteriormente a esta fecha.

Se han identificado, por tanto, aquellas zonas con unas comunidades de peces en mejor estado de conservación y de especial interés para el mantenimiento de la biodiversidad de peces de la cuenca. La inclusión de subcuencas completas, tal y como se muestra en la Figura 9 podría garantizar una solución más o menos óptima en términos de agregación espacial y costes. Sin embargo, puesto que la existencia de reservas inconexas no garantiza la preservación a largo plazo de la biodiversidad de peces (Meffe, 2002; Angermeier, 2000; Lindermayer et al., 2000) y dado el estado de fragmentación actual de la Cuenca del Guadiana, se hacen necesarios nuevos estudios que analicen más detalladamente la importancia de cada una de las áreas señaladas y el diseño más sistemático de una red de reservas.

Capítulo 5. Conectividad en la planificación para la conservación. Aplicación a sistemas fluviales mediterráneos.

Connectivity in freshwater systematic conservation planning.

La planificación para la conservación tiene como objetivo principal la selección de un conjunto de áreas que garanticen la conservación de la biodiversidad (Margules and Pressey, 2000). Estas áreas deben representar adecuadamente al conjunto de la biodiversidad y garantizar su persistencia a largo plazo, separándola de los procesos que la amenazan. De esta forma se resuelven los problemas encontrados por otras estrategias de planificación previamente utilizadas como las basadas meramente en métodos de ordenación de áreas en función de su riqueza o rareza (Pressey and Tully, 1994), o las estrategias ad hoc mencionadas en el capítulo anterior. Ninguno de estos sistemas garantiza, entre otras cosas, la representatividad del total de la biodiversidad a conservar, puesto que un punto de máxima riqueza no tiene por qué contener el total de la diversidad, como tampoco uno de alta rareza, aunque ambos tengan un elevado valor. A diferencia de éstos, los mecanismos de selección que aquí emplearemos, basados en el principio de complementariedad, aseguran la representación adecuada del total de los elementos a conservar (Possingham y Ball, 2000).

Para realizar el ejercicio de planificación sistemática se utilizó el programa Marxan, una herramienta creada específicamente para abordar este tipo de asuntos (Possingham y Ball, 2000). Este programa se basa en la aplicación de un algoritmo de selección que considera la complementariedad y el diseño espacial de las reservas. El proceso de selección se basa en la minimización de una función de objetivos en la que se consideran diferentes parámetros relativos a penalizaciones de soluciones óptimas que no incluyen adecuadamente el total de taxones a conservar, el diseño espacial de las reservas o los costes de protección (Fórmula 1). El objetivo final es representar adecuadamente un conjunto de especies en el menor y más compacto área posible, reduciendo así la fragmentación. Con ello reducimos los costes de conservación mientras que aumentamos la probabilidad de persistencia de la biodiversidad, puesto que como ha sido demostrado, el efecto de los agentes perturbadores externos disminuye a medida que aumenta la compacidad de la reserva (ver Possingham y Ball, 2000 para más detalles sobre Marxan).

$$\mathit{Función\ de\ objetivos} = \sum_{\mathit{taxones}} \mathit{Penalizaciones\ por\ taxones} + \sum \mathit{Longitud\ límites} + \sum_{\mathit{unid\ planif}} \mathit{Costes}$$

Fórmula 1. Función de objetivos a minimizar en el proceso de diseño de una red de espacios protegidos mediante Marxan.

El procedimiento de minimización aplicado en Marxan, denominado *simulated annealing*, consiste en un proceso iterativo de introducción y extracción de unidades de planificación a partir de un conjunto aleatorio inicial. Así se reduce al máximo la magnitud de la función de objetivos comentada. A diferencia de otros procedimientos iterativos que buscan continuamente la reducción de esta función, el *simulated annealing* permite aleatoriamente la entrada de unidades que no conlleven directamente una reducción inmediata de la función, pero que podrían suponer nuevas vías para la búsqueda de soluciones aun más óptimas. El número de iteraciones puede ser controlado, aunque el uso de un número excesivamente elevado de ellas puede ralentizar el proceso de selección.

Sin embargo, la identificación de una única solución supone una estrategia bastante rígida que no aporta ningún tipo de información sobre el valor relativo de cada área, incluso de aquellas no incluidas en esta solución óptima (Pressey et al., 2004; Cabeza and Moilanen, 2006). Para flexibilizar el resultado final se utiliza el concepto de *irreemplazabilidad*, que indica la probabilidad de una determinada área de ser requerida para conservar un conjunto determinado de taxones (Pressey et al., 1994; Ferrier et al., 2000).

La adaptación de la planificación sistemática para la conservación de medios fluviales requiere la consideración de las características especiales de estos medios (Dunn, 2003). Entre otras debería considerarse especialmente la naturaleza continua y conexa de los ríos (Pringle, 2001; Linke et al., 2007). Para considerar este hecho y atenuar en la medida de lo posible los efectos adversos de tramos perturbados aguas arriba (Pringle, 2001), se utilizó la regla espacial de selección propuesta por Linke et al. (2007). Según esta regla, un tramo fluvial no puede ser seleccionado independientemente por sí mismo sin incluir en dicha selección todos los tramos aguas arriba. Puesto que ésto puede influir excesivamente le proceso de diseño de la red de espacios protegidos, se atenuó la exigencia espacial en función de la distancia, es decir, fue más importante incluir las zonas más cercanas que las más alejadas aguas arriba.

Para el diseño de la red de áreas a proteger se utilizó el algoritmo de selección provisto en Marxan sobre la probabilidad de presencia de 9 especies nativas en un conjunto de 2170 unidades de planificación en toda la cuenca del río Guadiana. Una unidad de planificación es cada una de las pequeñas subcuencas en las que se dividió el área de estudio mediante el empleo de ARC Hydro (Maidment, 2002) provista en ArcGIS 9.1 (ESRI, 2002) a través de un mapa de elevaciones digitales. Las probabilidades de presencia utilizadas en este caso fueron obtenidas a través de modelos MARS (Tabla 7). Se trata de probabilidades de presencia real, puesto que para la construcción de los modelos fueron usadas todas las localidades de muestreo, independientemente del estado de conservación en el que se encontrasen, así como variables potencialmente afectadas por la acción humana (Tabla 2).

Tabla 7. Resultados de la fortaleza del modelo MARS-GLM. La devianza explicada (valores brutos y %, tanto por uno, entre paréntesis) indica la reducción de la devianza para cada especie con respecto a un modelo nulo. La capacidad del modelo para localizar correctamente las presencias-ausencias de cada especie se indica a través del AUC de la curva ROC. Se calculó mediante un procedimiento de remuestreo K-fold. La DE de los valores obtenida en el mismo análisis se muestra también entre paréntesis. La prevalencia está expresada como %, tanto por uno, de unidades de planificación ocupadas por cada especie (el número de unidades de planificación total corresponde a todas aquellas unidades en las que se encontró al menos una especie nativa).

Especies	Devianza explicada	ROC	Prevalencia (n=151)
Anaecypris hispanica	33,6 (0,41)	0,72 (0,11)	0,05
Luciobarbus comizo	44,7 (0,27)	0,75 (0,15)	0,24
Luciobarbus microcephalus	46,6 (0,25)	0,70 (0,20)	0,31
Luciobarbus sclateri	34,6 (0,34)	0,79 (0,21)	0,10
Iberochondrostoma lemmingii	42,9 (0,30)	0,73 (0,15)	0,28
Pseudochondrostoma willkommii	53,2 (0,24)	0,70 (0,20)	0,17
Cobitis paludica	32,2 (0,02)	0,57 (0,08)	0,66
Salaria fluviatilis	47,8 (0,42)	0,69 (0,28)	0,13
Iberocypris alburnoides	3,8 (0,18)	0,66 (0,12)	0,66
Squalius pyrenaicus	25,5 (0,42)	0,69 (0,16)	0,13
Media	36,5 (0,26)	0,71 (0,16)	0,16

Dada la ausencia total de cualquier información sobre costes de conservación de cada unidad de planificación, se optó por dar a todas ellas el mismo valor (1), centrando los esfuerzos en el resto de parámetros a controlar. La compacidad de la reserva fue afrontada a través del máximo *Boundary Length Modifier* (BLM) posible. Puesto que se ha demostrado que el área total de la solución óptima aumenta a medida que se incrementa el BLM (Carwardine et al., 2007), se probaron diferentes configuraciones hasta encontrar el mayor posible sin que el área total a proteger creciese excesivamente siguiendo las recomendaciones de Carwardine et al. (2007). La compacidad fue medida medida como la comparación de la relación area-perímetro, con la de un círculo del mism área, que es la solución más compacta posible. Vaores próximos a uno indican soluciones muy compactas. Además se utilizaron diferentes niveles de target, para evaluar el posible efecto de éste sobre la estructura espacial de la reserva, puesto que es esperable que a mayores niveles de target exigidos, mayor será el área necesaria a proteger.

Los valores de irreemplazabilidad corresponden a la proporción de soluciones en las que aparece cada unidad de planificación cuando el proceso fue repetido 100 veces (cada una de ellas con 1 millón de iteraciones).

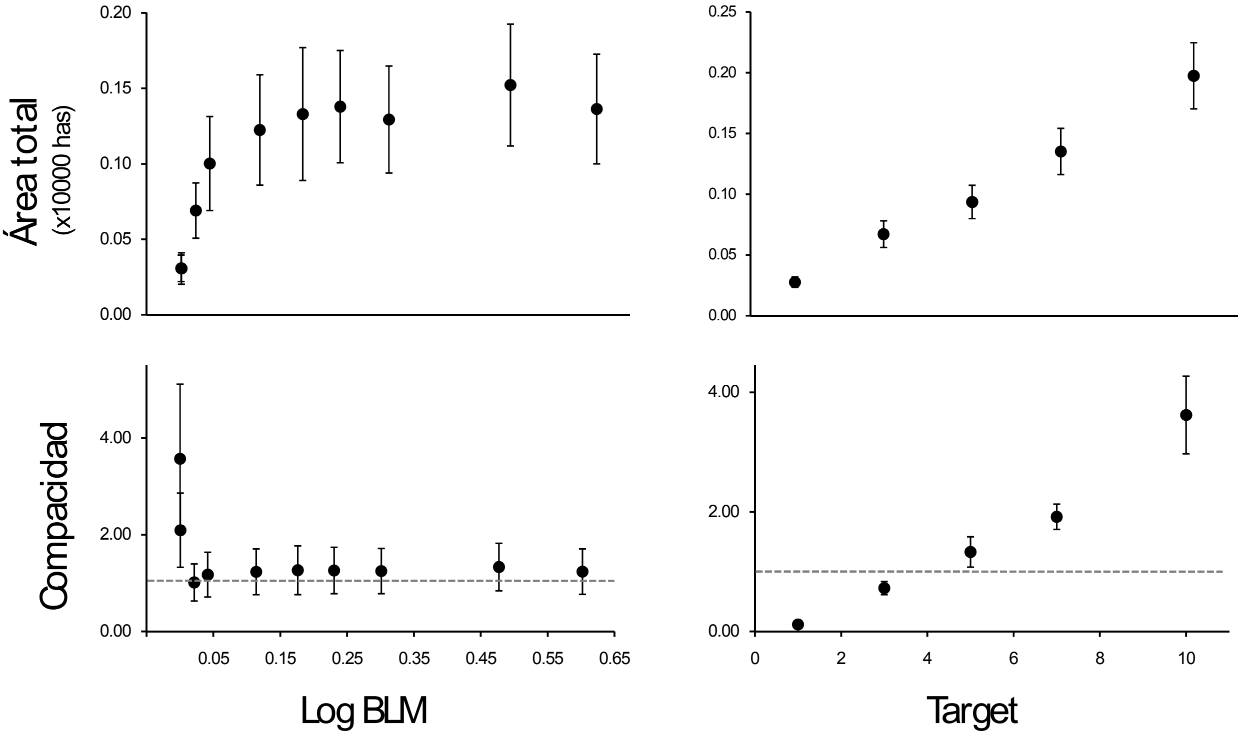

Figura 11. Área total y Compacidad (Media ± EE) para diferentes valores de Boundary length Modifier (BLM) y target (n= 49 combinaciones diferentes de BLM y target). La línea punteada indica el valor óptimo de compacidad (1).

Tanto el BLM, com el target influyeron sobre el área total a proteger y la compacidad de la reserva, aunque con respuestas de diferente tipo (Fig. 11). Además el BLM favoreció el correcto funcionamiento de la regla espacial, ya que cuando no fue usado (BLM=0) fueron incluidas el la mejor solución áreas muy desconexas localizadas incluso en el cauce principal del río Guadiana (Fig. 12).

Los resultados obtenidos centran tanto la mejor solución como los mayores valores de Irreemplazabilidad en la subcuenca del río Chanza (Fig. 11). Además aparecen otras pequeñas zonas cercanas a cabeceras como lugares de interés para la conservación de la biodiversidad total. Estos resultados concuerdan con los presentados en el capítulo anterior, donde el río Chanza aparecía como una de las zonas prioritarias para la conservación, dado el buen estado de conservación de sus comunidades de peces a lo largo de todo su recorrido. El hecho de encontrar una subcuenca completa como zona a reservar es bastante interesante de cara a dotar de sentido práctico a la solución. Puesto que, como se ha demostrado en estudios anteriores, las comunidades de peces en el área de estudio se organizan a lo largo del gradiente longitudinal (Blanco-Garrido et al., 2006), la inclusión de una subcuenca completa en la que podamos encontrar todo el gradiente ecológico garantiza la atenuación de posibles problemas de fragmentación, que en cualquier caso es muy grave dado el elevado grado de regulación de la cuenca.

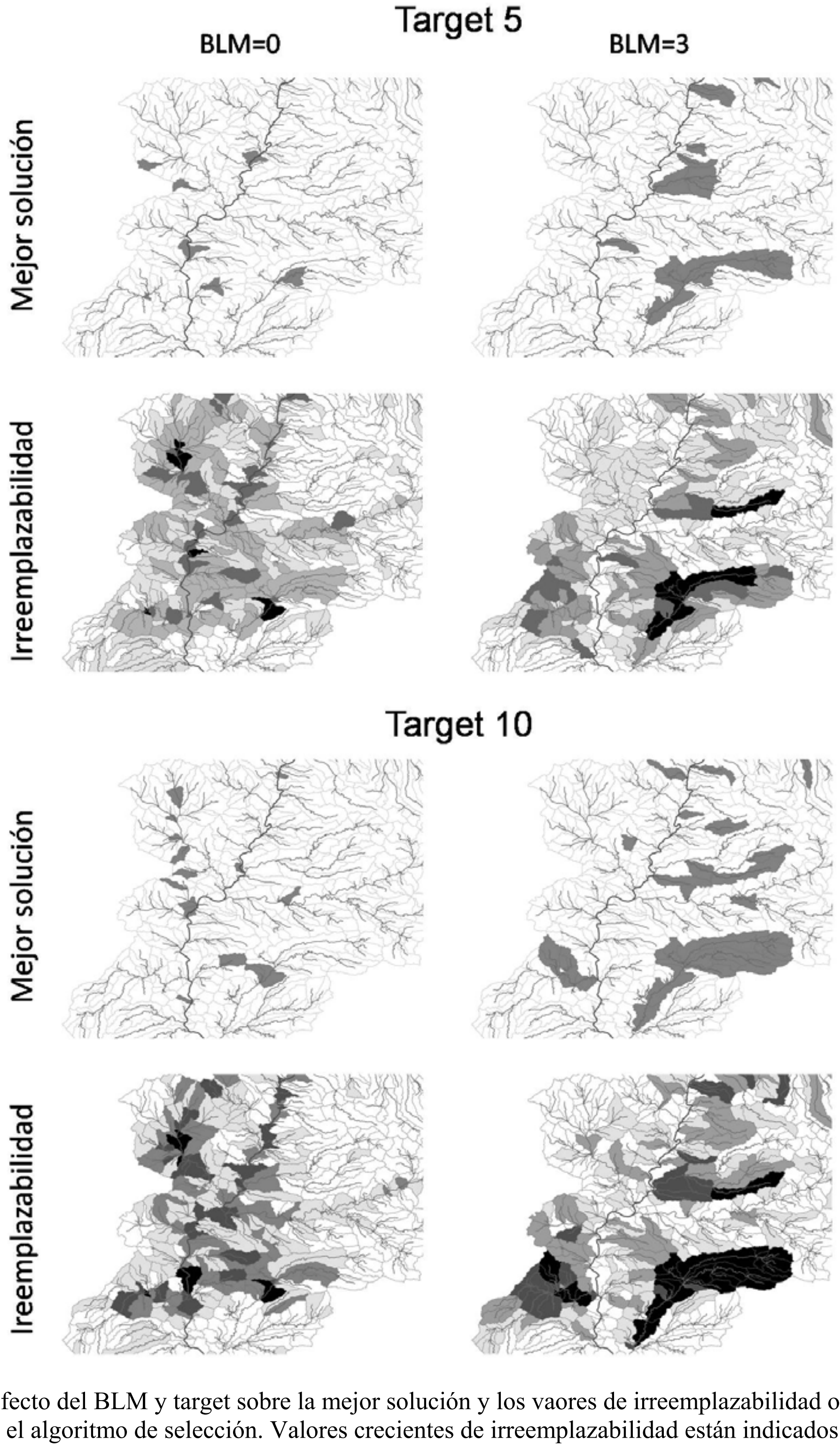

Fig 12. Efecto del BLM y target sobre la mejor solución y los vaores de irreemplazabilidad obtenidos mediante el algoritmo de selección. Valores crecientes de irreemplazabilidad están indicados en colores más oscuros. Para facilitar la comprensión del efecto de ambos factores, sólo se ha representado una porción de la cuenca, que es además la zona donde se concentran las áreas con mayores valores de irreemplazabilidad y las seleccionadas como mejores soluciones.

El empleo de distribuciones reales basadas en probabilidades de aparición amplia adicionalmente las garantías de persistencia de las especies estudiadas (además del diseño espacial de la reserva) (Cabeza, 2003; Cabeza et al., 2004). Estas probabilidades tienen en consideración diversos factores determinantes de la distribución de las especies como sus requerimientos de hábitat o la vulnerabilidad a amenazas (sensibilidad ante diferentes agentes de perturbación) (Araújo and Williams, 2000), por lo que la selección de las áreas con las mayores probabilidades de presencia garantiza su persistencia en dichas zonas al menos si las condiciones generales se mantienen estables. Por tanto, el esquema actualmente presente de múltiples usos y aprovechamientos de las zonas destacadas podría seguir siendo mantenido sin poner en peligro la conservación de la biodiversidad que aun mantienen en sus cauces. Sin embargo, esto no debería significar la falta absoluta de control y manejo del área, como han indicado algunos autores en otros casos analizados (Cowling et al., 2003; Lindermayer et al., 2000), puesto que el conocimiento del estado de las principales amenazas es imprescindible para garantizar el mantenimiento de la diversidad de especies de una cuenca (Willson et al., 2005). Por el contrario, ya que se ha destacado la zona como un lugar de especial interés para la conservación en el río Guadiana, sería muy recomendable prestar especial atención al seguimiento de las poblaciones de peces nativos y la atenuación, en la medida de lo posible, de los factores que amenacen su persistencia. En concreto, se recomienda mantener un adecuado control sobre la proliferación de especies exóticas puesto que es una de las mayores amenazas para los peces nativos.

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CAPÍTULO 1

ARE LARGE-SCALE LANDSCAPE CLASSIFICATIONS DERIVED FROM THE WFD ADEQUATE TO EVALUATE THE ECOLOGICAL STATUS OF IBERIAN RIVERS?

¿Son las clasificaciones derivadas de la DMA adecuadas para evaluar el estado ecológico de los ríos ibéricos de régimen mediterráneo?

(En revisión en Freshwater Biology)

Are large-scale landscape classifications derived from the WFD adequate to evaluate the ecological status of Iberian rivers?

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SUMMARY

1. The European Water Framework Directive (WFD) requires the protection and restoration of biological integrity as part of the water quality standards of all European water bodies. It establishes a type-specific approach to define reference conditions to be used in the evaluation of the ecological status by using a priori landscape classifications. However, these classifications systems are not related to biological data at all, despite the accuracy of the assessment relying on it.

2. We tested the biological significance of the WFD landscape groups by relating groups in a Mediterranean basin to freshwater fish assemblages in reference sites and examined its influence on predictive models performance. We compared predictive models using two different classification approaches (a priori vs a posteriori) within the same predictive model (RIVPACS) and the improvement when avoiding the classification (ANNA).

3. The WFD classification did not group reference sites better than randomly, with low within-group heterogeneity and high between-group homogeneity. However,as a correspondence analysis showed, there was a real underlying gradient. Instead of being patchily distributed, the fish community underwent gradual changes through a longitudinal habitat gradient.

4. As a consequence, the classification-based predictive methods failed in fitting the biological data and predicting the expected fish community hence. Only the continuous predictive approach succeeded and was accurate enough to be used in bioassessment.

5. Synthesis and applications: This study shows two major points: a) the landscape classifications do not always coincide with biotic assemblages (in our case they do not at all), b) this non-concordance has severe consequences for bioassessment which is one of the main purposes of the classifications and the WFD itself. Before using landscape classifications as a basis for bioassessment, we suggest to verify the accuracy of the classifications towards the chosen

bioindicator. Alternatively, a continuous approach without pre-defined groups should be considered.

Keywords: ANNA, bioassessment, continuous, Mediterranean, predictive model, reference condition, RIVPACS.

INTRODUCTION

Landscape classifications are increasingly being used in conservation planning and biodiversity management, although there is a lack of studies showing concordance between such classifications and biodiversity patterns (Heino & Mykrä, 2006; Oliver et al., 2004; MacNally et al., 2002). The popularity of classifications is based on the assumption that each subdivision of streams has some consistent biological significance (e.g. Frissell et al., 1986) and the use of these subdivisions will somehow decrease the biological variance of the studied communities and make it easier to study, understand or manage (Rabeni et al., 2002).

These landscape classifications are frequently used to predict the expected conditions that should occur at individual sites through predictive models. The idea that site condition can be inferred from landscape features is based on the view that local ecological attributes of aquatic ecosystems are strongly influenced by their catchments (Johnson & Goedkoop, 2000; Johnson, 1999; Hynes, 1975). However if used as the only stratification of biological condition, large-scale regionalization will only be of limited use in aquatic bioassessment, where it is critical to specify expected conditions as accurately and precisely as possible (Hawkins et al., 2000a).

To be useful in bioassessment, landscape classifications should satisfy two main conditions, i) correspond to the distribution of species and hence identify recurring biotic assemblages (Heino & Mykrä, 2006), and ii) portray relationships between biological and physical features including environmental classifiers which reflect ecologically important factors for the biota (Angermeier & Schlosser, 1995; Karr & Chu, 1999). If a classification satisfies all of the above, both type I (inferring impairment when it does not exist) and type II (not detecting impairment when it does exist) statistical errors are reduced (Hawkins et al., 2000a) increasing its value as a water bioassessment tool. For that reason a rigorous evaluation of how landscape classifications are able to capture and characterize natural biotic variability (biological significance of landscape classifications) is needed and highly recommended.

The main aim of the European Water Framework Directive (WFD) is the establishment of a framework to protect and improve the quality of all the European aquatic ecosystems (E.C., 2000). To achieve biological goals within the WFD, the ecological status of all the water bodies needs to be characterised, identifying the main pressures and recommend the most suitable actions with which these water bodies will reach a *good* ecological status before 2015. This ecological status should be defined relative to its deviation from a reference condition in the absence of human disturbances. The WFD establishes a type specific approach to define reference conditions which are currently described for each different water body type, previously identified by a landscape classification system. However, these landscape groups are not validated using the biological data which is going to be used in the establishment of the ecological status.

In this study we examine the biological significance of the WFD landscape classification in a Mediterranean basin in south-west Spain. After examining the concordance between the classification and the freshwater fish assemblages, we highlight the implications for future bioassessment programs. Since most of actual bioassessment tools rely on predictive models, we compare the effect of the a priori WFD classification on predictive model performance against an a posteriori classification method and a continuum predictive approach.

STUDY AREA

The Guadiana River basin is located in the South-West of the Iberian Peninsula. It drains a total area of 67039 Km^2, shared by Spain (88.8%) and Portugal (17.2%), to the Atlantic Ocean. The main river flows through a smooth relief with an average altitude of 450 m. Only in the north and southwest boundaries where the most mountainous areas are located, the catchment reaches a maximum altitude of 1600 m. The Mediterranean climate follows a clear spatial gradient related to the Atlantic (West)-Continental (East) influence. Mean air temperature ranges from 13 to 18.1 ºC, with a strong intra-annual variation in extreme temperatures which in some areas reach up to 60 ºC (similar to Siberian and Saharan climates). Mean annual precipitation ranges from 350 to 1200 mm (with a mean of 450 mm).

For the implementation of the WFD, Spanish rivers were classified according to their physical and chemical characteristics following the specifications of *system B* (E. C., 2003; Ministerio de Medio Ambiente, 2005). The Guadiana River basin included six out of the 29 different landscape water body types identified through this methodology (Table 1).

METHODS

Fish community and habitat characterization

Fish communities were characterized in 242 sites throughout the whole basin using backpack electrofishing, covering all the 6 water body types. Sampling was conducted once at each location without block-nets. This sampling effort has been proved to be sufficient to capture most species present, except for large rivers as Felipe et al. (2004) suggest on a study in the same basin. All fish were released after we identified the individuals to species level.

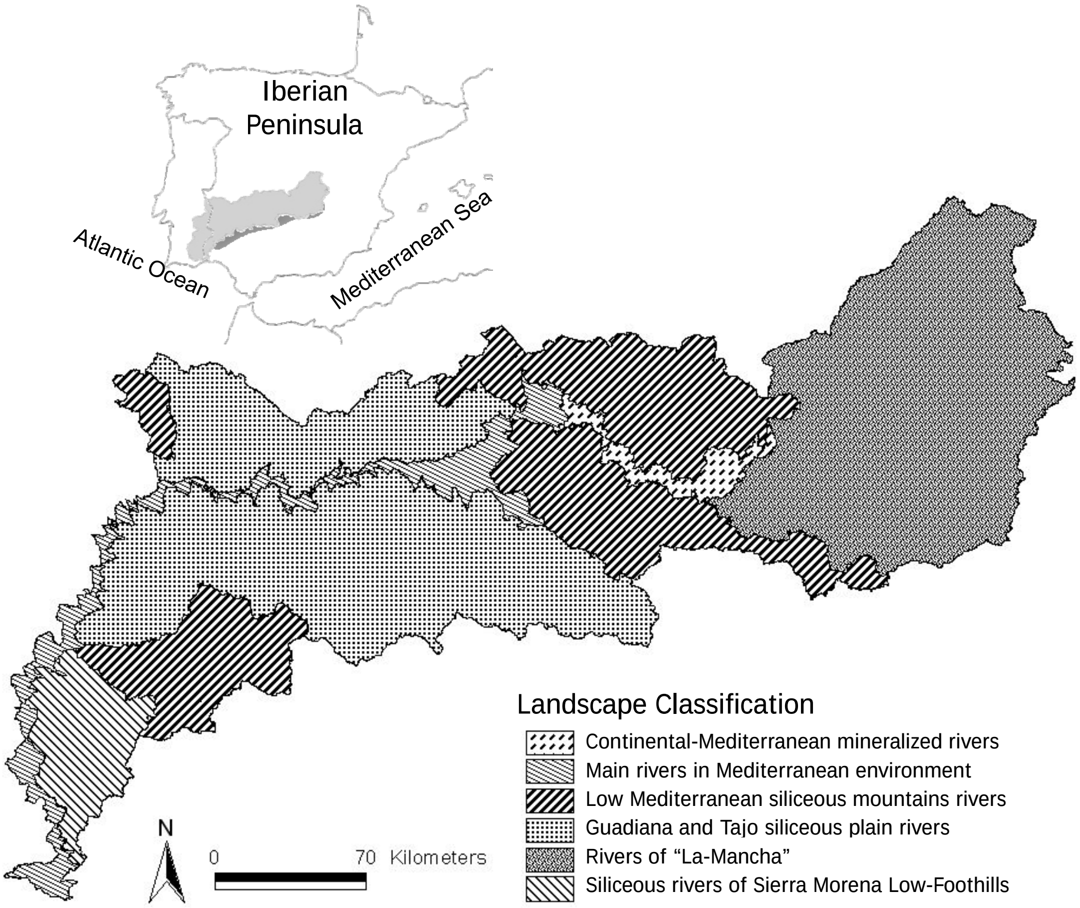

Fig 1. Location of the Guadiana River basin and the extent of the six WFD water bodies types included in this basin.

Since an ecoregional comparison with a fish community altered by human influences does not make sense, we only considered those sites less affected by human activities (reference sites hereafter). Reference sites are characterised by low urban or agricultural land uses within the whole basin and at the reach scale (500 m around the sampling point). Furthermore, bank and channel structure as well as the riparian zone should be in natural condition (Pont et al., 2004). To ensure that reference sites are not impacted by invasive fish, all sites where exotic species accounted for more of 5% of total fish abundance were also discarded (Kennard et al., 2006). We finally considered 55 reference sites distributed through all the landscape groups except *Continental-Mediterranean mineralized rivers.* Rivers in this group were omitted

Table 1. Set of environmental variables which better discriminated the six landscape water body types described in the Guadiana River basin through the WFD system B. Mean values of environmental variables within each group are shown.

Water body Type	Altitude (m. a. s. l.)	Annual mean temperature range (ºC)	Basin area (Km^2)	Mean flow (m^3/s)	Water Conductivity (µS/cm)	Stream order (Stralher)	Mean basin slope (%)	Mean air temperature (ºC)	% months with no flow
Continental-Mediterranean mineralized rivers	554	18.7	6,445	20.8	571	5	4	13.8	0
Main rivers in Mediterranean environment	286	18.0	34,132	164.8	390	6	3	15.3	2
Low Mediterranean siliceous mountains rivers	470	18.7	237	1.1	156	2	4	15.4	46
Guadiana and Tajo siliceous plain rivers	348	18.7	255	1	19.6	2	2	15.6	43
Rivers of “La Mancha”	736	19.7	763	1.1	656.6	3	1	13.8	7
Siliceuos rivers of Sierra Morena Low-Foothills	153	16.9	464	2.2	218	2	3	17.4	41

* Data source: Ministerio de Medio Ambiente, 2005

because of the high human pressure to which they are submitted and their poor conservation status hence. This group is exclusively located in the main Guadiana River channel through a highly intensive-agriculture transformed landscape (Fig. 1). We then tested the biological significance of landscape classifications for the remaining 5 water body types. To reduce noise introduced by very rare species, we did not consider species present in less than 5% of localities in the analysis above.

Habitat was characterised by 17 environmental variables, covering three different spatial scales: site, reach and basin. All of them were selected from a larger group of habitat variables for their relative independence of human perturbations. Two approaches were used in this characterization: field measures to characterize the habitat at the reach scale and remotely sensed measures to record data from available digital maps (Table 2). All these variables were tested for normality and conveniently transformed when necessary for further analysis.

Table 2. Set of environmental variables used to describe habitat characteristics. * Denotes those variables possibly affected by human perturbation and not included as predictors in ANNA and RIVPACS models. The transformations applied to environmental variables to ensure a normal distribution is also given.

Scale	Variable	Method	Code	Tranf.
Site	Water depth (cm)	*In situ*	DEP	Log(x+1)
	Water temperature *	*In situ*	TEM	
	River width *	*In situ*	WID	Log(x+1)
	Substrate coarseness (Wentworth scale) *	*In situ*	SUS	
	Shelter availability (m^2 of shelter/river width) *	*In situ*	SHE	
	Annual precipitation (mm/m^2)	GIS	PRE	Log(x+1)
	Solar radiation (10 KJ/m^2*dia*µm)	GIS	RAD	Log(x+1)
	Average annual air temperature (ºC)	GIS	ATEM	
	Elevation (m)	GIS	ELE	Log(x+1)
	Relative position within the sub-basin (dist. to the most headwater point/total length of the stream)	GIS	POR	Arcsin(x)$^{0.5}$
	Stream order (Strahler)	GIS	ORD	
	Distance to headwater (Km)	GIS	HED	Log(x+1)
	Distance to main river (Km)	GIS	MRD	Log(x+1)
Reach	Slope ($^0/_{00}$)	GIS	SLO	Log(x+1)
	Sinuosity	GIS	SIN	
Basin	Gravelius index	GIS	GRA	Log(x+1)
	Basin area (Drainage surface in each site, Km^2)	GIS	ARE	Log(x+1)

Testing the biological significance of WFD landscape classifications

Non-Metric Multidimensional Scaling (NMDS) was used to examine fish assemblage structure based on presence-absence data. We decided to use presence-absence since it is more suitable for comparisons between streams within a whole basin in a large spatial context, as our case. Abundance data is specially recommended for smaller areas where many taxa are widely distributed. Presence-absence data gives more weight to species with a reduced distribution than abundance data (Rabeni et al., 2002). NMDS was carried out on a Bray-Curtis similarity matrix, recommended because it ignores joint absences, the predominant case in site x species matrices (Faith et al., 1987). To test for significance in fish community composition among the landscape classification a Multiresponse Permutation Procedure (MRPP) was carried out. This is a nonparametric method designed for testing differences in assemblage structure among *a priori* defined groups (Mykrä et al., 2004) making few assumptions about the distribution structure of the data (Zimmerman et al., 1985). Based on the Sørensen dissimilarity coefficient, the statistic used in MRPP (A) compares observed within-group homogeneity to that expected by chance (obtained by 1000 Monte Carlo permutations). Thus, when all assemblages within-group are identical, the statistic reaches its maximum value of 1. If the within-group heterogeneity is identical to the homogeneity expected by chance then $A=0$, and if heterogeneity within-groups is higher than expected by chance $A<0$ (Paavola et al., 2003).

Finally, the Classification Strength (CS) of the WFD landscape groups was tested using the mean similarity approach (Van Sickle and Hughes, 2000). In our study CS is a measure of biological homogeneity within pre-defined groups ,calculated as the difference between mean of all within-group similarities (W) and the mean between-group similarity (B) ($CS=W-B$). Strong classifications are characterised by large values of CS (high within-group similarity and low between-group similarity).

Influence of landscape continuum on biota

To quantify the influence of local habitat and basin attributes on fish assemblages and compare this to the relationships with the landscape classifications, we correlated biotic and habitat gradients. For the habitat gradients we performed a Principal Component Analysis (PCA) on an environmental attributes x sites matrix. These environmental attributes were not influenced by human activity. To cover as much habitat heterogeneity as possible in our analysis we used all the prospected localities ($n=242$ sites). In the case of biotic gradients we carried out a Correspondence Analysis (CA) on a presence-absence x sites matrix. Here we only considered reference localities, to obtain a biotic gradient free of human influence. To evaluate the relationship between both gradients we calculated Pearson’s correlation coefficient (r).

Influence of landscape classifications on the accuracy of the assessment of the ecological status

Ecological status is commonly assessed by comparing current assemblages to expected biotic communities in the absence of human perturbation obtained from predictive models

(Oberdorf et al., 2002; Hawkins et al., 2000b). RIVPACS models (Clarke et al., 2003; Wright, 1995) are one of the most widespread and accepted methods for bioassessment. It predicts the probability that a particular taxon is present at a given site as a weighted average of its probability of occurrence in a set of pre-defined classifications groups (Clarke et al., 2003). Thus the accuracy of the bioassessment depends on the ability of the predictive methods to correctly predict the expected fauna.

To test the influence of imprecise classification systems on model accuracy, we built three different predictive models using the same data:

- A forced RIVPACS model in which we avoided the initial classification step by using the *a priori* WFD derived landscape groups
- A standard RIVPACS model, in which the groups were derived by a UPGMA classification (an a posteriori classification), based on the Bray-Curtis similarity of fish assemblages (Clarke et al., 2003).
- An existing ANNA model (Linke et al., 2005) for the region (see Hermoso et al., unpublished), in which sites are treated as a continuum and in which prediction is derived from the environmentally most similar sites. ANNA models finds the set of reference sites most similar to the target site and predicts the community composition of the target site based on the community composition of those nearest neighbours (Linke et al., 2005).

Hereby, we first compared the influence of the WFD classification on model performance through the same modeling approach (RIVPACS models). We then evaluated the improvement when avoiding the effect of the classification step (ANNA model) considering more precisely the continuum nature of rivers (Vanote et al., 1980).

Since the original number of reference sites we got within the Guadiana River basin was not large enough for model construction and validation, some reference sites from adjacent basins in the same biogeographical region (Tinto, Odiel and Guadalquivir basins) were used. All new reference sites belonged to the same landscape classes than the Guadiana sites, so they did not introduce additional variability for classification based approaches. Models were built on a reference set of 65 sites and validated on an independent data set (n=20 sites) by plotting the observed vs the expected species richness. As these sites are reference sites, a 1:1 correlation between observed and expected is anticipated. Model fit was checked through the regression line slope, intercept and R^2 (Linke et al. 2005) and the standard deviation of O/E values (Van Sickle, 2005) in the validation data set.

In case of a strong concordance of WFD classifications with fish assemblages, we expect the WFD-derived model to perform as strongly as a RIVPACS model based on biota-driven classifications and the very-well performing ANNA model (Hermoso et al., unpublished).

RESULTS

Biological significance of landscape classifications

Overall, no correlation with fish data was found for the landscape classification in any of the analysis carried out, indicating no biological significance. Mean within-group similarity was quite similar to between-group similarity which showed a very low CS value (Table 3). The high within-group heterogeneity is reflected in the scattered spatial distribution of reference sites in the NMDS plot (Fig. 2). MRPP showed that the internal heterogeneity displayed by the classification groups was similar to that expected if localities would be grouped randomly (A=0.12, P<0.001). Furthermore, the extensive overlap between groups in the NMDS ordination space indicated that WFD groups do not correspond to the real underlying gradients (Fig. 2). However, fish fauna distribution patterns followed a clear habitat gradient, indicated by the high correlation between the most informative CA axis (DIM1 accounted for almost one third of original fish community variance) and PCA component (PC1 explained more than a half of habitat variance) (Fig 3).

Table 3. Results of the biological significance of landscape classification analysis. It is shown the partial within-group similarity for each water body type considered (Wi) (all except Continental-Mediterranean mineralized rivers with no reference locality), mean all within-group (W) and between-group (B) similarity and the derived CS value.

Water body type	Wi	B	CS
Main rivers in Mediterranean environments	0.71	0.48	**0.07**
Low Mediterranean siliceous mountains rivers	0.50		
Guadiana and Tajo siliceous plain rivers	0.57		
Rivers of "La Mancha"	0.85		
Siliceuos rivers of Sierra Morena Low-Foothills	0.47		
W	0.56		

Influence of landscape classifications on model accuracy

The WFD groups did not deliver an adequate RIVPCAS model, with almost random predictions (Table 5). Although the standard model outperformed the WFD RIVPACS model, it was still very poor as the slope and intercept of the O/E line for the validation data set exceeded the limits proposed by Linke et al (2005) (Table 5). This steep slope increases the risk of Type I error when assessing ecological status, since the expected species richness will always be higher than the observed in reference conditions (Fig. 4). Thus the O/E score will be below 1 even in reference sites. These will be wrongly labelled as perturbed. Moreover, the predictive range was very narrow and never over four species, which increases the probability of Type II errors as well. RIVPACS approach would be inefficient detecting species loss in the richest sites and thus potentially fails to detect perturbation.

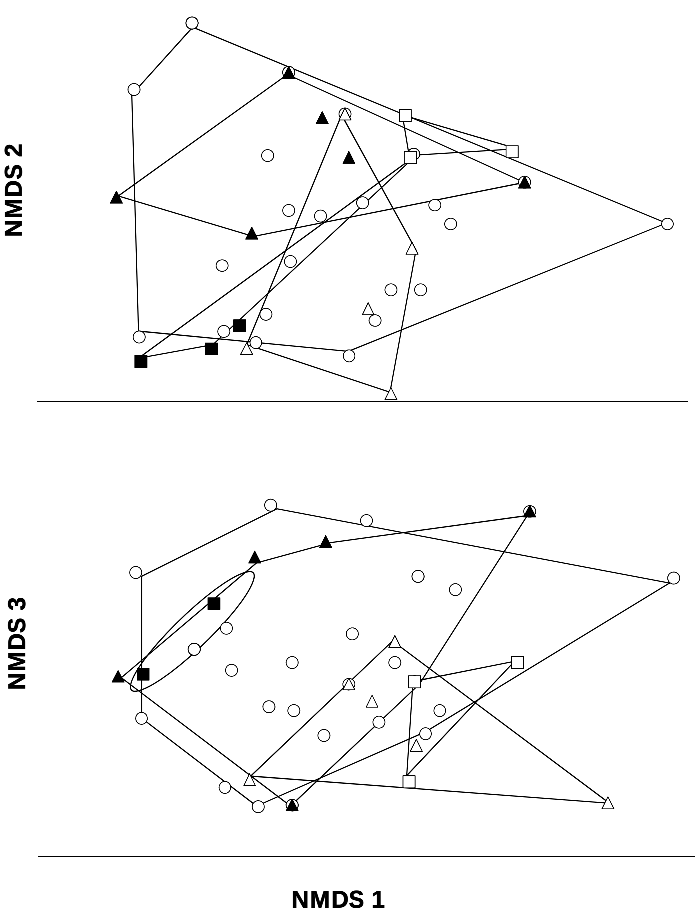

Fig. 2. NMDS ordination plots for reference sites. Different water bodies types are denoted as follow: Main rivers in Mediterranean environments, black squares; Low Mediterranean siliceous mountains rivers, white circles; Guadiana and Tajo siliceous plain rivers, black triangles; Rivers of "La Mancha, white squares; Siliceous rivers of Sierra Morena Low-Foothills, white triangles.

These problems are not an issue in the ANNA model. Its slope (not different from 1, b=1.063, t-test, p=0.58), intercept (not different from 0, a= 0.058, p=0.95), R^2 and SD (Table 5) are within the standard range of models (see Linke et al., 2005). This ensures that Type I and Type II errors in the bioassessment are lower than in the other models (Fig. 4). Furthermore, ANNA was the only model which correctly predicted the presence of all the species at least once.

DISCUSSION

Our study clearly shows that the WFD landscape classification is not concordant at all with fish assemblages, as the WFD landscape groups are not more biologically homogeneous than random groups (Table 3). Several studies have analysed the concordance between environmental classifications and biological communities and its implications on bioassessment. According to these studies, landscape classifications sometimes portray the variability of stream assemblages to some degree, although the strength of these classifications is often weak (Hawkins et al., 2000a; Van Sickle & Hughes, 2000; Heino et al., 2002). Particularly, Sanchez-Montoya et al. (2007) found that the correspondence between an *a priori* top-down classification and an *a posteriori* bottom-up classification approach based on macroinvertebrate assemblages for Mediterranean rivers was weak. Some of their a priori groups did not displayed significant biological differences with clear implications for the establishment of reference conditions. Since the accuracy of biological assessments depends on how well we can describe expected conditions at a test site our ability to detect and quantify biological degradation is largely limited by the extent to which we can match degraded sites with reference sites which

Table 4. Environmental gradients extracted from the PCA carried out on the environmental variables x sites matrix. Is shown Pearson correlation coefficients between each environmental variable and the first three Principal Components extracted from the PCA carried out on the environmental variables x sites matrix (n=242). *p<0.05; **p<0.01; ***p<0.001. Variables codes are shown in Table 1.

Variable Eigenv.	**PC1** (52.2%) 8.9	**PC2** (13.4%) 2.7	**PC3** (11.5%) 1.8
HED	0.30*	-0.32*	0.89***
POR	-0.19	-0.29*	0.93***
MRD	0.49***	0.55***	0.13
ARE	0.98***	-0.10	-0.04
GRA	0.26	-0.06	-0.19
SIN	0.92***	0.14	0.02
PRE	0.98***	0.11	0.01
RAD	0.99***	0.09	0.01
ATEM	0.99***	0.00	-0.02
TEM	0.91***	-0.03	-0.04
SLO	-0.23	0.56***	0.05
ELE	-0.95***	0.07	-0.05
ORD	-0.26	-0.83***	-0.20
DEP	0.62***	-0.39**	-0.11
WID	0.03	-0.89***	-0.21
SUS	-0.94***	-0.16	-0.02
SHE	-0.78***	0.40***	0.11

represent the biological potential of test sites (Hawkins et al, 2000b). The WFD establishes a type-specific approach to define reference conditions via a landscape classification system for each different water body type. However this landscape classification was far from an ideal classification in our study, as characterised by low classification strength values and a high within group similarity (Dodkins et al., 2005) what could disqualify it from attaining the goals for which it was originally conceived.

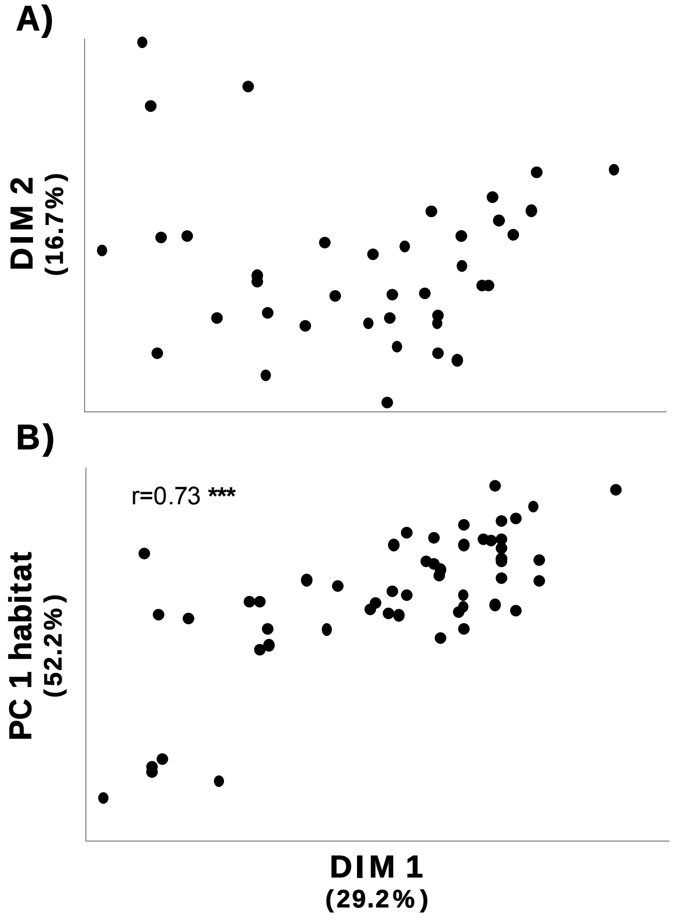

Fig. 3. Ordination plot of the 55 reference sites included in the Correspondence Analysis A), and Pearson correlation between the most informative habitat and biotic gradient axes (PC1 and DIM1 respectively) with indication of the Pearson´s coefficient. B). The % of original variance explained for the different gradients are also shown between brackets. *** denotes p<0.001.

The lack of discrete grouping on the ordination has traditionally been linked to the effect of many taxa varying independently and weakly in response to environmental gradients (Heino & Mykrä, 2006; Sheldner et al., 2004; Heino et al., 2003). However this seems not to be the reason of the poor performance of the landscape WFD groups since a clear community response to the main environmental gradient did exist. It is largely known that biotic communities undergo gradual responses to habitat gradients (Clavero et al., 2005; Welborn et al., 1996;

Allan, 1995; Rahel & Hubert, 1991). In this respect, we found a continuous change in community composition along a longitudinal (headwater-mouth) environmental gradient. The effects of these gradual biological changes in response to habitat gradients on grouping performance have extensively been reported in scientific literature: (i) Van Sickle & Hughes (2000) showed that a classification based on the proximity of sites was stronger than any other a priori classification. (ii) McCormick et al (2000) concluded that similarities among sites declined as a function of the distance between sites. (iii) Hawkins & Vinson (2000) showed that classifications in which sites within a class were located relatively close to one another were stronger than classifications in which sites were located in geographically non-contiguous classes. Thus, the inability of the WFD landscape groups to account for the observed longitudinal zonation of biotic communities (Hawkins & Vinson, 2000; Snelder et al., 2004) seems to be the main reason of the poor grouping performance that they displayed.

Table 5. Parameters describing the O/E regression line for the validation data set (n=20 sites) in three different predictive approaches. SD denotes Standard Deviation. The SD of the best possible model and the null model sensu Van Sickle et al. (2005) is also given between brackets. Since the validation data set was the same for all the models, these values are shared by all of them.

Predictive method	Slope	Intercept	R^2	SD (0.38-0.45)
WFD-RIVPACS	0.28	1.37	0.05	0.41
Standard RIVPACS	1.36	-0.89	0.22	0.40
ANNA	1.06	0.06	0.43	0.39

Different approaches are been used for predicting the expected conditions against to which compare the real observed data (Wright, 1995; Simpson & Norris, 2000; Linke et al., 2005). Here we compared not only the relative performance of an *a priori* vs an *a posteriori* classification approach through the same predictive model (RIVPACS), but also the improvement when avoiding the classification step through a new predictive method which relay on the concept of streams as biological continua (Linke et al., 2005).

As expected, the absence of biological concordance of the WFD landscape groups had immediate consequences on model performance. The WFD RIVPACS model did not predict species richness at new sites better than randomly and would thus be completely useless in determining the degree of impairment of these sites if it was incorporated in a future bioassessment tool. This is not a surprising conclusion as the high within group heterogeneity displayed by the WFD landscape groups did not allow the WFD RIVPACS model to match particular fish assemblage compositions to specific environmental conditions. However this habitat-biota relationship did exist (Fig. 3), but was veiled by the inaccurate WFD classification as was discussed above. As Hawkins & Vinson (2000) concluded for macroinvertebrate assemblages and Newall & Magnuson (1999) showed for freshwater fish, a priori systems of landscape classification are weak predictors of the biological structure of streams and, when used alone, cannot provide a reliable basis for establishing the biotic conditions expected in the absence of human disturbance. Thus, given its proven inaccuracies this WFD derived classification should not be used in fish-based bioassessment in the current mode at least. This also highlights the need for biological validation of landscape classifications before their inclusion in any bioassessment tool.

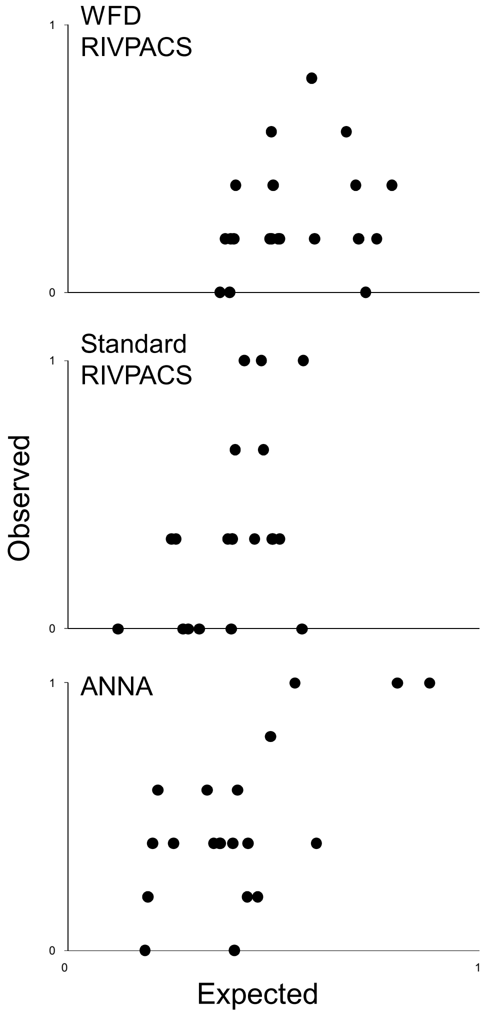

Fig. 4. Plot of observed vs. expected native species richness for the validation data set (n=20) in three predictive approaches. Data on predicted and observed species richness has been rescaled (0,1) to allow an easier comparison.

The a posteriori classification approach (standard RIVPACS model) had better model results than the a priori classification (WFD RIVPACS), although both were outperformed by the continuous approach through the ANNA model. Given the clear biotic pattern we found, assemblages would not be expected to show much tendency to cluster into discrete groups, and any classification of species into a few groups would thus allow a coarse estimation of the actual fauna expected to occur at a site (Hawkins & Vinson, 2000) even when using a posteriori classifications as the standard RIVPACS did. That is the main reason why the standard RIVPACS model deviated from to the rule of thumb by Linke et al. (2005). Its predictions entailed a high risk of type I and II statistical errors, hindering its use for assessing the degree of impairment of sites. On the other hand, the ANNA model was an average/good model. As expected from the biological patterns uncovered in the first section of the paper, a model which accounted for the continuous nature of streams would better fit the biological data and would deliver more accurate predictions - as the ANNA model did. The main advantage of using this predictive approach is its flexibility in selecting the reference sites used to predict the expected assemblage at a given site. Thus, predicting assemblage structure benefits from using continuous approaches instead of stream type classes as Linke et al. (2005) and Heino & Mykrä (2006) recommended.

The implementation of bioassessment programs for the protection and restoration of biological integrity as part of the water quality standards required by the WFD supposes a great advance in the management of European´s freshwater ecosystems. However, although the WFD is not very specific and it only provides general guidance in key factors (for example on how to define and determine the ecological status), it sets very strict rules on other essential aspects, such as the use of a classification system to establish reference conditions. As our results and other studies suggest, this could be a major obstacle for the efficient implementation of the WFD itself.

In summary, two key lessons are to be learnt from this study (and are backed up by the literature):

1. Environmental classifications often have no biological meanings. Then bioassessment methods that classify sites into a few discrete groups are likely to be limited in how precisely they can specify expected conditions, and thus de degree of biological impairment that they can detect.
2. Continuous systems are sometimes more accurate than classifications. They avoid the effects of imprecise classifications and better portray the continuous nature of rivers.

So when stream classifications affect the precision of bioassessment as severely as our analysis indicates it is highly recommended the use of methods that do not rely on site classification for specifying reference conditions (Hawkins & Vinson, 2002). Instead of that we recommend the use of methods which avoid artificial classifications and recognise streams as biological continua through site-specific measures which allow more refined predictions of the expected fauna and more sensitive assessment of its biological impairment hence.

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CAPÍTULO 2

ASSESSING FRESHWATER FISH SENSITIVITY. THE NEED TO ACCOUNT FOR DIFFERENT SOURCES OF COVARIATION

Evaluación de la sensibilidad de los peces continentales. Necesidad de tener en cuenta diferentes fuentes de covariación

(En revisión en Ecology of Freshwater Fish)

Assessing freshwater fish sensitivity. The need to account for different sources of covariation.

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ABSTRACT

There is an urgent need to assess the ecological status of rivers due to the generalized degradation to which freshwater ecosystems are submitted. The European Water Framework Directive and other international laws require the protection and restoration of biological integrity as an essential aspect of water quality standards through the implementation of bioassessment programs. The accuracy of such programs is highly limited by the precision of the systems used to derive sensitivity values for the organisms used as indicators. We provide quantitative support to the objective evaluation of 10 native freshwater fish species´ sensitivity to human disturbances. This supposes and important advance to the use of freshwater fish within bioassessment programs, since expert judgment has traditionally been applied for deriving freshwater fish sensitivity values to be integrated in fish-based biotic indices. Presence-absence data was used to avoid the inaccuracies related to wrong abundance estimations and to discard them from future bioassessment tools. The need to account for covariation not only between stressors and natural gradients but also between different stressors are considered to increase the reliability of the sensitivity values and to deep on the knowledge on the question, sensitive to what? With this aim, species´ responses to a global perturbation gradient and a set of independent stressors were checked. River size effects and predictive model inaccuracies were the responsible for the observed differences in sensitivity values between our approach and two alternative methods. All analyzed species were found to be sensitive to any of the main stressors described in the study area and good indicators of specific sources of human impairment hence. This enables the use of these species in future bioassessment tools to make effective evaluations and accurate diagnostics.

Keywords: Bioassessment, freshwater fish, Mediterranean, tolerance, Water Framework Directive.

INTRODUCTION

Freshwater ecosystems are submitted to an accelerated rate of transformation due to the intensive human use they suffer (Vitousek 1994, Collares-Pereira and Cowx 2004, Prenda et al. 2006) which implies a critical threat to a substantive quote of the global biodiversity they hold (Abell 2002). As an example, only freshwater fish comprise one-fourth of all living vertebrate species (Abell 2002) and recent assessments suggest that over 30% of them are seriously threatened (World Conservation Union 2000). Thus, there is a critical need to assess the ecological status of freshwater ecosystems and determine how they are being affected by human transformations (Revenga and Kura 2003). Many international laws as the Clean Water Act in the US or the European Water Framework Directive (WFD) (E.C. 2000) try to address this problem requiring protection and restoration of biological integrity as part of the water quality standards. The WFD warrants the application of such principles through the development of bioassessment programs using four different biotic water quality indicators (diatoms, macrophytes, macroinvertebrates and fish). These programs must lead all the European rivers to a good ecological status by 2015.

Freshwater fish assemblages have been used to assess river condition for more than 100 years (Simon 1999) through different approaches (e. g. Karr 1981, Oberdorff et al. 2002). The position of fish at or near the top of food webs makes them potentially good integrators of ecological processes of streams (Hynes 1970). The ability of fish to act as indicators over large spatial (because of their mobility) and temporal scales (because of their longevity) increase their utility for bioassessment (Harris 1995). Despite all these advantages, the use of fish as impairment indicators requires an accurate knowledge on species' sensitivities to such sources of impairment. Many studies have dealt with the assessment of sensitivities for macroinvertebrate taxa (Yuan 2004, Cao and Hawkins 2005, Carlisle et al. 2007), but there is a general dearth on empirical studies which specifically address this essential issue for freshwater fish species. Moreover they only partially face this essential question, lacking answers to the question sensitive to what? through incomplete assessments related to specific sources of perturbation (Meador and Carlisle 2007).

Tolerance to impairment refers to the degree to which an organism can withstand stressors related to human disturbance (Yuan 2004). Therefore more tolerant organisms can withstand more highly disturbed environments, but this does not necessarily imply that they could persist in a broad range of conditions, as Shelford´s law of tolerance suggests (Shelford 1911). The designation of tolerance values is based on interpreting characteristics of different taxon-environment relationships, in a manner similar to that used in investigations of ecological niches (Yuan 2004). Different procedures are being used to estimate sensitivity values of aquatic organisms, including expert judgment (Oberdorff et al. 2002), empirical analysis (Meador and Carlisle 2007, Carlisle et al. 2007) or modelling approaches (Armitage et al. 1995, Yuan 2004, Cao and Hawkins 2005). Then these values are incorporated into biotic indices which mainly compare the expected community composition in the absence of human perturbation with that observed, following the Reference Condition Approach (Wright et al. 1984, Reynoldson et al. 1997). When a sensitive species is expected to be present with a high probability but not observed, the effect of any human perturbation can be inferred. However, species' diagnostic power is infra-used when using sensitivity values simplified in two subjective categories (tolerant/intolerant) (Oberdorff et al. 2002, Pont et al. 2006). Moreover, most of previous studies (usually centered on macroinvertebrates) have defined organisms' sensitivities with respect to a single gradient of anthropogenic disturbance or source of perturbation (Armitage et al. 1983;,Lenat 1993). A finer knowledge on species sensitivity to particular sources of perturbation would allow managers to face more accurate diagnostics of potential causes of impairment (Norton et al. 2000) and to start efficient corrective programs hence. However the interpretation of sensitivity values for individual stressor factors should be made with caution because all the stressor can co-vary among them (Meador and Carlisle 2007) and along natural gradients (Yuan 2004). River size (or longitudinal gradient) has been pointed out as a key factor explaining freshwater ecosystems ecology in general (Vannote et al. 1980, Pringle 2001) and community composition (Angermeier and Schlosser 1989, Matthews 1998, Magalhães et al. 2002). The effect of natural gradients on sensitivity values is expected to increase as species´ (or any other taxa level) home range does, since the larger the home range, the broader environmental conditions they occupy. So this is a special issue to be considered in the case of fishes which usually display medium-large spatial domains.

The motivation of the present study is the urgent need for implementing biotic indices based on freshwater fish communities to evaluate the conservation status of worldwide rivers in a systematic and effective way. Our main aim is to estimate species´ sensitivities to human disturbances facing the question of co-variation and trying to derive sensitivity values accurate enough to make reliable diagnostics. These results could then be integrated in future bioassessment programs ensuring objective evaluations and allowing accurate diagnostics.accurate diagnostics.

METHODS

Study area

The Guadiana River basin is located in the South-Western Iberian Peninsula draining a total area of 67039 Km^2 to the Atlantic Ocean. It features a typical Mediterranean climate, with high intra and interannual discharge variation, with severe and unpredictable floods between autumn and spring and persistent summer droughts (Gasith and Resh 1999). Mean air temperature ranges from 13 to 18.1 ºC, with a strong intra-annual variation in extreme temperatures. Mean annual precipitation ranges from 350 to 1200 mm (with a mean of 450 mm).

Although it is not an overpopulated area (28 hab/Km^2), landscape has been deeply transformed during the last century by agricultural activities. Almost a half of the basin (49.1%) is currently used for agriculture (30.6% occupied with intensive agriculture as irrigated lands and 18.5% occupied with extensive agriculture, like olive groves or fruit trees). As a consequence, about 8300 Hm3 of water is retained in 86 large reservoirs (>1 Hm3) and more than 200 small ones (<1 Hm3) for water supply. Other common human perturbations are related to river channel modifications due to river channelization and degradation and even completely depletion of the riparian forest.

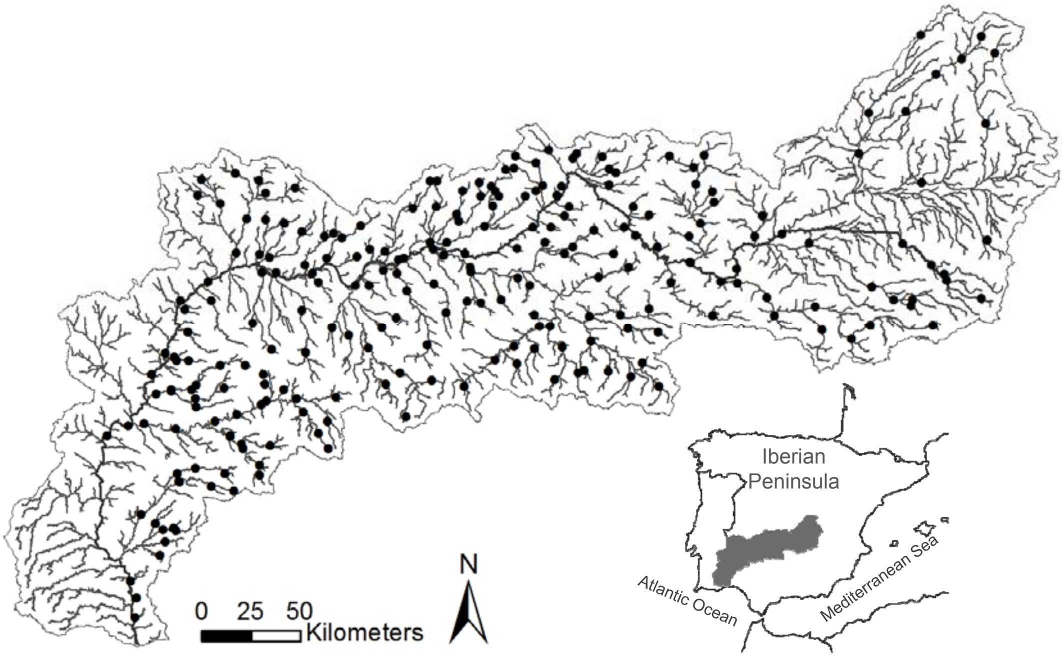

Fig. 1. Guadiana River basin and location of sampling sites.

Fish community and habitat characterization

Fish community was characterized in 241 localities through the whole basin, using electrofishing. Sampling was conducted once at each location without block-nets along 100 m long stretches. This sampling effort has been proved to be sufficient to capture most species present, except for large rivers, as Collares-Pereira et al. (2004) suggest on a previous study. All fish were identified to species level and then returned to the water. Only native species were tested for their sensitivity, since exotics presences are highly dependent on human introductions and a particular site may have introduced species simply because they were introduced there.

Table 1. Environmental variables used to characterize the sampled sites. * Denotes human potentially perturbed variables and used to describe stressor gradients.

Scale	Variable	Method	Code	Mean	Range
Site	Water depth (cm)	*In situ*	DEP	42.8	7.0-200
	Shelter availability (m^2 of shelter/river width)	*In situ*	SHE	5.6	0.0-60.6
	Elevation (m)	GIS	ELE	384.1	7.1-974.9
	Relative position (dist. to the most headwater point/total length of the stream)	GIS	POR	0.47	0.04-1.00
	Stream order (Strahler)	GIS	ORD	2.1	1.0-6.0
	Distance to headwater (Km)	GIS	HED	68.1	3.6-1,036.1
	Distance to Guadiana River (Km)	GIS	GUA	58.2	0.0-196.0
	River width (m) *	*In situ*	WID	10.8	1.4-123.0-1.4
	Substrate coarseness (Wentworth scale) *	*In situ*	SUS	5.3	1.0-9.0-1.0
	Riparian Quality Index (QBR, Munne et al., 2003) *	*In situ*	QBR	61.8	0-100-0
	NH_4^+ (mg/L) *	*In situ*	AMO	1.38	0.02-51.60
	NO_2^- (mg/L) *	*In situ*	NTI	0.10	0.01-2.00
	NO_3^- (mg/L) *	*In situ*	NTA	4.09	0.50-55.90
	PO_5^{3-} (mg/L) *	*In situ*	PHS	1.00	0.05-23.20
	SO_4^{2-} (mg/L *	*In situ*	SLF	110.1	10.0-2380.0
	Cl^- (mg/L) *	*In situ*	CLR	56.1	2.0-834.0
	Water temperature (ºC) *	*In situ*	WTE	20.5	9.4-32.6
	Conductivity (µS/cm) *	*In situ*	CND	624.7	38.0-3230.0
	pH *	*In situ*	PH	7.84	2.21-10.63
	Annual precipitation (mm/m^2)	GIS	PRE	593.1	370.2-1114.5
	Solar radiation (10 KJ/m^2*day*µm)	GIS	RAD	2033.9	1646-2227
	Average annual air temperature (ºC)	GIS	ATEM	15.85	13.0-18.0
	Distance to the nearest reservoir upstream (Km) *	GIS	DUP	41.1	0.0-196.0
	Distance to the nearest reservoir downstream (Km) *	GIS	DWN	25.9	0.2-115.8
Reach (500 m)	Slope ($^0/_{00}$)	GIS	SLO	5.92	0.00-58.03
	Sinuosity	GIS	SIN	1.23	1.00-2.79
	Land uses				
	Urban/Industrial (%) *	GIS	RUI	1.0	0.0-36.0
	Intensive agriculture (%) *	GIS	RIA	29.0	0.0-100.0
	Extensive agriculture (%) *	GIS	REA	7.0	0.0-100.0
	Natural (%) *	GIS	RNA	63.0	0.0-100.0
Basin	Basin area (Drainage surface in each site, 10^3 Km^2)	GIS	ARE	260.1	0.9-5919.1
	Gravelius index	GIS	GRA	1.68	1.14-2.68
	Land uses				
	Urban/Industrial (%)*	GIS	BUI	0.4	0.0-6.7
	Intensive agriculture (%)*	GIS	BIA	22.5	0.0-97.0
	Extensive agriculture (%)*	GIS	BEA	11.0	0.0-89.1-0.0
	Natural (%)*	GIS	BNA	65.8	0.9-100.0
	Reservoir (%)*	GIS	BRS	0.32	0.0-21.2
	Population density (Hab/Km^2)*	GIS	POP	21.0	0.0-459.3

Habitat was characterised through 38 environmental variables, covering three different spatial scales: site, reach and basin. Two approaches were used in this characterization: *in situ* measures, which described micro and mesohabitat characteristics at each locality, and remote GIS measures used to record variables from digital maps (Table 1). These measures could be split in two categories: a) predictors that described the natural habitat variability in the basin and b) descriptors of human perturbation (Table 1). All variables were checked for normality and transformed when necessary prior to analysis.

Evaluation of species tolerance to human impairment.

We assessed species´ sensitivity through two different approaches: i) checking the responses of species' presences to a global stressor gradient including all the set of environmental variables, as it has traditionally been done (Armitage et al. 1983, Lenat 1993), and ii) exploring the partial responses of each species' presence to a set of independent stressors (human and biotic). Additionally, we compared our results with other two commonly used approaches to assess species sensitivity (see bellow). We discarded from the analysis all native species with very low prevalence (<5%) (*Anguilla anguilla*, *Alosa alosa*, *Gobio lozanoi* and *Luciobarbus guiraonis*) due to the difficulty to differentiate their presences-absences from a random distribution. Although other authors have pointed out their value for bioassessment (Cao et al. 1998), this is not the aim of this work and the results on their tolerance/sensitivity would have low interpretable value and it may have immediate negative consequences on bioassessment as Van Sickle et al. (2007) recently pointed out. Thus we finally considered 10 species in the analysis.

Definition of Environmental, Human Impairment and Fish Community gradients

To face the general problem of co-variation in species´ sensitivity assessment, we carried out a set of multivariate analysis to extract independent stressor gradients. In a first approach a Principal Component Analysis (PCA) was applied to the environmental variables x sites matrix (Table 1), including even variables not related to human impacts to account for the natural variability in the analysis. A varimax rotated PCA was used in this case to clarify the sense of the extracted gradients (Table 2). The first two PCs accounted for the 42.7% of the original variance of our data. The PC1 was mainly related to variables describing land uses at the basin and reach scales, chemical alterations and the quality of the riparian forest (Table 2). This gradient was also related to some climate variables as mean air temperature and rainfall as well as altitude. All the variables describing river size scored highly in the PC2 (Table 2). Thus, two independent gradients were identified: a stressor-climatic gradient in PC1 and a longitudinal natural gradient in PC2. Given the orthogonal nature of these two PCs the species´ response to the global perturbation gradient could be tested avoiding the effect of the longitudinal gradient, as Kennard et al. (2005) suggested.

A second PCA was carried out exclusively on human impairment related variables (Table 1) to obtain a set of independent stressor gradients. This would allow to deep on species´ responses to specific stressors considering co-variation issues among stressors and stressors-longitudinal gradient. The first 6 PCs extracted from this PCA with eigenvalues>1 (McGarigal et al. 2000) explained more than two thirds of the original variance (68.6%). Each of them was related to a particular source of human impairment as the portion of the basin in a natural condition (with low agriculture or urban uses levels) (PC1_Nat), phosphorous enrichment probably related to urban waste water (PC2_Phs), effects of downstream river regulation (PC3_Dwn), a raise in nitrates concentration due to agriculture fertilizers (PC4_Nta), other effects related to agriculture at the reach scale (PC5_Agr) and upstream river regulation (PC6_Ups). Only basin naturalness and effects of downstream river regulation gradients were significantly related to the longitudinal gradient (Pearson´s r=0.3, p<0.001, and r=0.5, p<0.001, for basin naturalness and downstream river regulation respectively).

As freshwater fish communities tend to vary along longitudinal gradients (Magalhães et al. 2002), their spatial location within it must be accounted for in species sensitivity analyses. Then a Correspondence Analysis (CA) was performed in a species´ presence-absence x sites matrix to identify community variations. The first dimension (DIM 1 which accounted for 21.9% of the fish community variance) was strongly correlated to the longitudinal gradient (Pearson´s r=0.61, p>0.001), meaning a clear spatial fish community gradient. So some species tended to appear predominantly in headwaters while others occurred mainly in low stretches.

Species´ sensitivity to global human disturbance.

The global perturbation gradient (PC1) was split into five equivalent portions to evaluate the hypothesis of non-randomness of species distribution along it. The intensity of use of each portion (measured as the number of localities where each species was present) was compared to its availability (measured as the total number of localities within each portion). The null hypothesis of random association between the amount of habitat available and used was tested through a Chi-square test (Prenda et al. 1997, Morán-López et al, 2005). If rejected, a partitioned Chi square test was conducted to determine those portions that contributed to the statistical significance, i.e., in which perturbation class the species was over- or under-represented. An overuse of low impacted portions and infra-use of degraded ones would be expected for sensitive species, the opposite pattern for tolerant species, while insensitive species should exhibit a random use of gradient's portions. Then an index of species sensitivity (Available/used index) was built as the difference in over/infra use of both extremes of the stressor gradient. Positive values (the species overused the less degraded portion and rejected or disappeared from the degraded portion) indicate sensitive species while negative values (the species infra-used the less degraded portion and/or overused the degraded) are related to tolerant species.

Table 2. Set of multivariate analysis used to define Environmental, Human Impairment and Fish Community gradients. Only loadings >0.6 (when possible) are shown. Variable codes in Table 1.

Aim	Technique	Variables	Extracted gradients	% expl. var. (Eigenvalue)	Negative extreme	Positive extreme	Denomination
Extract a global human perturbation gradient free of natural variation	PCA	All listed in Table 1	PC1	20.9 (8.14)	**RNA** (-0.77) **BNA** (-0.72) **ATEM** (-0.71) **PRE** (-0.62) **QBR** (-0.60)	**BIA** (0.78) **ELE** (0.63) **RIA** (0.62) **SLF** (0.60)	***Global perturbation gradient***
			PC2	13.8 (5.39)		**HED** (0.89) **ARE** (0.87) **ORD** (0.83) **POR** (0.77) **WID** (0.76) **SUS** (0.67)	***Longitudinal gradient***
Obtain patterns in fish community distribution	CA	Species´ presence-absence	DIM1	21.9			***Biotic gradient***
Identify independent and relevant human perturbation gradients	PCA	Human impairment in Table 1	PC1_Nat	28.9 (5.48)	**BNA** (-0.81) **RNA** (-0.75) **QBR** (-0.61)	**BIA** (0.67) **POP** (0.64) **BUI** (0.61)	***Basin naturalness***
			PC2_Phs	12.0 (2.29)	**PHS** (-0.70)		***P Enrichment***
			PC3_Dwn	8.6 (1.63)	**DWN** (-0.83)	**BRS** (0.81)	***Downstream river regulation***
			PC4_Nta	7.2 (1.37)	**NTA** (-0.48)		***N Enrichment***
			PC5_Agr	6.2 (1.17)	**REA** (-0.57)		***Surrounding agriculture***
			PC6_Ups	5.7 (1.08)	**UPS** (-0.55)		***Upstream river regulation***

Species´ sensitivity has been assessed through many different indices and approaches (Cao and Hawkins 2005, Lenat 1993). We applied two of these indices focused on the evaluation of species´ sensitivity to global stressor gradients, the Tolerance Value (TV) from Knapp et al. (2005) and the RD/TD index of Hawkins et al. (2000), to check for parallelism with our results. TV is based on observed vs expected presences (O/E) in test sites derived from RIVPACS models. TV values larger than 1 identify tolerant species while those TV<1 indicate sensitive taxa. We used the outcomes from an ANNA (Linke et al. 2005) model instead

RIVPACS given its higher performance on our data set (Hermoso et al. submitted). The RD/TD index, which we adapted to be used with presence-absence data, measures species' tolerances as the relationship between the proportional difference in mean taxon abundance between reference and test sites. In our index, RP and TP (RD and TD in Hawkins` index [Hawkins et al. 2000]) measure the proportions of reference and test sites where the species is present respectively. In this modified index the higher the value the more sensitive the species is and the closer to 0 the more tolerant. Reference sites were selected from the original data set as the less affected by human perturbation (low urban or agricultural land uses at the basin and reach scale -500 m around the sampling point-, bank and channel structure in natural condition, a naturalized riparian forest and exotic species accounting for less than 5% of total fish abundance). We considered 70 reference sites which were located along the whole longitudinal gradient, though not homogeneously distributed along it. Finally, we analyzed the possible relationship between both indices scores and the relative position of each fish species along de longitudinal gradient. For this purpose each species´ scores in all the three indices were correlated to their loadings in the DIM1. No significant correlation would be expected if the indices were completely independent of river size effects.

Species´ sensitivity to specific sources of human and biotic disturbance.

To face the question "sensitive to what?" we explored the relationship between species´ presence-absence and the set of independent stressor gradients defining the main sources of human disturbances. We also used two additional measures of biotic perturbation dealing with the degree of exotic fish dominance in the community (percentage of both total exotic abundance and species richness).None of these biotic perturbation measures was highly influenced by the longitudinal gradient (Pearson´s $r<|0.2|$ for both the percentage of total exotic abundance and species richness) and any other human stressor (Pearson´s $r<0.17$ for all possible perturbation gradients-biotic variables combinations), so they did not introduce any additional source of co-variation in the analysis. We used percentages instead of original data (total exotic abundance and species richness) to avoid the effect of local abundance and species richness in the analysis.

We tested the effects of species presence-absence on each independent stressor gradient, aiming to detect significant differences in the stressors gradients between occupied and unoccupied sites. When stressors were independent from the longitudinal gradient we used t-test to compare occupied and unoccupied sites. Whenever we detected a significant relationship between stressors and the longitudinal gradient we used ANCOVA models to test for these differences. We used the longitudinal gradient as covariate, testing the influence of each target species presence-absence of each species (factor) on each stressor gradient (dependent variable).

We run ANCOVA analysis using a two-step procedure: i) first, we tested the homogeneity of slopes assumption through the significance of the interaction term (presence-absence × longitudinal gradient), in case of significance we kept this complete model; ii) . When the interaction was not statistically significant, it was deleted from the models and standard ANCOVA analyses were run.

The ratio between the ranges of each stressor in which the species was present/absent was used as a measure of their sensitivity in this case. All of them were tested for their relationship with the longitudinal gradient in the same way as was previously done with the global perturbation sensitivity indices.

RESULTS

Species´ sensitivity to global human disturbances.

The analysis of available/used through the 5 equivalent segments of the global perturbation gradient pointed out the sensitivity of each species to this stressor gradient (Fig. 2 and Fig. 3). Some species showed a clear sensitivity, over-using the best preserved portions, avoiding (or disappearing from) the perturbed portions and showing the highest values for the Availability/used Index hence (*Luciobarbus sclateri*, *Pseudochondrostoma willkommii* and *Anaecypris hispanica*). Other species showed an intermediate sensitivity as they used the best preserved portions as they were available and infra-used only the worst portions with intermediate values for the Index (*Luciobarbus microcephalus*, *Luciobarbus comizo* and *Salaria fluviatilis*). Insensitive species were characterized by a global use as available (*Iberociprys alburnoides*, *Cobitis paludica*) or an over-use of both extremes of the gradient (*Squalius pyrenaicus*) and tolerant species by a higher over-use of the worst portion (*Iberochondrostoma lemmingii*).

The two alternative tested indices (RP/TP and TV) were highly correlated (Pearson´s r=0.78, p=0.008) showing similar patterns in species tolerance, but not concordant with our previous results (Fig. 3). Additionally each species sensitivity scores were related to their respective loading within the CA ordination gradient (Table 3). Thus, the species present at lower reaches tended to show higher tolerance values (*L. comizo* and *S. fluviatilis*) than the species present in headwaters-middle reaches (*S. pyrenaicus*, *I. lemmingii*, *L. sclateri* and *A. hispanica*) (Fig. 3). This effect was not detected in the Available/used Index (Table 3).

Species´ sensitivity to specific sources of human and biotic disturbance.

The t-test/ANCOVA analysis allowed to deep on each species sensitivities to particular sources of human perturbation at a finer scale. Given the high number of partial analysis (one per species and stressor gradients), these results are summarized in Figure 4.

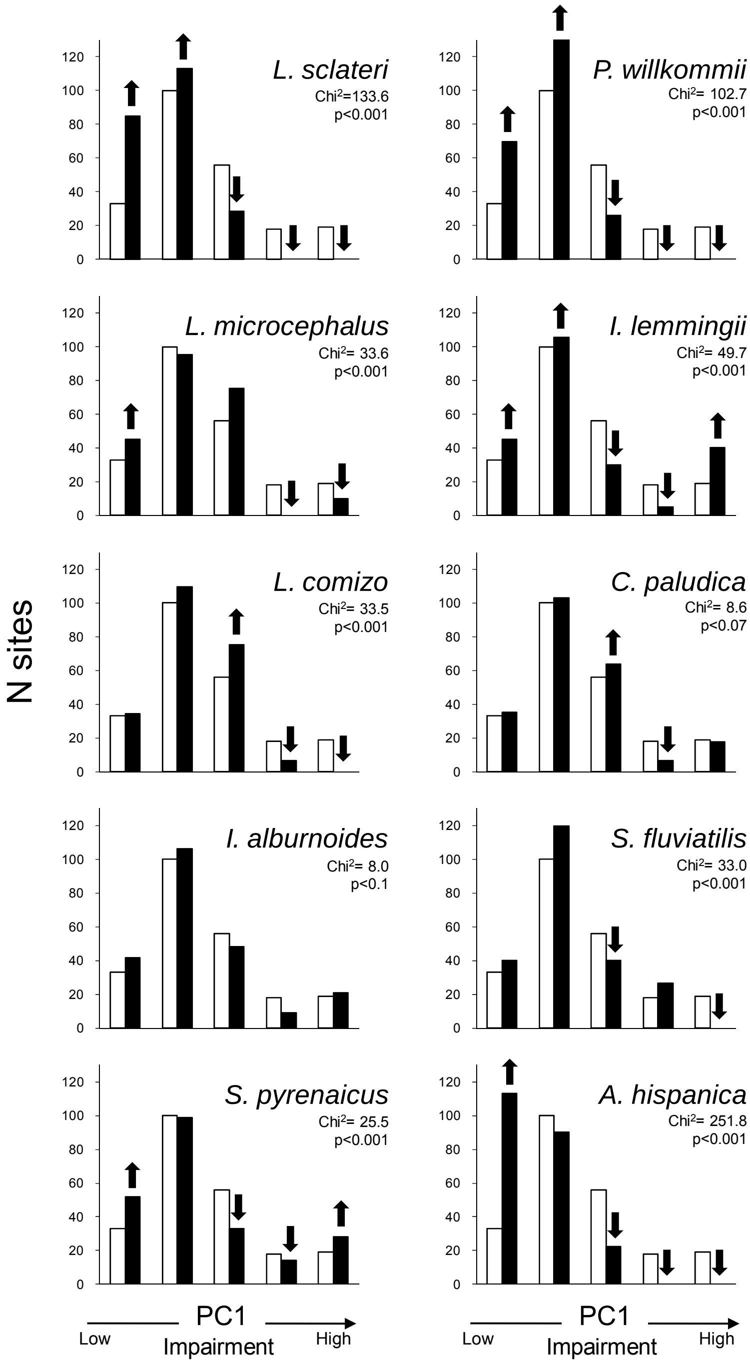

Fig. 2. Analysis of preference for the five equivalent portions in which the stressor gradient was split. The available number of sites is represented in white columns and the adjusted number of used in black columns. The Chi^2 statistic and its associated p value are also given. Significant differences were interpreted as overuse (up arrow) or infra-use (down arrow).

As expected the most sensitive species to the global stressor gradient showed the stronger responses to particular sources of perturbation (*L. sclateri* to nutrient enrichment due to both P and N, *P. willkommii* to P enrichment, the effects of agriculture at the reach scale and upstream river regulation; *A. hispanica* mainly responded to the basin naturalness status and the enrichment in nitrates). However, the finer scale of this approach allowed noticing responses for the species labeled above as insensitive. *S. pyrenaicus* showed significant responses to nitrates enrichments and the upstream river regulation, *I. lemmingii* to upstream river regulation and *I. alburnoides* to surrounding agriculture stress gradient and upstream river regulation. In the same

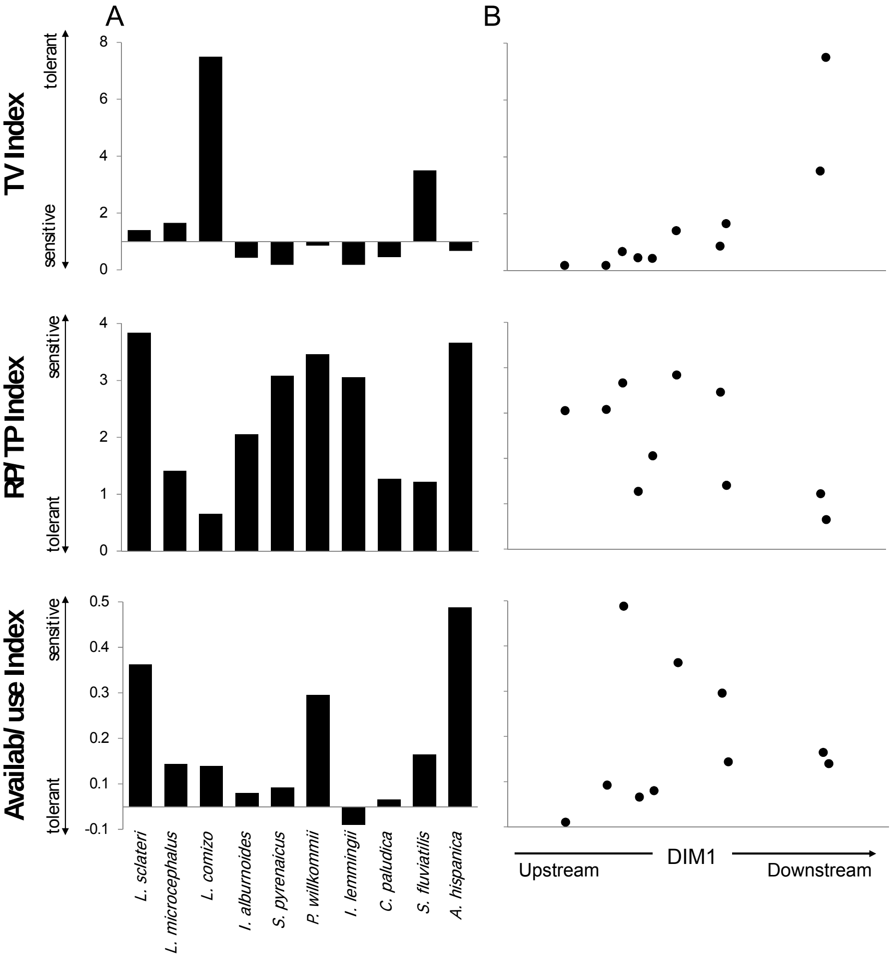

Fig. 3. Scores of three indices used to evaluate species tolerance to the global stressor gradient A). TV measures the O/E relationship in test sites for each species (predictions were derived from ANNA models); RP/TP is the ratio proportion of reference/ test sites where each species were present. The Availability/use Index measures the difference in over/infra use of the best and worst portions (1 and 5 respectively) of the global stressor gradient pointed out in Fig. 2. Relationship between tolerance values and scores of each species in the first axis of the Correspondence Analysis (DIM1) ordination is showed in B). Both, the indices and CA were carried out in the same data matrix (n=242 sites: 70 reference and 172 test sites).

way, stronger responses were found for intermediate sensitive species as *S. fluviatilis*, *L. comizo* and *L. microcephalus* to surrounding agriculture. No species was found to be sensitive to downstream river regulation after the effect of river size had been accounted for. All species, except *L. comizo* and *S. fluviatilis,* showed a significant response to the biological degradation. Additionally no significant relationship was found between each species´ sensitivity score and their position within the longitudinal gradient (Table 3).

DISCUSSION

In this study we provide quantitative support to the evaluation of fish species tolerance to human disturbances in a Mediterranean basin. A deep knowledge on species sensitivity is fundamental for a successful diagnostic of human disturbances affecting a target area and the ability to undertake effective remedial programs as the WFD requires.

Table 3. Species´ sensitivity values. The Pearson correlation coefficients between species´ sensitivities and their location within the longitudinal gradient (DIM1, see Table 2) are also shown. *** denotes $p<0.001$ and ** $p<0.01$. The first three indices correspond to the study of species´ responses to a global perturbation gradient. The second group of indices shows the species´ sensitivities to specific sources of perturbation.

Species	RP/TP¶	TV§	Avail/used	B. Natural	P Enrich	N Enrich	Agricult	Upst. regulation	Exotic Abund	Exotic S
Anaecypris hispanica	0.2	3.7	0.4	0.3	0.9	0.9	0.5	0.4	0.2	0.2
Cobitis palúdica	0.8	0.7	0.1	1.1	0.9	1.3	0.8	0.7	1.0	0.9
Iberocypris alburnoides	0.1	1.4	0.1	0.8	0.9	1.0	0.6	0.7	1.0	0.8
Iberochondrostoma lemmingii	0.2	3.8	0.3	0.8	0.6	1.0	0.6	0.6	0.8	0.6
Luciobarbus comizo	0.2	3.1	0.0	0.5	0.8	0.7	0.5	0.7	1.0	0.7
Luciobarbus microcephalus	0.4	1.3	0.0	0.8	0.3	1.0	0.6	0.6	1.0	0.6
Luciobarbus sclateri	0.3	3.5	0.2	0.7	0.5	0.2	0.4	0.5	0.9	0.8
Pseudochondrostoma willkommii	0.3	2.1	0.0	0.6	0.5	1.0	0.6	0.6	1.0	0.6
Salaria fluviatilis	0.8	1.2	0.1	0.4	0.8	0.4	0.4	0.7	1.0	0.6
Squalius pyrenaicus	0.1	3.1	0.0	0.7	0.9	1.0	0.6	0.7	1.0	0.8
Pearson´s r	**0.84*****	**0.77****	0.08	0.41	0.50	0.52	0.37	0.25	0.03	0.36

The ability to develop accurate bioassessment programs based on bioindicators is highly limited by the precision of the systems used to derive tolerance values for the target taxa. Although many efforts have been focused on the evaluation of macroinvertebrate sensitivity to different human perturbation (Armitage et al. 1987, Yuan 2004, Carlisle et al. 2007), little attention has been put on freshwater fish (Meador and Carlisle 2007) and there is no information concerning to European freshwater fish species. Instead of empirical-derived values of species tolerance or sensitivity, expert judgment has been traditionally being applied in freshwater fish based bioassessment programs (Karr 1981, Oberdorff et al. 2002, Pont et al. 2006). The expert judgment relies on subjective appreciations that have proved to be unreliable in previous studies (Lenat 1993). Therefore the empirical approach we followed in this study ensures an objective evaluation of freshwater fish sensitivity and supposes an important contribution to the future implementation of fish based bioassessment programs.

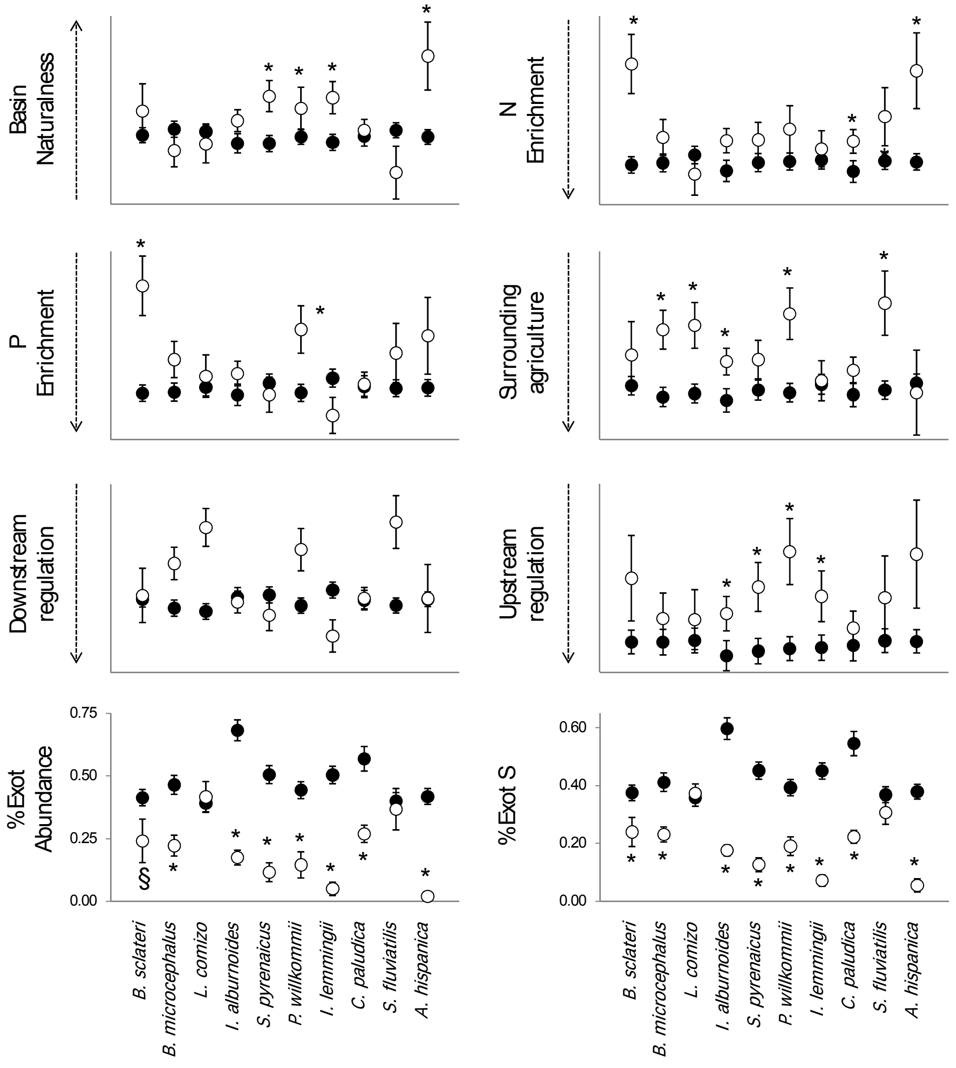

Fig. 4. Mean ± SE for species presence (white dots) and absence (black dots) at the 6 independent stressor gradients and the biotic perturbation variables. * Denotes significant differences found in the ANCOVA or t-test analysis when avoiding the effect of river size (p<0.05).

Presence-absence data is the basis of some of the more widespread an applied bioassessment methods all around the world, as RIVPACS or AUSRIVAS (Wright et al. 1993, Norris 1996). It could be expected these methods to be insensitive to many stressors, because individual populations of some species can suffer a considerable degradation before going locally extinct. However, at the assemblage level, presence-absence data appears to be sufficiently robust to allow the detection of reasonably subtle differences among sites (Hawkins et al. 2000). Only slightly differences have been reported in taxa tolerance values when using presence-absence data instead of abundance (Yuan 2004). Population densities are submitted to a greater seasonal and annual variation rates than presences-absences does, especially in harsh environments as Mediterranean (Magalhães et al. 2007). Moreover this simpler approach has additional advantages vs abundance-based ones, since the characterization of taxa's abundances is largely more complicated and difficult to standardize than the checking of species presences. Sampling accuracy could be reflected not only on bioassessment results, but also in sensitivity values (Lenat 1993). Additionally many efforts have been focused on the study of the optimal sampling effort to adequately characterize the presence of species in Mediterranean environments (Collares-Pereira et al. 2004) rather than abundance. Therefore, if presence-absence data allow a reasonable accurate assessment of species sensitivity it would not be necessary to find ways of detecting abundance changes and all the other drawbacks previously emphasized of using abundance data could be avoided.

In a first step we faced the importance of accounting for natural environmental gradients in sensitivity analysis. The broad range of environmental (natural and stressor) variables gathered in this study ensures that the sensitivity results can be extrapolated, and used hence in a wide area as suggested Whittier and Hughes (1998). We applied an available/used analysis through a stressor gradient free from stream size effects, which has been described as a key factor determining the presence-absence of species (Vanote et al. 1980, Pringle 2001, Magalhães et al. 2002). Most of the species showed response to this stressor gradient with different intensities, as not all of them over-used with the same intensity the less impaired portion of the gradient, or even used the perturbed portion as it was available. These results were not concordant with the outcomes of two alternative methods. A clear relationship between the sensitivity values derived for each species and their pattern of spatial distribution pointed out the influence of river size on species´ sensitivity values in both methods. Different causes may be responsible for this result. The exclusion of the natural covariates had been previously reported as an important cause of differences in sensitivity measures in certain taxa (Yuan 2004). We did not remove the effect of river size in Hawkins´ RP/TP index, since although reference localities were defined along the whole longitudinal gradient, they were not homogeneously distributed being mainly located in headwaters-middle reaches, while few of them were found in low reaches. As an immediate consequence species which mainly appeared in low reaches tended to have over-estimated tolerance values, since the ratio RP/TP could not gather their selectivity for the scarce number of reference localities in this low reaches. On the other hand species mainly present in headwaters showed infra-estimated tolerance values. In the second method Cao and Hawkins computed species tolerance through the ratio O/E presences in perturbed sites in their TV index. River size effect is *a priori* accounted for in this approach as predictive models included variables describing the longitudinal gradient, but this hypothesis was not supported by our results. In this case the effect of predictive models inaccuracies may be the cause of such result, although the ANNA model was the one that best fitted the data in previous studies in the same study area. Therefore, the effect of river size must be considered when evaluating species sensitivity to human perturbation and special care must be put if predictive models are used.

In most studies, sensitivity has been defined with respect to a single gradient of anthropogenic disturbance (Hilsenhoff 1987, Armitage et al. 1983) lacking the evaluation of species´ sensitivities to particular sources of human perturbation (sensitive to what?). Given the urgent need of more accurate diagnostics of human impairment the utility of this kind of studies would be greatly enhanced if they were focused on specific sensitivities to a wide number of independent stressors. In this complex scenario an additional factor as stressors co-variation must be considered (Meador and Carlisle 2007). We tackle this issue through the generation of a number of independent axes representing the most significant stressors within the study area avoiding river size effects using a longitudinal gradient as covariate in the analysis when necessary. In this finer analysis clearer responses of species to particular sources of human impairment were found, even for species previously classified as low sensitive or insensitive. Thus, through more detailed approaches like ANCOVA analysis or GAM models used in similar studies (Yuan 2004) the need of more in depth information on species sensitivity for an accurate site specific diagnostic on human disturbances can be addressed.

Significant effects were found for every stressor factor at least on one species. *A. hispanica* showed the strongest response to the degree of land and riparian transformation at the basin and reach scales, appearing predominantly in the most natural sites. *L. sclateri* was the most sensitive species to changes in water quality (nutrient enrichment), *P. willkommii* to upstream river regulation and *S. fluviatilis* to effects derived of agriculture at the reach scale (possibly an increased rate of sedimentation according to the ecological requirements of the species). Other lower responses were found for the remaining species which ensures the capability of future fish-based bioassessment tools to detect the main environmental impairment causes and make accurate diagnostics. Additionally all the species except *L. comizo* and *S. fluviatilis* responded to the degree of biotic impairment.

Tolerance values are commonly simplified to ordinal scales for being used within biotic indices in bioassessment (Yuan, 2004, Carlisle et al. 2007). As Whittier and Huges (1998) noted there is a considerable variability in the published literature in the number of tolerance classes ranging from one to ten. Additionally there is not a standardize criteria for selecting the limits in tolerance values between classes. For that reason we suggest to avoid these classifications and use a continuum indicator of the relative species sensitivity value. Further studies are needed to introduce this continuum concept on the biotic indices used in bioassessment.

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CAPÍTULO 3

ASSESSING THE ECOLOGICAL STATUS IN SPECIES-POOR SYSTEMS: A FISH-BASED INDEX FOR MEDITERRANEAN RIVERS

Evaluación del Estado Ecológico en sistemas pobres en especies a través de un índice basado en peces para los ríos mediterráneos

(En revisión en Aquatic Conservation: Marine and Freshwater Ecosystems)

Assessing the Ecological Status in species-poor systems: a fish-based index for Mediterranean rivers.

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ABSTRACT

The assessment of the ecological status of freshwater ecosystems is a key issue for many international laws as the Water Framework Directive in light of the actual impoverished status of such ecosystems. Different multimetric approaches have been successfully developed all around the world in different freshwater environments. However multimetric indices are difficult to apply in Mediterranean rivers basins, where freshwater fish communities feature very low species richness per site and high number of endemics with generalist and opportunistic life strategies. A site-specific approach was followed to develop an adaptation of the multimetric concept in the Index of Community Integrity. The presence-absence of ten native freshwater fish species was modeled and used to assess the deviation of the observed and expected community composition at reference condition. These deviations were transformed into probabilities to belong to a reference site and species by species measures were then integrated in a final score. The use of presence-absence only data reduces the possible errors associated to incorrect estimations of species´ abundance and its seasonal changes. The index was sensitive to both habitat and biotic disturbances while irresponsible to natural sources of variation. To our concern, this is the first index specifically tested to be responsible to biotic perturbations, which traditionally have been *forgotten pressures* in Indices of Biotic Integrity.

KEYWORDS: ANNA, bioassessment, community, freshwater fish, integrity, invasive species, site-specific indices, type-specific indices.

INTRODUCTION

Assessing the ecological status of freshwater ecosystems has been a key issue in freshwater management for the last decades as a consequence of their poor conservation status. The importance of these ecosystems to human culture, welfare and development has lead them to a poor status (Malmqvist & Rundle, 2002). Five main sources of perturbations are responsible for this situation: i) species introductions and translocation, ii) impoundment of rivers and water abstraction, iii) water quality deterioration (pollution or eutrophication), iv) habitat degradation and fragmentation and v) overexploitation (Allan & Flecker, 1993; Collares-Pereira & Cowx, 2004; Prenda et al., 2006). As a consequence, many freshwater fish species have become extinct or are highly endangered. This situation is particularly worrying in rivers of arid and semi-arid regions (Collares-Pereira & Cowx, 2004). To face this serious situation many international laws as the Clean Water Act in the US or the European Water Framework Directive (WFD, E.C., 2000) try to address the problem requiring the protection and restoration of biological integrity as part of water quality standards.

According to the WFD the present status of all European rivers must be assessed and classified to five predefined levels of ecological integrity based on four biotic elements including freshwater fish. This bioassessment should help to evaluate the potential problems affecting rivers and to lead them to a good ecological status before 2015 by imposing effective corrective plans.

Many efforts have been devoted to the development of efficient tools to measure the ecological status of these systems based on freshwater fish. Karr (1981) originally designed an Index of Biotic Integrity (IBI) for north-eastern American rivers that followed a multimetric approach. The index was originally composed by twelve metrics reflecting important components of community ecology: taxonomic richness, habitat and trophic guild composition, individual health and abundance which were summed up in a final score. Following this multimetric approach many other indices have been developed throughout the world and adapted to specific conditions and requirements (Roset et al., 2007). Two different trends can be distinguish within recent European efforts: spatially-based or type-specific methods (Melcher et al., 2007; Schmutz et al., 2007) and site-specific methods (Oberdorff et al., 2002; Pont et al., 2007) both based on the original Karr´s multimetric index and the reference condition approach (Hughes et al., 1986; Reynoldson, 1997, Bailey et al., 1998). These methods compare an ecosystem potentially exposed to a stress against a similar ecosystem free from any perturbation or in the best possible condition (its maximum ecological potential or reference conditions), but differ in the way they find the reference conditions for a given site. The type-specific approach relies on grouping techniques to cluster reference sites in a set of homogeneous landscape or biological groups. Then a given site only has to be compared against the reference conditions of the group in which it is included, which are normally referred as the characteristics of the best preserved sites within each group. The site-specific approach does not require any classification and it simply finds specific reference conditions for every new given site according to its environmental characteristics. A site-specific index was the option selected for the development of the European Fish Index (EFI) which arose from the European FAME project (FAME, 2004) and is being applied to most of European rivers.

Type-specific predictive methods have been developed for other taxonomic groups, such as RIVPACS (Clarke et al., 2003) or AUSRIVAS (Simpson and Norris, 2000) on benthic macroinvertebrate communities. Instead of relying on elaborated metrics as the multimetric approaches do, these methods directly compare the observed and expected communities using presence-absence only data. However they have received little attention in freshwater fish indices (Hawkins, 2006; Kennard et al., 2006a; Carlisle et al., 2008).

Despite the EFI was not validated for Mediterranean rivers (Pont et al., 2007) and no similar index is available for this area, scarce efforts have been focused on the adaptation of an IBI to this area (Ferreira et al., 1996; De Sostoa et al., 2004; Ferreira et al., 2007). Mediterranean fish communities share common problems with other warm-water streams which make difficult to establish IBIs as a reduced number of species per site, a high number of endemisms per basin and high spatial and temporal changes in fish communities (Moyle and Randall, 1998; Moyle and Marchetti, 1999). Moreover, Mediterranean freshwater fish species have evolved in harsh environments (e.g. facing severe droughts and floods) and have generally developed short lifespans, generalist habitat use, opportunistic feeding strategies, high fecundity and early sexual maturity (e. g. Velasco et al., 1990; Vila-Gispert and Moreno-Amich, 2002). All these drawbacks may impose serious limitations to the development of an effective index in Mediterranean rivers, at least in its traditional approach, as in other similar environments even with richer freshwater fish communities (Shields et al., 1995; Harris and Silveira, 1999). All the previous attempts to the development of a bioassessment tool in the Mediterranean region followed a type-specific approach (e. g. Ferreira et al., 2007) while a site-specific has never been tried in this complex environment.

For all these reasons, in this study we develop a new approach to the assessment of the ecological status in Mediterranean rivers. A mix of site-specific and multimetric approaches was made to overcome the drawbacks commonly associated to them in scientific literature. Special care was put in the evaluation of the sensitivity of the index to biotic disturbances since, though they suppose a major recognized threat to the conservation of native communities it has traditionally received little attention.

METHODS

Study area

The Guadiana River basin is located in the South-Western Iberian Peninsula draining a total area of 67039 Km^2 to the Atlantic Ocean (Fig. 1). It features a typical Mediterranean climate, with high intra and interannual discharge variation, with severe floods and droughts. Mean air temperature ranges from 13 to 18.1 ºC, with a strong intra-annual variation in extreme temperatures. Mean annual precipitation ranges from 350 to 1200 mm (with a mean of 450 mm).

Although it is a sparsely populated area (28 hab/Km^2), landscape has been deeply transformed during the last century by agricultural activities. Almost a half of the basin (49.1%) is currently being used for agriculture (30.6% occupied with intensive agriculture as irrigated lands and 18.5% occupied with extensive agriculture, like olive groves or fruit trees). As a consequence, about 13000 Hm^3 of water is retained in 88 big reservoirs (>1 Hm^3) and more than 200 small ones (<1 Hm^3) for water supply. Other common human perturbations are related to river channel modifications due to river channelization and degradation and even completely depletion of the riparian forest.

Fish community and habitat characterization

Fish community was characterized in 241 localities through the basin, using electrofishing (Fig. 1). Sampling was conducted once at each location without block-nets along 100 m long stretches. This sampling effort has been proved to be sufficient to capture most species present, except for non-wadable rivers, as Filipe et al. (2004) suggest. This was not a major problem since no more than 2% of sites were non-wadable. Alternative methodological approaches similar to that used in other European countries for this kind of environments (Kestemont and Goffaux, 2002) were followed. All fish were released after we identified the individuals to species level.

Habitat was characterised through 38 environmental variables, covering three different spatial scales: site, reach and basin. These measures could be split in two categories: a) predictors that described the natural habitat variability in the basin and b) descriptors of human perturbation (Table 1). Two approaches were used in this characterization: *in situ* or remote measures, which described micro and mesohabitat characteristics in each locality, and GIS measures used to record variables from digital maps (Table 1). All the variables were checked for normality and transformed when necessary prior to analysis.

The original data was divided in two independent sub-sets: a reference data set used for building and calibrating the predictive model and a test data set used in conjunction with the reference to establish quality classes. Reference sites were characterised by low urban or agricultural land uses within the whole basin and at the reach scale (500 m around the sampling point). Furthermore, bank and channel structure as well as the riparian zone should be in natural condition (Pont et al., 2007). To ensure that reference sites were not impacted by invasive fish, all sites where exotic species accounted for more of 5% of total fish abundance were also discarded (Kennard et al., 2006b).

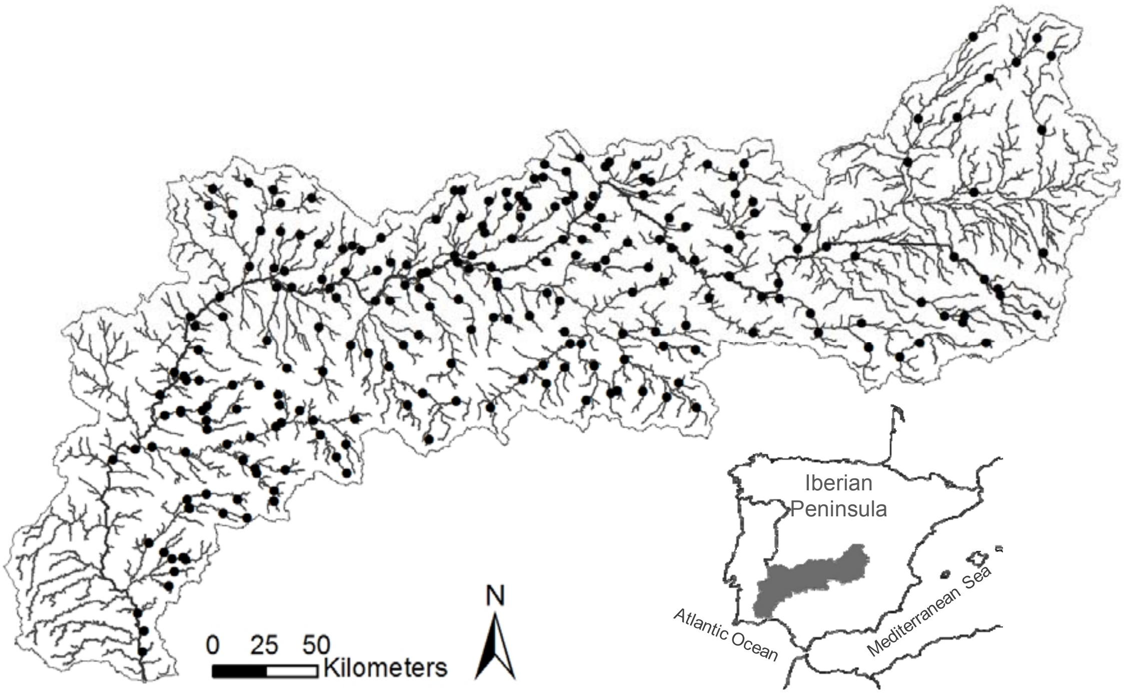

Fig. 1. Guadiana River basin and location of sampling sites.

Development of the Community Integrity Index.

The Index of Community Integrity measures the general deviation of the observed community composition from an expected community in total absence of any source of perturbation (human or biotic) following the reference condition approach (Hughes et al., 1986; Reynoldson et al., 1997; Bailey et al., 1998). The index summarizes the partial evaluations made species by species for each site to be a reference site.

We first built and validated a predictive model describing the presence-absence of 10 native species in relation to environmental variables not affected by human perturbations (Table 1) only in reference sites. These set of non perturbed environmental variables were used to match test sites with the most similar environmental reference sites allowing site-specific predictions of expected taxonomic composition. An ANNA model (Linke et al., 2005) was used

Table 1. Environmental variables used to characterize the sampled sites. * Denotes human-biotic potentially perturbed variables and used to describe stressor gradients.

Scale	Variable	Method	Mean	Range
Site	Water depth (cm)	*In situ*	42.8	7.0-200
	Shelter availability (m^2 of shelter/river width)	*In situ*	5.6	0.0-60.6
	Elevation (m)	GIS	384.1	7.1-974.9
	Relative position (dist. to the most headwater point/total length of the stream)	GIS	0.47	0.04-1.00
	Stream order (Strahler)	GIS	2.1	1.0-6.0
	Distance to headwater (Km)	GIS	68.1	3.6-1,036.1
	Distance to Guadiana River (Km)	GIS	58.2	0.0-196.0
	River width (m) *	*In situ*	10.8	1.4-123.0-1.4
	Substrate coarseness (Wentworth scale) *	*In situ*	5.3	1.0-9.0-1.0
	Riparian Quality Index (QBR, Munne et al., 2003) *	*In situ*	61.8	0-100
	NH_4^+ (mg/L) *	*In situ*	1.38	0.02-51.60
	NO_2^- (mg/L) *	*In situ*	0.10	0.01-2.00
	NO_3^- (mg/L) *	*In situ*	4.09	0.50-55.90
	PO_5^{3-} (mg/L) *	*In situ*	1.00	0.05-23.20
	SO_4^{2-} (mg/L) *	*In situ*	110.1	10.0-2380.0
	Cl^- (mg/L) *	*In situ*	56.1	2.0-834.0
	Water temperature (ºC) *	*In situ*	20.5	9.4-32.6
	Conductivity (µS/cm) *	*In situ*	624.7	38.0-3230.0
	pH *	*In situ*	7.84	2.21-10.63
	Annual precipitation (mm/m^2)	GIS	593.1	370.2-1114.5
	Solar radiation (10 KJ/m^2*day*µm)	GIS	2033.9	1646-2227
	Average annual air temperature (ºC)	GIS	15.85	13.0-18.0
	Distance to the nearest reservoir upstream (Km) *	GIS	41.1	0.0-196.0
	Distance to the nearest reservoir downstream (Km) *	GIS	25.9	0.2-115.8
	% Exotic abundance	*In situ*	39.6	0-100
	% Exotic species richness	*In situ*	36.1	0-100
Reach (500 m)	Slope ($^0/_{00}$)	GIS	5.92	0.00-58.03
	Sinuosity	GIS	1.23	1.00-2.79
	Land uses			
	Urban/Industrial (%) *	GIS	1.0	0.0-36.0
	Intensive agriculture (%) *	GIS	29.0	0.0-100.0
	Extensive agriculture (%) *	GIS	7.0	0.0-100.0
	Natural (%) *	GIS	63.0	0.0-100.0
Basin	Basin area (Drainage surface in each site, 10^3 Km^2)	GIS	260.1	0.9-5919.1
	Gravelius index	GIS	1.68	1.14-2.68
	Land uses			
	Urban/Industrial (%)*	GIS	0.4	0.0-6.7
	Intensive agriculture (%)*	GIS	22.5	0.0-97.0
	Extensive agriculture (%)*	GIS	11.0	0.0-89.1-0.0
	Natural (%)*	GIS	65.8	0.9-100.0
	Reservoir (%)*	GIS	0.32	0.0-21.2
	Population density (Hab/Km^2)*	GIS	21.0	0.0-459.3

for this purpose which showed the best performance in our data in previous studies (data not shown). ANNA is a whole community predictive model (like RIVPACS) allowing the prediction of rare species which should be discarded in other traditional predictive methods like logistic regression. Logistic regression needs the number of presences-absences to be balanced and the number of predictors that can be used is highly limited by the number of available cases (e.g. Filipe et al., 2004). That makes the development of predictive models for species with very low prevalence impossible. In ANNA even rare species can be modelled following a continuous approach as a solution to the drawbacks of artificial grouping strategies (see Linke et al., 2005 for more details on ANNA models).

The ANNA model was built on a set of 70 reference sites and validated in an independent data set of 20 reference sites (calibration data set). Since the original number of reference sites found in the study area (n=55 sites) was not sufficient for both model construction and validation, some reference sites were also chosen from adjacent basins in the same biogeographical region (Tinto, Odiel and Guadalquivir basins). Although all these basins share most of their native species we introduced the variable basin as an additional predictor. Model performance was assessed through different tests carried out in the calibration data set. First, the slope and intercept of the O/E line was not different from 1 and 0 respectively (b=1.063, t-test, p=0.58 and intercept= 0.058, p=0.95). Second, the prediction success measured as the area under the curve (AUC=0.79) of the Reciever Operating Characteristic (ROC) (Fielding and Bell, 1997) and the Standard Deviation of the O/E values ($SD_{O/E}$=0.39), which improved that displayed by the null model (SD_{null}=0.45) and set close to the best possible model (SD_R=0.38) according to Van Sickle et al. (2005). These two analyses showed the model to be valid and accurate enough to be used in the index minimizing the probability of committing type I and II statistical errors according to Linke et al. (2005). Once the model was validated, independent sub-models were built to predict the expected fauna in the reference localities used in the model construction to allow their inclusion in the index. To avoid pseudo-replication errors in these predictions each reference site used for building the model was run through it, without itself.

The basic unit used to construct the index was the probability of a given site to be a reference site, which depends on the deviation of the observed and the expected community composition from a reference site with similar environmental characteristics. The deviation of the observed presences-absences against the expected probabilities in absence of perturbations (O-E) was measured for each species in each site (referred as residuals hereafter). Note that since the index compares the community composition species by species we had ten different residuals for a given site. Negative values indicate species loss (the species was predicted to be present with a certain probability but it was absent). The lower the residuals, the higher the probability of presence unconfirmed hence. In the opposite extreme, positive residuals owe to observed presences with low predicted probabilities. These residuals were standardized to a (0,1) normal distribution (x-mean/SD in the reference data set) and then transformed into probabilities to belong to a reference site (Fig 1). Since we assumed that residuals decreased with disturbance, we used only one-tailed evaluations for probabilities' calculations. With this approach we assumed all the 10 native species to be sensitive to any source of perturbation according to previous studies on species tolerances (data not shown). The probability of a site to be a reference site having a standardized residual x is obtained from the cumulative normal distribution function corresponding to that x value [pnorm(x), according to Pont et al. (2007)]. Each species measure was then summed up in the final index score. It ranged between 0 (the site has a null probability to be a reference site according to all the partial species´ evaluations) and 10 (the site has a high probability to be a reference site according to all the partial species´ evaluations) (Fig 2).

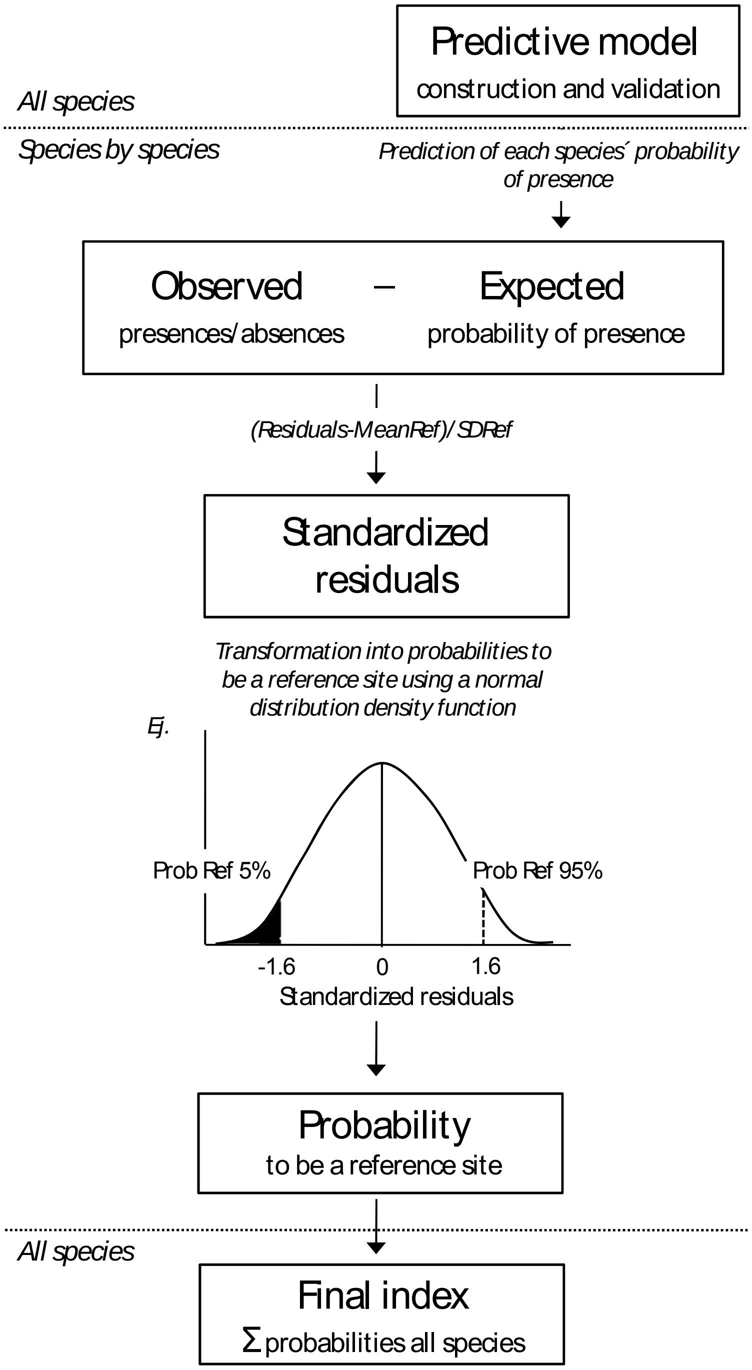

Fig. 2. Flowchart for the development of Community Health Index. An example of the transformation of the standardized residuals into sites´ probabilities to be a Reference site is showed. The accumulated probability for a low residual (-1.6) is very low (5%) while for a large one (1.6) is high (95%). Dotted lines separate steps where the whole community (all species) or partial evaluations (species by species) are used.

Scoring and validating the Index

To validate the index and to establish the cut-off points between quality classes we checked its relationships with impairment gradients and its response vs. other accepted indices.

A Principal Component Analysis (PCA) was carried out on the environmental matrix (Table 1) to identify a set of independent gradients representative of the main sources of environmental variation in the study area. The first three Principal Components (PC) accounted for the 37% of the original variance recording a longitudinal headwater-mouth gradient (PC1), a habitat impairment gradient (PC2) and a biotic perturbation gradient (PC3) (Table 2). As a first approach, the effects of these gradients on the index scores were explored through a General Linear Model (GLM), where each gradient was used as independent variables and the index score as dependent. The limits between quality classes were established to maximize the difference among the perturbation gradients while not responding to the longitudinal gradient (to avoid the effects natural variability) and reducing the risk of committing Type I (inferring impairment when it does not exist) and Type II (not detecting impairment when it does exist) errors. Statistical differences among quality classes along the gradients were tested through ANOVA analysis. Once established, quality classes were tested for concordance with other

Table 2. Principal Component Analysis carried out in the whole habitat-biotic variables data set listed in Table 1 except QBR. Only variables with loadings >0.6 in any Principal Component (PC) are shown. % of explained variance (in brackets) and eigenvalues are also shown. Denomination denotes the name for each PC in the text.

Variable	PC1_Alt	PC2_Alt	PC3_Alt
	(13.0%)	(13.0%)	(11.0%)
Eigenv.	5.4	5.4	4.5
Denomination	Longitudinal gradient	Habitat impairment	Biotic perturbation
Basin area	0.9		
Distance to headwayer	0.9		
River order	0.9		
River width	0.8		
Gravelius Index	0.6		
Water depth	0.6		
Reach Int. Agr.		-0.9	
Basin Int. Agr.		-0.9	
SO_4^{2-}		-0.9	
NO_3^-		-0.8	
Substrate coarseness		-0.6	
Basin Natural land		0.6	
Precipitation		0.9	
Reach Natural land		0.9	
% Exotic Richness			-0.9
% Exotic Abundance			-1.0

widely used indices measuring human disturbances in freshwater ecosystems. The Iberian Biomonitoring Working Party (IBMWP, Alba-Tercedor et al., 2002) is an index specially designed to measure water quality based on benthic macroinvertebrate communities. The QBR index (Munné et al., 2003) is used to evaluate the quality of riparian forest and riparian-zone habitats. Final scores of both indices were used in this analysis instead of their respective quality classes since we mainly aimed to explore concordant responses of the present classes in other indices´ scores despite an intercalibration. An ANOVA analysis was used for this purpose. Although intercalibration exercises between the quality classes for different indices (ensuring concordance in the assignation of quality classes within different indices) are highly recommended (Sandin and Hering, 2004; Birk and Hering, 2006) this it is not the aim of this study. Significant differences in the scores of both indices among the Community Integrity Index quality classes would be expected if a good concordance between them existed and similar information was offered.

RESULTS

The Index of Comunity Integrity ranged between 1.7 and 8.3 and clearly discriminated between reference and test sites (t-test, t=5.5 p<0.001). The GLM analysis showed that both perturbation gradients (habitat and biotic) had significant effects on the scores of the index, but no effect was found for the natural longitudinal gradient (Table 3). The cut-off points fulfilled the goals we demanded. The limits for the bad-poor and high-good quality classes (Table 4) reduced the probability of Type I and II errors minimizing the probability of labelling a reference site as *in bad condition* and perturbed sites as *in high condition*. Different percentiles were used in both extremes to maximize the discriminatory power. The mean index´s scores in the reference data set was used as limit between moderate-good quality classes, ensuring that

Table 3. Effect of natural and perturbation gradients summed up in Table 2 on the Index of Community Integrity. Sum of squares (SS), F and associated p values are given for all the independent variables used in the General Lineal Model (GLM). The model displayed a global $R^2_{multiple}=0.35$, $p<0.001$. Significant effects are characterized by $p<0.05$.

	SS	d. f.	F	p
Intercept	1526.08	1	2228.03	<0.001
Longitudinal gradient	0.04	1	0.06	0.81
Habitat impairment	3.23	1	4.71	0.03
Biotic perturbation	53.37	1	77.92	<0.001
Error	108.22	158		

only sites with a score higher than an average reference site could be labelled as *in good condition*, while *in moderate condition* otherwise. Similarly the limit between poor-moderate was set at the mean value of the perturbed sites. In this way only sites in a condition higher than an average perturbed site could be labelled as *in moderate condition* while *in poor condition* otherwise (Table 4). There were no significant differences in the longitudinal gradient among quality classes (ANOVA, F=0.5, p=0.7), while these were marginal in the habitat impairment gradient (F=1.8, p=0.1) (Fig. 3). Although this marginal statistical significance, mean habitat impairment values followed a reasonable increasing tendency along the quality classes (Fig. 3). The values of the biotic perturbation gradient were clearly different among quality classes (F=22.5, p<0.001) (Fig. 2).

Table 4. Community Health Index quality classes. The cut-off points were established to maximize the index response to habitat and biotic impairment and reduce the risk of Type I and II errors.

Quality class	Cut-off point	Aim	Interval
HIGH			(6.46-10]
	Percentil 95 Perturbed sites	Less than 5% of Perturbed sites are misclassified as in high condition (Reduced Type II error)	
GOOD			(5.51-6.46]
MODERATE	Mean Reference sites	Only sites with a score higher than the mean scores within sites in Reference condition are classified as Good	(4.36-5.51]
POOR	Median Perturbed sites	Only sites with a score higher than an average perturbed site are classified as Moderate	(3-4.36]
BAD	Percentil 1 Reference sites	Less than 1% of Reference sites are misclassified as in bad condition (Reduced Type I error)	[0-3]

The Index of Community Integrity showed a high concordance with the other two tested indices (Fig. 2). A previous analysis proved no redundancy between both indices and the pressure gradients used to calibrate the present index (Pearson´s r <|0.5| for all the relationships between the gradients in Table 2 and the QBR and IBMWP), avoiding circularity effects in the validation. There were significant differences in the scores of both alternative indices among present quality classes (ANOVA, F=13.5, p<0.001 for IBMWP and F=4.6, p<0.001 for QBR). This result not only validates the present index but also supports and enhances the slight response it displayed when assessing the habitat quality impairment. Both QBR and IBMWP are especially dedicated to the assessment of riparian forest-environment quality (physic habitat) and water quality respectively. Given the high concordance they displayed we can ensure that these two sources of habitat degradation can be correctly assessed through the Index of Community Integrity, while co-variation issues between land uses and water quality could be masking the response of the index to both sources of perturbation in the habitat impairment gradient.

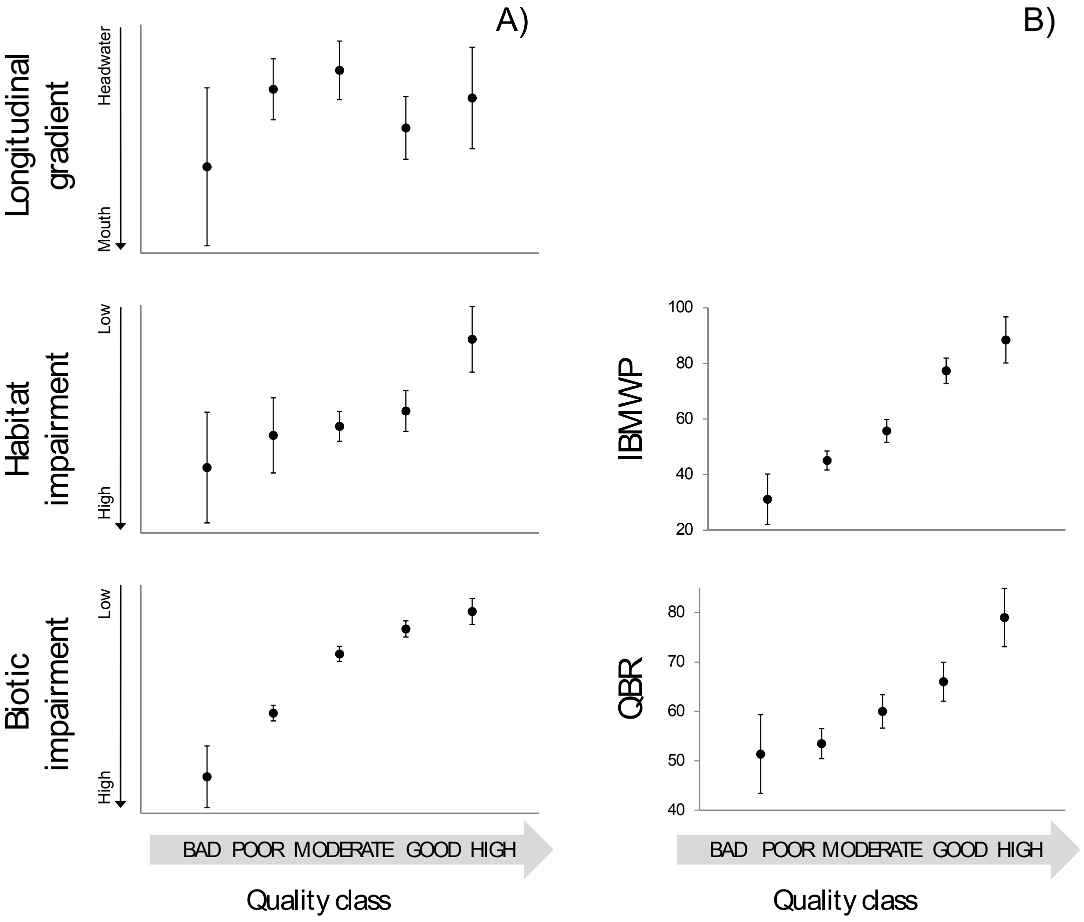

Fig. 2. Community Health Index calibration A) and validation B). For the calibrating process the Principal Components detailed in Table 2 were used. The response of the index vs. other two common indices was used for validating it ability to give at least the same information. Mean ± SE values are shown.

DISCUSSION

The Index of Community Integrity follows a site-specific comparison of community composition based on the reference condition approach. The presence-absence of ten native species was successfully modeled and used to evaluate the deviation between the observed and expected community composition. Species by species probability measures of each site to be a reference site were summed up in the final score of the index.

The reference condition approach (Hughes et al., 1986; Reynoldson et al., 1997, Bailey et al., 1998) in bioassessment defines biological integrity in terms of compositional similarity between present and expected optimal situations so it is assumed that human and biotic impairment affects the local community composition. Multimetric indices quantify the biological integrity through several community attributes (as changes in guilds-based metrics) rather than compositional comparisons. Since taxa are the basic unit of communities and hence aggregated biological organizations, alterations in taxonomic composition may occur before changes at other structural levels (Norris and Hawkins, 2000; Hawkins et al., 2000). Moreover, different members of the same ecological aggregation may not respond in the same way to a given disturbance showing independent responses to different types and degree of degradations (Thiollay, 1992; Lindermayer et al., 1999; Lindermayer et al., 2000). This could make indices insensitive to specific disturbances. Additional problems have been linked to multimetric approaches, such as circularity in the selection of sensitive metrics to conform the final index or the tendency to make Type I errors associated also to metrics selection (Norris and Hawkins, 2000). In addition, the development of complex indices with numerous metrics in rivers with a small number of native species, such as the Mediterranean rivers is difficult (Miller et al., 1988) and a good knowledge on species basic ecology aspects is needed for developing multimetric indices (Norris and Hawkins, 2000). However, this information is lacked at the moment and most of the ecological classifications are based on expert judgment. As an example a half of the species included in the European FAME project could not be correctly classified into different guilds because missing information, according to Schmutz et al. (2007a). A predictive approach which considers species composition instead of elaborated metrics seems a more efficient way to face the assessment of the ecological status hence.

However some predictive approaches based on species composition as RIVPACS or AUSRIVAS (Simpson and Norris, 2000; Clarke et al., 2003) simplify the complex effects of natural or perturbation induced changes on community composition through the use the O/E species richness ratio as a synthetic measure of community integrity. Some important changes in ecosystem structure or function may not be detected through this simplistic approach (Karr and Chu, 2000; Norris and Hawkins, 2000). To overcome this drawback, changes at the whole community were considered in this study through species by species comparisons making the index more powerful and flexible according to Pont et al. (2006). Thus solutions to potential problems in both multimetric and richness-based approaches have been faced and overcome in this index.

Presence absence data has been used as the basis for the comparison between the observed and the expected communities. It could be expected these method to be insensitive to many stressors, because individual populations of some species can suffer a considerable degradation before going locally extinct. However, at the assemblage level, presence-absence data appears to be sufficiently robust to allow the detection of reasonably subtle differences among sites (Hawkins et al., 2000) and has widely been used in other common indices, such as RIPVPACS (Clarke et al., 2003). Population densities are submitted to greater temporal (seasonal and inter-annual) and spatial rates of change than presence-absence data even under natural conditions in Mediterranean and other similar harsh environments (Meffe and Minckley, 1987; Matthews and Marsh-Matthews, 2003; Magalhães et al., 2007). Species presences have been proved to be more persistent than abundance through natural dramatic climatic events (frequent in this kind of environments) as severe droughts or floods (Magalhães et al., 2007). This temporal variability usually forces researchers to validate their indices through time series to account for the effect of these natural changes (Pont et al., 2006; Collier, 2008). Although a temporal validation of the index would be desirable, it seems of less concern in a presence-absence index than when using highly seasonal dependent metrics. Furthermore, the characterization of species´ abundances is largely more complex and difficult to standardize than the description of species´ presences-absences. Key issues in sampling methodologies like sampling effort or methods have immediate consequences on bioassessment results (Lenat, 1993; Reynolds et al., 2003), so the simplest the information needed the more confident the results. This also has economic implications for the implementation of bioassessment programs, since the more information needed the higher the cost of recording it while a cost-efficient method is always desired (Schmutz et al., 2007a). Thus the use of presence-absence data supposes not only a considerable simplification for the implementation of the index but also a way to overcome other weakness related to the use of abundance data.

The summing of metrics or measures to produce a final index score cannot be recommended unless it could be demonstrated every single partial measure to vary in the same direction and with the same magnitude of response to damage (Norris and Hawkins, 2000). The present index fulfils this exigency since the residuals between the observed presences-absence and the expected probabilities tend to decrease with human-biotic impairment. Sensitive species will disappear at perturbed sites deriving high negative residuals in case of being predicted to be present while tolerant species will remain at perturbed sites implying higher positive residuals. Then all this partial evaluations are transformed into probabilities of a site to be a reference site all ranging between 0-1. Every species´ evaluations had the same weight within the final index score since all their derived probabilities for the site to be a reference site were summed up without any weighting given that every species was proved to be sensitive to any source of perturbation in a previous study (data not shown). Even rare species with very low prevalence were successfully modelled through the ANNA model and included in the index overcoming the potential weakness of this no-multimetric predictive approach according to Pont et al. (2007). They justify the use of elaborated metrics instead of real data due to the unfeasibility to model rare species. The use of all the species with no weights and the wide range of environmental conditions accounted for through data from the whole basin ensured the index to respond to a broad range of perturbations.

The Index of Community Integrity showed to be sensitive to both human and biotic while not at all to the natural spatial variation. The ability to distinguish between natural and impairment-induced changes in community composition is a crucial point in bioassessment (Fausch et al., 1990; Huges et al., 1998; Norris and Hawkins, 2000). The lack of concordance of the present index and the longitudinal gradient was managed through the predictive model which accounted for a substantial portion of the spatial variability of species´ presence-absence making its scores independent of natural variations. A site-specific approach was used in this study following the river continuum concept (Vanote et al., 1980) avoiding artificial classifications and their derived consequences on bioassessment. The effect of inaccurate *a prioi* top-down landscape classification and even *a posteriori* bottom-up biological classifications used in type-specific approaches (Schmutz et al., 2007b; Melcher et al., 2007) on predictive model performance and bioassessment results are being pointed out in Hermoso et al. (submitted). On the other hand good responses were found for all the sources of perturbation tested. A strong response was found for different sources of habitat perturbation given the concordance of this index with other two commonly used indices to assess water quality and the status of the riparian forest and the naturalness of the riparian zone. Is also remarkable the response showed to the biotic disturbance measured as the relative dominance of exotic species within the whole fish community. Attending to relative measures the effect of local species richness and abundance were discarded. Exotics are considered one of the major threats to the conservation of native fish communities and to the ecological status of rivers hence (Kaufman, 1992; Godinho and Ferreira, 2000; Clavero et al., 2004). However it has not been extensively considered in recent works (Oberdorff et al., 2002; Pont et al., 2007; Ferreira et al., 2007) while was emphasized as a main issue to consider when assessing the ecological status (Pont et al., 2006). Moreover impacts of exotic species on the ecological status are difficult to prove and assess through physic-chemical habitat quality measures since exotics neither alter any other ecosystem attribute besides from biotic communities nor may be related to other human impacts (a site in very good physic-chemical condition may be completely dominated by exotics) though it is difficult to distinguish between both sources of perturbation (Light and Marchetti, 2007). That makes especially important for any index to be sensitive to biotic disturbances. Thus the present index is capable to detect not only commonly measured habitat perturbations but also and more important the degradation status of the native freshwater fish community. Furthermore the relative weight of different factors can be assessed in a post-hoc diagnostic using each species partial evaluations which may help to the development of efficient corrective plans to lead the assessed freshwater ecosystems to a good ecological status as the WFD requires.

The use of the present index in a broader area would not be a complicated task since the only requisite needed is the development of accurate predictive models for local faunas. In this way the Index of Community Integrity could be adapted and validated in other Mediterranean basins which at the moment lack of efficient fish-based bioassessment tools. Through a site-specific approach the limitations imposed by river types (the index is only applicable to a river previously included within the same type) are overcome. Given the special characteristics of the Mediterranean freshwater fish communities (high basin endemicity) a basin approach seems to be the best option for site specific predictive methods. A multi-species predictive approach is highly recommended for other Mediterranean basins given the reduced distribution areas of some endemics. Finally a deeper characterization of the different sources of perturbation would allow to better study the index response and potential.

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CAPÍTULO 4

IDENTIFYING PRIORITY SITES FOR THE CONSERVATION OF FRESHWATER FISH BIODIVERSITY IN A MEDITERRANEAN BASIN WITH A HIGH DEGREE OF THREATENED ENDEMICS

Identificación de lugares prioritarios para la conservación de la biodiversidad de peces de agua dulce en una cuenca mediterránea con un elevado grado de endemismos amenazados

(En revisión en Hydrobiologia)

Identifyng priority sites for the conservation of freshwater fish biodiversity in a Mediterranean basin with a high degree of threatened endemics

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ABSTRACT

The Guadiana River basin’s freshwater fish species richness and its threatened status in the circum-mediterranean context highlights the need for a large scale study to identify priority areas for their conservation. One of the most common problems in conservation planning is the quantification of a site’s relative value for the conservation of local biodiversity. Here we used a two-tiered assessment approach, which integrates an assessment of biodiversity loss and the evaluation of conservation value through site-specific measures. These measures based on the reference condition approach introduce the ability to make objective comparisons throughout the Guadiana River basin avoiding *a priori* target areas. We identified a set of biodiversity priority areas with less perturbed fish communities and that contain rare taxa of special conservation significance because of their outstanding contribution to basin’s biodiversity. The inclusion of complete sub-basins in these biodiversity priority areas ensures the consideration of the main conservation planning principles: representation of all the species and their persistence. Additionally, these areas may guarantee the existence of an optimal solution in terms of spatial aggregation, and cost. However the high fragmentation which the Guadiana River basin is submitted to makes necessary further studies to evaluate the capability of the priority areas pointed out in this work to maintain the Guadiana´s freshwater fish biodiversity.

Keywords: Guadiana River basin, freshwater fish, priority areas, conservation.

INTRODUCTION

Freshwater ecosystems are among the richest and more diverse ecosystems on earth (Revenga & Mock, 2000) and fish account for a large part of this biodiversity (Saunders et al., 2002). However, the importance of these ecosystems to human culture, welfare and development has lead to increasingly severe and complex impacts to freshwater biodiversity and ecology (Malmqvist & Rundle, 2002). Five main sources of perturbations are responsible for this situation: i) species introductions and translocation, ii) impoundment of rivers and water abstraction, iii) water quality deterioration (pollution or eutrophication), iv) habitat degradation and fragmentation and v) overexploitation (Prenda et al., 2006; Collares-Pereira & Cowx, 2004; Allan & Flecker, 1993). As a consequence, many freshwater fish species have become extinct or are highly endangered. Particularly rivers of arid and semi-arid regions (Collares-Pereira & Cowx, 2004) are submitted to an accelerated rate of change - with synergistic effects between the sources of disturbance sources cited above and the effects of climate change. This is especially important within the Mediterranean basin with its high level of endemism. Here, 56% of endemic freshwater fish species are threatened, 18% critically endangered, 18% endangered and 20% vulnerable. Only 52 species (21%) are assessed as least concern (Smith and Darwall, 2006).

Despite the urgent need for efficient conservation planning in the face of continuing land use changes (Malmqvist & Rundle, 2002), little effort has been spent on applying systematic conservation planning in freshwater ecosystems. Formal protection in reserves tends to be *ad hoc*, favouring the conservation of the biodiversity of areas that are less valuable for commercial uses, easiest to reserve and with least need for short-term protection (Margules et al., 2002; Knight, 1999; Pressey, 1994). Additionally, most of these reserves were designed for terrestrial conservation purposes, based on inefficient criteria for freshwater biodiversity management (Filipe et al., 2004).

Conservation planning deals with the design of reserve systems to ensure not only the representation of the all target biodiversity attributes but also its persistence promoting their long-term survival (Margules & Pressey, 2000). The identification of priority areas where the conservation efforts should be focused on has traditionally been based on methods which used rarity or species richness as a measure of their relative contribution to the global target (Knight et al., 2007, Darwall & Vié, 2005; Eken et al., 2004). However, all these methods lack of comparative systems at the management unit which make priority setting more systematic and explicit. The use of site-specific measures enables a comparative evaluation at this scale. To address this, Linke & Norris (2003) developed an efficient methodology, based on the reference condition approach, which ensures the comparability of the results for macroinvertebrate communities in Australia through site-specific scores for each target taxon.

In this work we identify priority areas for the conservation of the Guadiana River basin's freshwater fish biodiversity to guide the future conservation planning in the area. We apply the methodology developed by Linke & Norris (2003) to the freshwater fish community of the Guadiana River, a specially endangered basin identified by the IUCN as regionally important for endemism and a centre of threatened species (Smith & Darwall, 2006). In this study, we integrate assessment of the fish community health and evaluation of the conservation value to identify the most suitable areas to focus the conservation efforts on. We analyse natural and human induced causes for the areas in need of restoration. The effect of one of the most important threats to the conservation of the Mediterranean freshwater biodiversity is also discussed.

METHODS

Study area

The Guadiana River basin is located on the South-Western Iberian Peninsula between Spain (88.8%) and Portugal (17.2%). The total drainage area is 67039 Km^2, flowing into the Atlantic Ocean. This study was mainly focused on the Spanish portion of the basin, characterised by a typical Mediterranean climate, with high intra- and interannual discharge variation, with severe droughts and floods (Gasith & Resh, 1999).

Although it is not an overpopulated area (28 hab/Km^2), agricultural activities have transformed the landscape significantly during the last century. Almost half of the basin (49.1%) is currently used for agriculture (30.6% occupied with intensive agriculture as irrigated lands and 18.5% occupied with extensive agriculture, like olive groves or fruit trees). As a consequence of this, about 8300 Hm^3 of water is retained in 86 big reservoirs (>1 Hm^3) and more than 200 small ones (<1 Hm^3) for water supply which equals more than the average annual rainfall.

This situation is particularly worrying, since freshwater ecosystems have hardly ever been considered in traditional conservation planning. This tendency may change with the new *Natura 2000* network, the European network of protected areas. An important portion of the Guadiana River basin (14.7%) is planned to be included as a Site of Community Importance (SCI) of this network of reserved areas. However, most of the places initially proposed to be included in this network are still unprotected and submitted to disturbances. In practice, only about 3,150 Km^2 (5.2% of the basin) are officially stated as reserved areas and submitted to special management regimes (including two National Parks, three Natural Parks and 8 Natural Reserves).

Guadiana's freshwater fish fauna is especially relevant within the circum-Mediterranean context. Its species richness is only comparable to that found in Po River basin in northern Italy and the lower Orontes in south west Turkey (Smith & Darwall, 2006). All of these rivers contain between 11 and 17 native fish species. However the threat status of the freshwater fish fauna in this basin does not differ from the general situation in the remaining Mediterranean basins referred above with almost the 90% of total native species included in any of the IUCN threaten categories. Both factors, high species richness and threatened status, indicate the need for special attention.

Fish and Habitat Data

Sampling was carried out at 242 sites within 6 different types of water bodies previously defined by the Spanish Ministry of Environment (Ministerio de Medio Ambiente, 2005). These sites were homogeneously distributed among the types of water bodies -% of sites and % of Km per type of water bodies were highly correlated (Pearson correlation, r=0.96, p=0.002)-, ensuring a comprehensive characterization of the basin.

Fish communities were characterised at all of the 242 sites during the spring of 2002 (29 sites) and 2005-06 (213 sites) using backpack electrofishing. Every site was sampled once, covering all available habitats in a 100 m river stretch (83.1 ± 29.1 m, Mean ± SD), without block-nets. This sampling effort is sufficient to capture most species present, except for assemblages in large rivers as Felipe et al. (2004) suggest on a study in the same basin. All fish were released after we identified the individuals to species level.

Habitat was characterised by 33 environmental variables, covering three different spatial scales: site, reach and basin. These measures could be split in two categories: a) predictors that described the natural habitat variability in the basin and b) descriptors of human perturbation (Table 1). Two approaches were used in this characterization: *in situ* or lab measures, which described micro and mesohabitat characteristics in each locality, and GIS measures used to record variables from digital maps (Table 1).

Analysis overview

The analysis by Linke and Norris (2003) is conducted in two steps. First, a predictive model is constructed from a set of reference sites. To assess biodiversity loss, common taxa that are expected at a site are evaluated regarding their actual presence. To estimate conservation value, locally rare taxa are identified using the same models. Additionally the concordance between the results and the *Natura 2000* network and the effect of river regulation over time are explored. The following paragraphs illustrate the detailed flow of analysis (Fig. 1).

Table 1. Set of environmental variables used to describe habitat characteristics. * Denotes those variables possibly affected by human perturbation (pressure variables) and not included in ANNA models.

Scale	Variable		Method	Code
Site	Water depth (cm)		*In situ*	DEP
	Shelter availability (m^2 of shelter/river width)		*In situ*	SHE
	Elevation (m)		GIS	ELE
	Relative position (dist. to the most headwater point/total length of the stream)		GIS	POR
	Stream order (Strahler)		GIS	ORD
	Distance to headwater (Km)		GIS	HED
	Distance to Guadiana River (Km)		GIS	GUA
	River width (m) *		*In situ*	WID
	Substrate coarseness (Wentworth scale) *		*In situ*	SUS
	Riparian Quality Index (QBR, Munne et al. 2003) *		*In situ*	QBR
	NH_4^+ (mg/L) *		*In situ*	AMO
	NO_2^- (mg/L) *		*In situ*	NTI
	NO_3^- (mg/L) *		*In situ*	NTA
	PO_5^{3-} (mg/L) *		*In situ*	PHS
	SO_4^{2-} (mg/L *		*In situ*	SLF
	Cl^- (mg/L) *		*In situ*	CLR
	Water temperature (ºC) *		*In situ*	WTE
	Conductivity (µS/cm) *		*In situ*	CND
	pH *		*In situ*	PH
	Dissolved oxygen (mg/L and %) *		*In situ*	OXG
	Annual precipitation (mm/m^2)		GIS	PRE
	Solar radiation (10 KJ/m^2*dia*µm)		GIS	RAD
	Average annual air temperature (ºC)		GIS	ATEM
	Distance to the nearest reservoir upstream (Km) *		GIS	DUP
	Distance to the nearest reservoir downstream (Km) *		GIS	DWN
Reach	Slope ($^{0}/_{00}$)		GIS	SLO
	Sinuosity		GIS	SIN
	Land uses in a buffer of 500 m	Urban/Industrial *	GIS	RUI
		Intensive agriculture *	GIS	RIA
		Extensive agriculture *	GIS	REA
		Natural *	GIS	RNA
Basin	Basin area (Drainage surface in each site, Km^2)		GIS	ARE
	Gravelius index		GIS	GRA
	Land uses	Urban/Industrial *	GIS	BUI
		Intensive agriculture *	GIS	BIA
		Extensive agriculture *	GIS	BEA
		Natural *	GIS	BNA
		Reservoir *	GIS	BRS
	Population density (Hab/Km^2) *		GIS	POP
	Population index (Nº hab/dist for the nearest upstream populations) *		GIS	PIN

Predictive models

The reference data set, used for model construction and validation, was sorted from the initial database by identifying localities slightly or no affected by human perturbations. To select these reference sites, we constructed and evaluated a pressure or disturbance index (Pont et al., 2004). Six environmental variables related to human perturbations were coded from 1 (no pressure) to 5 and summed to get the index scores (Table 2). A site was considered in reference condition when none of the six impact variables were rated over 2 (total pressure index value not higher than 12) and exotic fish species did not account for more than 5% of total fish abundance (see Kennard et al., 2006). Since the original number of reference sites was not sufficient for both model construction and validation, some reference sites were also chosen from adjacent basins in the same biogeographical region (Tinto, Odiel and Guadalquivir basins). From the initial selection of 90 reference sites, a random subset of this reference data base (70 sites) was used to build an ANNA model (Linke et al., 2005) to predict the occurrence of the 10 native fish species present in more than 5% of sites. The model predicts the probability of occurrence of each modelled taxon at a new site using only the fish fauna composition of the most environmentally similar sites. For example, if a taxon is present in 6/10 most environmentally similar sites, the taxon gets an expected probability of 60%.

Table 2. Pressure variables used in the selection of the reference sites and their perturbation classes.

Pressure variable			**Pressure Class**
Distance to downstream reservoirs	No reservoir		1
	> 50 Km		2
	15-50 Km		3
	5-15 Km		4
	< 5Km		5
Modification in the river channel	No modification		1
	Fluvial terraces modified and constraining the river channel		2
	Channel modified by rigid structures along the margins		3
	Canalized river		4
	River bed with rigid structures (Wells) or Transverse structures into the channel (weirs)		+1
Connectivity and internal cohesion	Connectivity between the riparian forest and the woodland	Longitudinal cohesion of the riparian forest	
	Total	>50%	1
		<50%	2
	>50%	>75%	1
		50-75%	2
		<50%	3
	25-50%	>75%	2
		50-75%	3
		<50%	4
	<25%	>75%	3
		50-75%	4
		<50%	5
QBR	>90		1
	70-90		2
	50-70		3
	30-50		4
	<30		5
Land uses (Basin and Reach)	Urban/Industrial	Intensive Agriculture	
	>1%	>30%	5
		10-30%	4
		<10%	3
	<1%	>30%	4
		10-30%	2
		<10%	1

Only variables not affected by human perturbations were used for model building. In this way we ignored the effect of human alterations from our predictions to estimate pre-disturbance distribution of the species. Since the distribution range of some of the native fishes modelled here is restricted to the Guadiana basin, the variable *Basin* was also considered in the models as a predictor.

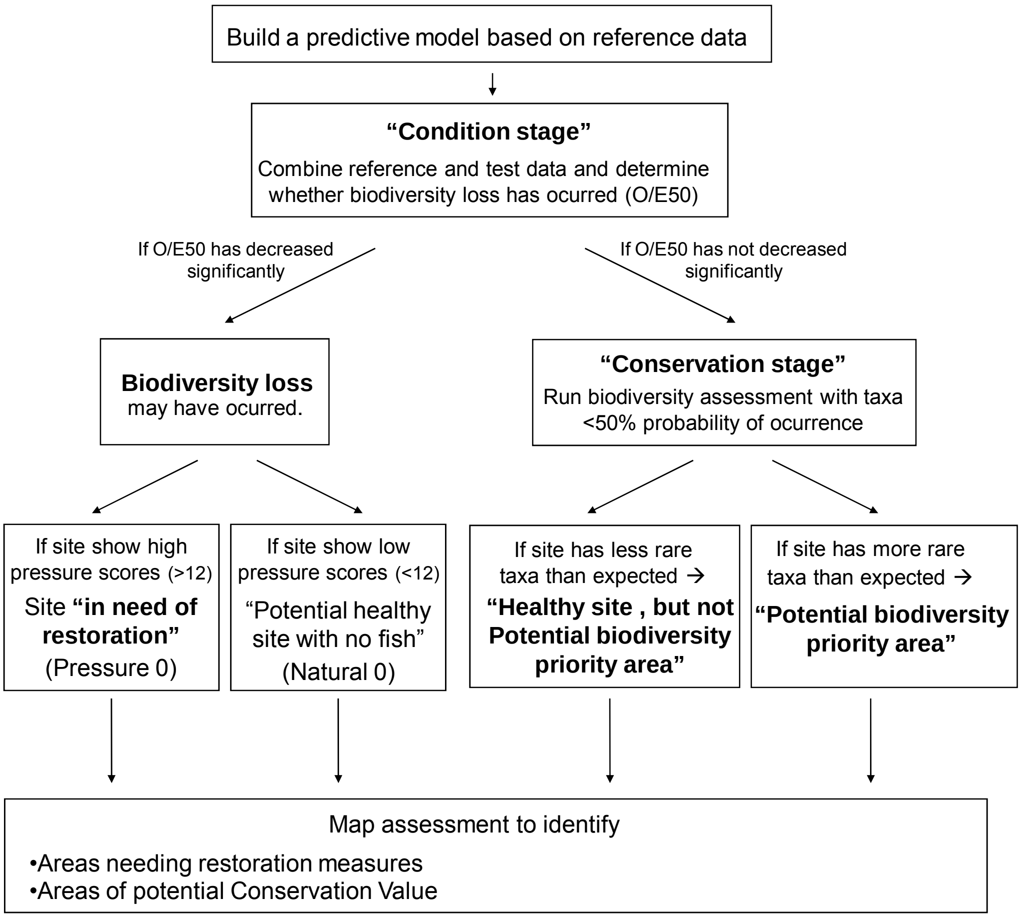

Fig. 1. Flowchart for the assessment of the condition and conservation value, adapted from Linke and Norris, 2003.

To validate the model, the expected species richness (sum of expected probabilities of each taxon) was compared to the observed richness in the validation reference sites (20 sites). If the model was valid, we would expect a 1:1 relation between the observed and expected taxa. Hence, a regression slope not different from 1 (t-test) and an intercept not different from 0 would be expected. An alternative measure was used to evaluate the precision of the model at species level, by establishing the best possible and the worst possible models (Van Sickle et al., 2005). The standard deviation of our model was compared to that derived from a model which took into account all possible non-sampled related variation which displayed the lowest possible

SD (SD_R) and a null model which was assumed to be the worst possible model, with the highest standard deviation hence (SD_{null}). Our model would be classified as good if its standard deviation in the validation data ($SD_{O/E}$) improves SD_{null} and end close to SD_R. Additionally, the area under the curve (AUC) of the Reciever Operating Characteristic (ROC) for the same data set was assessed as a measure of prediction success (Fielding and Bell, 1997).

Assessment of Condition and Conservation Value

Sites with a not or only slightly perturbed fish community - and those which contain rare taxa - are of special conservation significance, because of their outstanding contribution to basin's biodiversity. To identify these sites, a two-tiered approach was followed. First, the general condition of the native fish community was assessed through an index of biodiversity loss, the OE50 (Linke & Norris, 2003; Simpson & Norris 2000). It is a site specific coefficient which measures the potential loss of biodiversity and is calculated as the relationship between the observed and the expected species richness, considering only the common species (>50% probability of occurrence). To ensure a Type I error of 10%, the 10th percentile of the distribution of the reference sites was used as the cut-off for a significant loss of biodiversity (Linke & Norris, 2003; Simpson & Norris 2000). Only those sites with no significant loss of biodiversity were considered in the next step. With this pre-selection we ensured that sites that cannot be reasonably targeted for conservation purposes and labelled as "in need of restoration" (highly perturbed sites, where a healthy native fish community recovery would be complicated, Fig. 1) were removed from the set of potentially selected sites.

Second, an index of Conservation Value (CV) [called O/E (BIODIV), by Linke & Norris, 2003] was constructed analogously. However, only locally rare species as defined by the ANNA model (<50% probability of occurrence) were considered in the O/E. If the observed number of rare species was greater than the expected (CV>1), the site could be considered as a "potential conservation hotspot" (Fig. 1) (Linke & Norris, 2003).

Habitat harshness can strongly limit the colonization or the continued existence of fish species in freshwater ecosystems (Schlosser, 1995; Ross et al., 1985; Mathews & Styron, 1982). Thus a 0 score in the OE50 index could be related not only to human pressure but also to natural causes, as for example ephemeral hydrological regimens in headwaters. To identify sites that did not show mayor signs of human pressure, but did not have common native fishes and often no other fish (natural 0s hereafter) we carried out a PCA on a matrix of environmental variables x sites where the OE50 index scored 0. We then assessed the relationship between the main environmental gradient and the pressure index and used it as an indicator for distinguishing naturally low scoring sites and low scores due to human pressures. Sites located on the low pressure end of the disturbance gradient (mean pressure index below 12) could be hence labelled as “potential healthy site with no fish” (Fig. 1).

Finally we evaluated the effect of river regulation on the indices of biodiversity loss and the conservation value through time. Currently, this is one of the most important perturbation factors in Mediterranean environments with increasingly importance due to climate change. The mean value of both indices in regulated sub-basis thought big reservoirs (more than 100 Hm^3) in the same decade was compared along the last 50 years.

RESULTS

The ANNA model based on 70 reference sites was valid, since the regression slope in the validation subset was not significantly different from 1 (b=1.063, t-test, p=0.58) and the intercept not significantly different from 0 (Intercept= 0.058, p=0.95). This model used the closest 6 neighbour sites as a basis for its predictions. At an $SD_{O/E}$ (0.39), a substantial improvement over the null model (SD_{null}= 0.45) was observed. It was close to the optimal model´s SD_R (0.38) and could also be classified as fair-good by its AUC (0.79). This ensures that the model was strong enough to avoid under-prediction errors, which could invalidate the results of the indices below (Linke , 2006; Van Sickle et al., 2005).

The OE50 ranged between 0-1.57 (Mean ± SD, 0.49 ± 0.49). The cut-off point was set at 0.51 and a total of 145 sites failed it, showing a significant loss of biodiversity (Fig. 2A). By default, these included 7 reference sites (the 10th percentile). 94 from those 145 sites had an observed value of 0 - no common native species were found- (Fig. 2A).

The first component (PC1) of the environmental PCA carried out on the sites that scored 0 for the OE50 index showed a clear up-downstream/pressure gradient (Fig 3 and Table 3). It varied from upland reaches with no major signs of human pressure, to lowland reaches where the pressure variables reached their highest values within this subgroup of sites. This gradient was highly correlated to the pressure index (Pearson Correlation, r=-0.78, p<0.001). Thus, there is a group of sites that although scored 0 for the OE50 index, did not show major human disturbances. We selected all sites with a pressure index score below 12 (the benchmark used to differentiate between reference and perturbed sites), where the absence of any common species could be related to natural causes instead of human induced changes (Fig. 3). All localities included in this group were located in small ephemeral headwaters streams, which keep water only a few months a year. This set of sites could be labelled as “potential healthy site with no fish” (Fig. 1). The remaining sites with no pressure in addition to the set of sites which showed a significant loss of biodiversity pointed out those areas “in need of restoration” (Fig. 1).

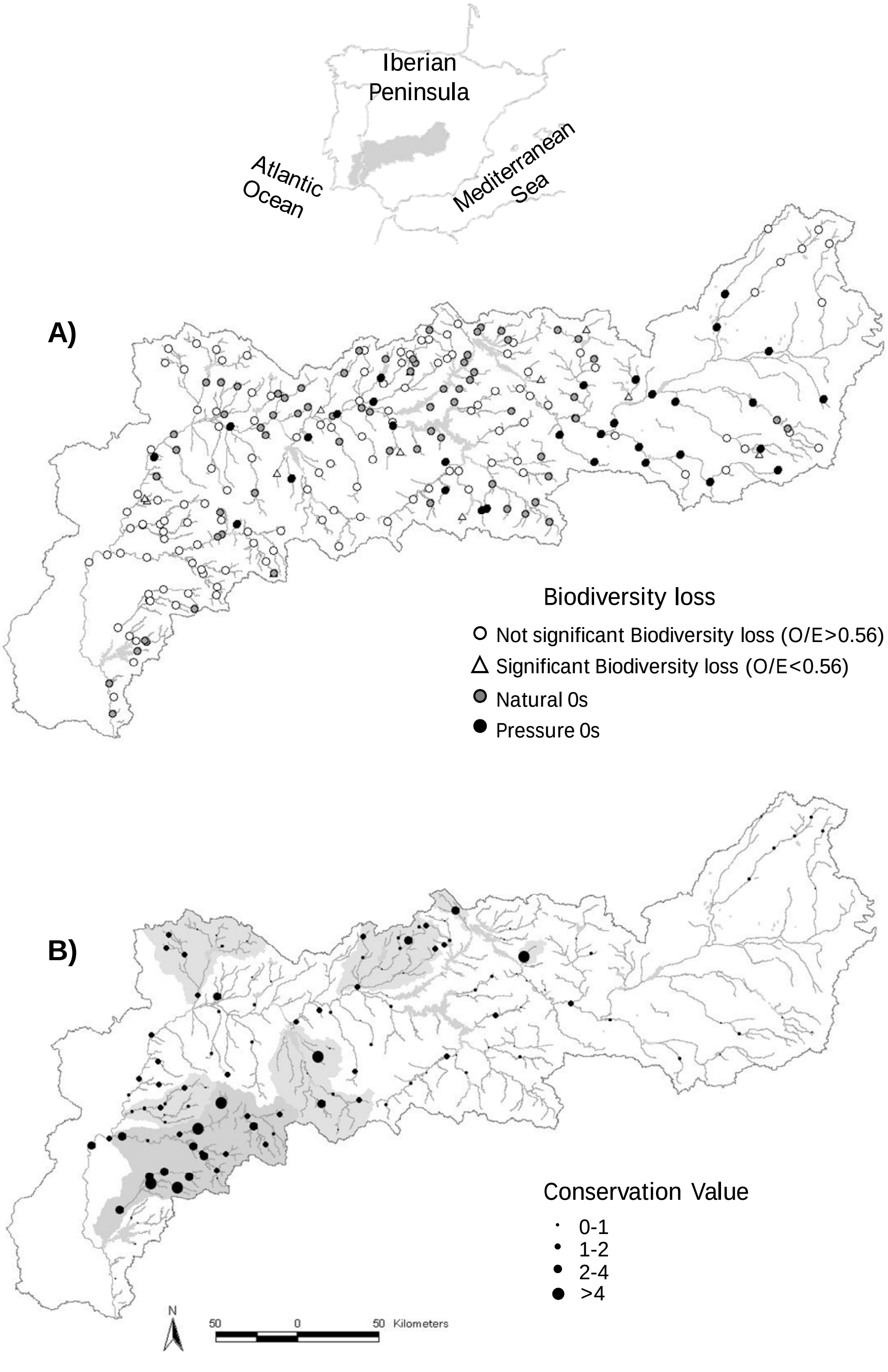

Fig. 2. A) Map of the index of Biodiversity loss for sampled sites (n=241). White dots denotes not significant biodiversity loss and hence sites that were considered in the second step. White triangles represent sites with significant loss of biodiversity, though any common native fish species was found. Grey and black dots refer natural and pressure 0s respectively. B) Scores of the Conservation value index of sites with no significant Biodiversity loss. The most relevant sub-basins are also highlighted. Only those rivers included in any of the studied sub-basins are shown.

We found a positive correlation between mean OE50 scores and the reserve extent in each sub-basin. When we considered all sites sampled within the same sub-basin, the mean scores of this index was positively correlated to the % of basin's area and the % of river (Km sub-basin/Km in a SCI) included in the Natura 2000 network (Pearson correlation, r=0.47, p=0.02, and r=0.39, p=0.06, n=25 respectively).

Table 3. Habitat gradients observed at sites with no common native species (O/E50=0) after a PCA (n=94 sites). r: Pearson correlation between the variables included in the PCA and the two principal components. *p<0.05; **p<0.01; ***p<0.001. The most influent variables (r>0.5) are highlighted.

Variable	**PC1** (24.4%)	**PC2** (11.6%)
BNA	**0.80** ***	0.25 **
PRE	**0.78** ***	-0.06
ARE	**-0.76** ***	-0.44 ***
BIA	**-0.74** ***	0.21 *
HED	**-0.73** ***	-0.48 ***
RNA	**0.68** ***	-0.31 **
GRAV	**-0.67** ***	-0.28 **
CLR	**-0.67** ***	-0.02
POP	**-0.66** ***	0.21 **
BUI	**-0.61** ***	0.19
QBR	**0.60** ***	-0.32 **
SLF	**-0.58** ***	0.1
ORD	**-0.56** ***	**-0.58** ***
SLO	**0.53** ***	0.29 ***
BEA	**0.53** ***	0.14
POR	**-0.50** ***	**-0.62** ***
PIN	-0.48***	-0.003
AMO	-0.48 ***	0.15
REA	-0.46 ***	0.29 ***
RIA	-0.43 ***	0.18
SUS	0.43 ***	-0.28 **
NTI	-0.38 ***	0.29 **
PHS	-0.37 ***	0.15
BRS	-0.36 ***	0.23 **
RUI	-0.34 **	-0.09
WID	-0.30 ***	**-0.71** ***
ATEM	0.30 **	**-0.51** ***
NTA	-0.28 **	0.33 **
WTE	0.25 ***	-0.40 ***
GUA	0.25 *	**0.56** ***
SIN	0.24 *	-0.01
RAD	0.14	-0.1
DWN	0.004	-0.15
SHE	-0.08	0.02
ELE	-0.1	0.58 ***
DEP	-0.12	**-0,63** ***
DUP	-0.17	0.19

CV was assessed for all sites with no significant loss of biodiversity (n=96). It ranged between 0, where no rare native species were found, and 9.2 (Mean ± SD, 1.03 ± 1.10). A value over 1 indicated that at least the same number of rare species as predicted were found. These sites (21.5% of 241 sites) had the healthiest fish communities, since they did not suffer significant loss of common species and the number of rare species observed were similar to or higher than predictions. When these results were mapped, extensive spatial differences were found. Most of sites with the highest CV scores were concentred in a reduced group of sub-basins (Ardila, Chanza, Alcarrache, Matachel, Gévora and Ruecas Rivers) (Fig. 2B). Furthermore, no significant relationships between the CV scores and the % of sub-basin area and river Km included in the *Natura 2000* network was found (Pearson correlation, r=0.07, p=0.79 and r=0.38, p=0.1 respectively, n=25).

The effect of river damming on OE50 and CV indices through time was significant. (ANOVA, F=4.32, p=0,003 for OE50, and F=4.28, p=0.003 for CV) (Fig. 4).

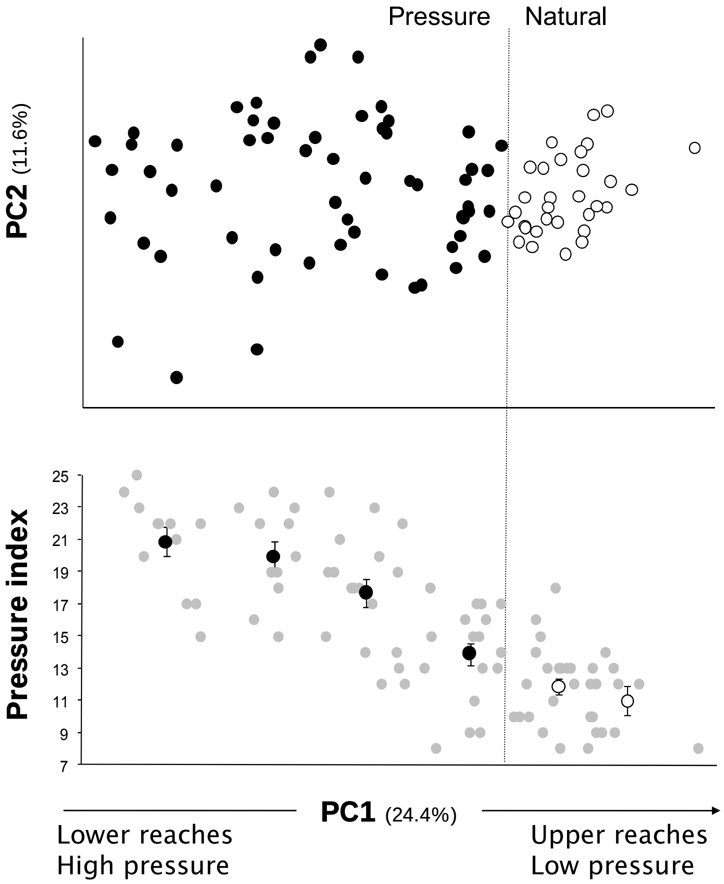

Fig. 3. Principal Component Analysis carried out on the environmental matrix of the 94 sites with 0 values for the OE50 index. Pearson correlation between PC1 and the Pressure index (Mean ± SE values for this index through the PC1 are also shown to visualise the difference between natural 0s -white dots- and pressure 0s -black dots- established in the porction of the PC1 gradient where the pressure index scored bellow 12).

DISCUSSION

Species-based criteria are employed in the majority of methods used to identify important sites for conservation of biodiversity (Darwall & Vié, 2005). One of the most common problems that have to be faced is the quantification of the relative value of a site for the conservation of the local biodiversity (Filipe et al., 2004; Root et al. 2002; Margules et al., 2002). Here we used a two-tiered assessment approach, which integrates an assessment of biodiversity loss and the evaluation of conservation value through site specific measures. These measures are based on the reference condition approach (Reynoldson et al., 1997), introducing the ability to make objective comparisons in biodiversity assessments throughout the study area (Linke & Norris, 2003). Additionally no *a priori* targets areas are selected in this study, giving the same opportunity to every river in the basin. This satisfies the criteria specified by Mace et al. (2000) avoiding *ad hoc* strategies.

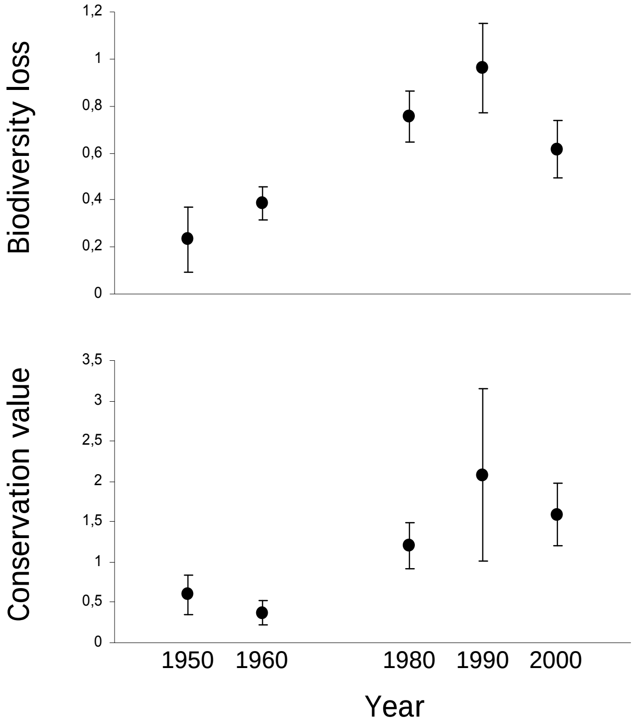

Fig. 4. Effect of basin regulation on the indices of Biodiversity loss and Conservation value through time. It is shown the scores (Mean ± ES) of the indices grouped in sub-basins regulated in the same decade. Only those sites located in a sub-basin containing a big reservoir (more than 100 Hm^3) were used (n=103 sites). For Alqueva and Pedrogao reservoirs (constructed in year 2002) only sites prospected after this year were included.

High performance models are characterised by a large AUC, with values between 0.7-0.9 (Manel et al., 2001; Swets, 1988) and other measures of model fit as slope, intercept and R^2 of the O/E regression line in the validation data set (Linke et al., 2005) or the standard deviation of O/E (Van Sickle, 2005). Our model performance was as good as other reviewed models (Elith et al., 2006; Linke et al., 2005; Van Sickle et al., 2005) as determined by AUC, the R^2, intercept and slope of the O/E, and its SD which was better than the null model and close to the best possible model. The risk of committing a Type I or II error was acceptably low, although not perfect - high CV scores pointed out local inaccuracies in the prediction of some taxa. However, we assumed that the predictions were accurate enough for using the model.

In a first step we selected the group of sites showing the fewest evidence of human disturbance on local biodiversity. These sites had similar richness of common native species compared to an expected richness value, estimated by their location within the basin and environmental characteristics. When they were grouped into sub-basins, a high correlation was found between the OE50 scores and the proportion of the sub-basin area and river length included in the *Natura 2000* network. The areas with the highest OE50 scores and hence with the best preserved fish fauna tended to be concentrated in zones with little potential for commercial exploitation or human habitation where the protected areas are usually gathered (Margules et al., 2002; Pressey, 1994). Thus, the actual *Natura 2000* network design seems to cover the areas with the less altered fish communities, but does not ensure the preservation of all the basin´s fish biodiversity as the OE50 index was based only on a portion of the total fish community.

For that reason in the second step, site-specific rarity *sensu* Linke & Norris (2003) was calculated and used to rank the sites which displayed not significant biodiversity loss. This ranking pointed out the priority sites for protection -sites with more rare species than expected in addition to be holding communities with no significant loss of biodiversity-. Ardila, Chanza and Alcarrache Rivers stood out among the reduced number of sub-basins with a dense concentration of high CV scores (Figure 2B), conforming the most suitable biodiversity priority areas. Although high scores were also found in other sub-basins (Gevora, Ruecas or Matachel Rivers), they were confined to upper reaches, while in the former three they occupied a wider range of the environmental gradient (headwater-middle-low stretches) within the whole sub-basin. The inclusion of a longer portion of the environmental gradient in these former biodiversity priority areas may ensure the consideration of the main conservation planning principles: representation of all the species and their persistence. Additionally, these areas may guarantee the existence of an optimal solution in terms of spatial aggregation, and cost hence, where the conservation efforts should be focused on to facilitate the effective development of the known limited resources intended for conservation issues (Knight et al., 2007).

These results are backed up by the findings of Filipe et al. (2004) for the Portuguese portion of the Guadiana River basin. They used an alternative method, based on predicted presences of native fish species, which were weighted by their threaten status into an index. They found that the rivers with the highest conservation value in their study area were Ardila River and other two close smallest rivers (Enxoe and Degebe Rivers). While the approach by Filipe et al. (2004) is not a site-specific assessment of conservation value and ignores the divergence between condition and biodiversity assessment, the concordance reinforces the result we present here, since the same area has been found to have the highest conservation values with two alternative methods.

River regulation may be behind the decrease of the OE50 and CV in many of the sub-basins we studied. The more time the sub-basin has been regulated, the higher biodiversity loss and the lower conservation value they displayed (Fig. 4.). Alqueva and Pedrogao were the last big reservoirs built in the Guadiana River basin, affecting the last big sub-basin which had not been regulated yet (Ardila River) and where the biodiversity priority areas are concentred. They have recently created more unsuitable habitat for most of native fish species by affecting their inter sub-basins movements and enhancing the population of exotic species as suggested Filipe et al. (2004) for this area and Clavero et al. (2004) found for the Iberian Peninsula. Thus, the establishment of discrete reserves, as would be the case in the Guadiana River due to the presence of multiple reservoirs in the basin, could not be enough to protect freshwater fishes (Meffe, 2002; Angermeier, 2000; Lindermayer et al., 2000) and must be deeply studied.

The actual reserve system seems to be the result of partial contributions of regional authorities instead of a global planned project. No significant relationship between the CV index and the *Natura 2000* network may suppose that some priority areas for conservation planning could be out of the final reserve system. This uncovers the need to review the current *Natura 2000* network applying complementarity criteria to check its competence to sustain all the Guadiana´s freshwater fish biodiversity in a whole basin context. However, the identification of biodiversity priority areas should imply neither the lack of active management regimes within them nor in off-priority areas (Cowling et al., 2003; Lindermayer et al., 2000). We highly recommended a mixed protection scheme where the conservation efforts are opened out to off-reserve management (Linke et al., 2007; Margules & Pressey, 2000) especially in the control of exotic fish species populations that may affect the contiguous reserved areas.

Thus, additional studies are needed to evaluate the capability of the biodiversity priority areas pointed out in this work to represent and ensure the persistence of the Guadiana's freshwater fish biodiversity, overcoming the limitations and threats that the reservoir fragmentation means. Further studies should also consider some key factors in conservation planning like threats and costs. This planning is especially important given the great value of Guadiana's endemic fish fauna and its highly threatened status.

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CAPÍTULO 5

CONNECTIVITY IN FRESHWATER SYSTEMATIC CONSERVATION PLANNING

Conectividad en la planificación sistemática para la conservación. Aplicación a sistemas fluviales mediterráneos.

(En revisión en Freshwater Biology)

Connectivity in freshwater conservation planning

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ABSTRACT

Freshwater conservation has received less attention than their terrestrial or marine counterparts, despite of holding an important quote of hearth´s biodiversity. Given the accelerated rate of change and intensive human use which freshwater ecosystems are submitted to, it is especially urgent to devote some attention to them. The application of tools traditionally used for systematic conservation planning, as Marxan, to river conservation necessarily needs some adaptations to consider the special nature of these systems. Connectivity has been pointed out as a key factor in freshwater systematic conservation planning to avoid the pernicious effect of degraded up-downstream stretches on reserved riverine areas.

To attain this goal we modified marxan´s boundary length penalty to account more specifically for the connected nature of rivers, which avoids the selection of isolated planning units in the reserve or forces the inclusion of its closer upstream neighbour ones favouring the selection of connected planning units within the reserve. The probability of occurrence of nine native fish species based on present distributions in a Mediterranean basin was used as target to select the more suitable set of planning units to conform the final reserve. Present probabilities of occurrence instead of potential probabilities were used to focus the efforts on identifying the more suitable areas for attaining persistence in reserves at the moment. These probabilities were obtained from MARS-GLM predictive models. Then, the simulated annealing algorithm provided in Marxan was used for designing the best possible reserve, which already accounts for essential complementarity and reserve spatial issues through the optimization of an objective function. Different Boundary Length Modifiers (BLM) and target levels to be held (set a priori) were tried to get the most clustered reserve.

The BLM had no direct effect on reserve compactness although it helped the connectivity rule to work properly. On the other hand, the target level had greater implications on the spatial parameters. An optimal combination of BLM and target ensured a compact solution with a high probability of species´ occurrence.

The probabilistic approach softens the interpretation of the reserve in terms of river length for a given target. A feedback process, where spatial needs of each conservation feature to develop healthy populations, with a high probability of persistence hence, led the reserve selection, would have beneficial results for the conservation planning task.

The best solution and the highest irreplaceability values were centred in a single sub-basin where all the species were expected to be present with a high probability according to present land uses-management. Thus, the current multi-use landscape scheme in this area could be maintained for future biodiversity conservation plans if general conditions do remain stable.

Keywords: Biodiversity, Guadiana River, irreplaceability, MARS-GLM, Marxan, native fish species.

INTRODUCTION

Despite of holding a considerable amount of Earth´s global biodiversity (Allan and Flecker, 1993) and being submitted to higher pressures and threats than adjacent terrestrial ecosystems (Malmqvist and Rundle, 2002; Nel et al., 2007), freshwater ecosystems have received less attention by the conservation community (Abell, 2002). There has been little emphasis on declaring protected areas for the primary purpose of conserving freshwater ecosystems and biodiversity (Saunders et al. 2002). Rivers have generally been inadequately dealt with in most assessments of terrestrial ecosystems unless they were considered important for terrestrial biodiversity patterns and processes (Nel et al., 2007). Matter of fact, most of the rivers included in terrestrial reserves are being used as mere reserves boundaries. This is clearly not enough to ensure an adequate long term preservation of their biodiversity..

The protection of all places which contribute to biodiversity conservation is impossible, since conservation usually competes with other human interests (Margules et al., 2002). Representativeness and persistence of biodiversity are two main goals in reserve design (Margules and Pressey, 2000). Once established reserves should promote the long term survival of the biodiversity they contain by maintaining natural processes and viable populations and by mitigating at least some of the proximate threats to their biodiversity (Margules and Pressey, 2000; Margules et al., 2002; Wilson et al., 2005a). Reserve design has traditionally involved either the use of subjective judgment of biodiversity value or the use of other completely extraneous criteria to biodiversity conservation such as scenic value, wilderness quality and inaccessibility, low primary production potential or simply availability (Margules et al., 1988; Pressey et al., 1996; Sarkar, 1999). These approaches lead to ad hoc conservation strategies focused on areas easiest to reserve and with least need for short term protection (Pressey, 1994; Knight, 1999; Pressey et al., 2000). In general these kind of approaches have had negative consequences for the conservation of biodiversity, as (i) they do not address representativeness, leading in some cases to the biodiversity most in need of strict reservation not to be protected (Pressey and Tully, 1994) and making the biodiversity to be uneven represented in existing reserves (Pressey et al., 1993, 2000), and (ii) a misuse of resources focused on areas containing relatively few biodiversity surrogates. To overcome these pitfalls, there must be an objective way to identify a set of areas capable of meeting the representation of all the targeted biodiversity. In the last two decades an explicit framework for systematic conservation planning has emerged (Margules and Pressey, 2000). Systematic conservation planning works on attaining biodiversity conservation goals identifying important areas where conservation efforts should be focused on (priority areas hereafter) to facilitate the effective development of the known limited resources intended for conservation issues (Knight et al., 2007).

Given the accelerated rate of land use change and because biodiversity protection competes with legitimate alternative human uses, methods for identifying priority areas have to be explicit, efficient, cost-effective and flexible (Margules et al., 2002). While traditional conservation tools rely on rank methods based on different criteria such as species richness, rarity value, naturalness, or size (Williams et al., 1996) modern conservation planning methods use complementarity-based algorithms. Under some circumstances a set of rich areas may contain no more species in total than a randomly selection of the same number of areas (Williams et al., 1999). This is because ranking methods fail to take into account the spatial heterogeneity and the turnover of features from area to area (Howard et al., 1998). Complementarity is defined as the gain in representativeness of biodiversity when a site is added to an existing set of areas (Possingham et al., 2000). Algorithms which incorporate complementarity lead to a more efficient representation of biodiversity features and better cost-effective solutions than ad hoc (Pressey and Tully, 1994), scoring or ranking strategies (Margules et al., 2002; Pressey and Nicholls, 1989). They look for areas that add as many under-represented surrogates (taxa or any other conservation feature) as possible to a network of protected areas (Pressey et al., 1997), achieving efficiency goal selecting as few areas as possible that together reach the representativeness goal (Pressey and Nicholls, 1989). However, the identification of single reserve solutions is a rigid strategy which gives no indication on the importance of each area in terms of their potential to be replaced by other available areas in the region (Pressey et al., 2004) and the value of unselected areas (Cabeza and Moilanen, 2006). To overcome this problem and to include flexibility in systematic conservation planning quantitative conservation tools often incorporate measures of irreplaceability. It indicates the likelihood that an area will be required to meet a given set of targets (Pressey et al., 1994; Ferrier et al., 2000).

Most terrestrial conservation planning has overlooked freshwater biodiversity, because incorporating freshwater species and habitats adds several layers of complexity to an already complicated effort (Abell, 2002). Nevertheless, systematic conservation planning specifically targeting freshwater ecosystems have begun to emerge (Nel et al., 2007; Linke et al., 2007, Moilanen et al., 2008) applying all the principles developed for terrestrial ecosystems, but recognizing the need for some refinements to consider the special characteristics of freshwater ecosystems (Dunn, 2003). Freshwater conservation planning must deal with the connected nature of rivers which is a key factor for the structure and conservation of freshwater biodiversity even more important than for terrestrial ecosystems. However in the context of freshwater biodiversity conservation, connectivity has not received the attention that it deserves (Pringle, 2001). Reserves located in middle-low watersheds often suffer the cumulative effects of hydrologic alteration and pollution originated in both upstream and downstream, imposing high threats to the conservation of its biodiversity.

Here we adapt an extensively used tool for terrestrial and marine systematic conservation planning to rivers peculiarities and needs, to attain a more effective reserve design in freshwater conservation planning. We specifically address connectivity issues within MARXAN software, to account for the connected nature of rivers, making special reference to the implications of our results in river habitat km terms.

METHODS

Study area

The Guadiana River basin is located in the South-Western Iberian Peninsula draining a total area of 67039 Km^2 to the Atlantic Ocean. It features a typical Mediterranean climate, with high intra and inter-annual discharge variation, with severe floods and droughts (Gasith and Resh, 1999). Mean air temperature ranges from 13 to 18.1 ºC, with a strong intra-annual variation in extreme temperatures which in some areas reach up to 60 ºC. Mean annual precipitation ranges from 350 to 1200 mm (with a mean of 450 mm).

Although it is not an overpopulated area (28 hab/km^2) agricultural activities deeply transformed the landscape during the last century. Almost a half of the basin (49.1%) is currently used for agriculture - 30.6% occupied with intensive agriculture as irrigated lands and 18.5% occupied with extensive agriculture, like olive groves or fruit trees. As a consequence, about 8300 Hm^3 of water is retained in 86 big reservoirs (>1 Hm^3) and more than 200 small ones (<1 Hm^3) for water supply. Other common human perturbations are river channel modifications due to river channelization and degradation and even completely depletion of the riparian forest. About 3,150 Km^2 (5.2% of basin´s area) are stated as reserves and submitted to special management regimes, though most of them arose from ad hoc or terrestrial planning.

Guadiana's freshwater fish fauna is especially important within the circum-Mediterranean context. Its species richness is only comparable to that found in Po River basin in northern Italy and the lower Orontes in south west Turkey (Smith & Darwall, 2006). All of these rivers contain between 11 and 17 native fish species. However, the threat status of the freshwater fish fauna in this basin does not differ from the general situation in the remaining Mediterranean basins referred above with almost the 90% of total native species included in some of the IUCN threaten categories.

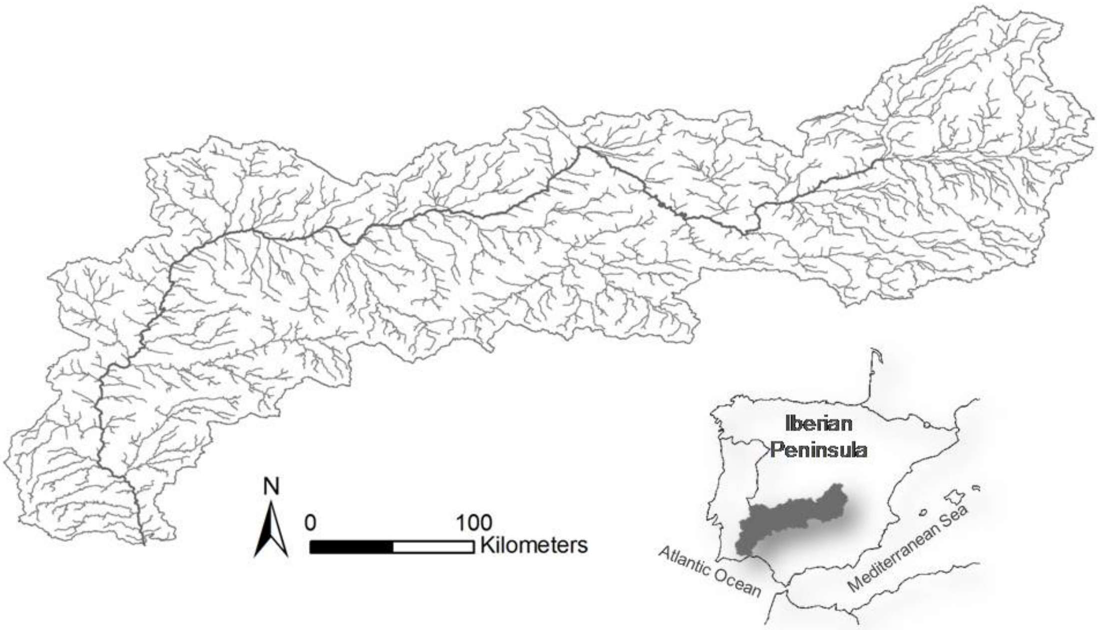

Fig.1. Guadiana River basin. The main river channel is pointed out in a thicker line.

Planning units

While in terrestrial systematic conservation planning equal sized grid cells are often used as planning units,, subcatchments are a more appropriate option for freshwater environments. This spatial approach accounts for the connected nature of rivers and natural boundaries of areas of influence (Linke et al., 2007). We hence derived 2170 planning units (Fig. 1) from a 90 m digital elevation model (Jarvis et al., 2006) through ARC Hydro (Maidment, 2002) within ArcGIS 9.1 (ESRI, 2002).

Environmental and biological data

Presence/absence of fish species was determined by electrofishing in 151 planning units. Sampling was conducted once without block-nets in stretches of 100 m when possible, as Filipe et al (2004) suggested for an accurate characterization of species´ presence-absence in the same study area. The sampling stretch was representative of all the habitats present in the area including pools and riffles. All fish were released once they were identified to species level. Given that freshwater fish display higher vital domains than other freshwater fauna (macroinvertebrates), it was assumed the observed data to be representative of the biodiversity present in each planning unit. .

We used two different spatial scales to characterize environmental attributes of our planning units, (i) subcatchment and (ii) catchment scale. In the former mean and accumulation across the whole upstream catchment area was considered. Only remotely sensed data was used in both approaches, to enable complete predictive coverage. The variables and data-sources are detailed in Table 1. All the variables were tested for normality and appropriately transformed when necessary for further analysis.

Prediction of biological data

Lack of complete coverage in areas which have not been systematically surveyed is a common problem to face in conservation planning (Margules and Pressey, 2000; Van Teeffelen et al., 2006; Linke et al., 2007). This is commonly faced by building predictive models of the distribution of conservation features throughout the landscape. A Multivariate Adaptive Regression Splines (MARS) model was used to predict the probability of occurrence for each species in the unsampled planning units. MARS is a method of flexible non-parametric regression modelling (Elith and Leathwick, 2007). It is useful for modelling complex non-linear relationships between response and explanatory variables with similar levels of complexity to that of a Generalized Additive Model (GAM) (Hastie, 1991). MARS fits a nonlinear function to the relationships between dependent and predictor variables by breaking the range of each predictor into a subset of portions or “knots”, and fitting linear relationships for each of them (basis functions). MARS allows the slope of the fitted linear segments between pairs of segments to vary while ensuring that the full fitted function is without breaks or sudden steps (Elith and Leathwick, 2007). The predictive function is finally composed of a series of connected straight line segments, rather the smooth curve of a GAM. Two interesting features made MARS models interesting for the present work. 1) It allows exploring interactions between predictors (Leathwick et al., 2006). 2) The possibility of fitting multiresponse models which simultaneously relates variation in the occurrence of all species to the environmental predictors in one analysis. Multiresponse models allow modelling even the occurrence of rare species, which are important in conservation planning exercises.

Table 1. GIS data used to characterize the planning units in the Guadiana River basin.

Catchment scale	Subcatchment scale
% Land uses[1]:	% Land uses[1]:
Urban	Urban
Intensive agriculture	Intensive agriculture
Extensive agriculture	Extensive agriculture
Naturalized	Naturalized
% Geology[2]:	% Geology[2]:
Actual alluvial deposits	Actual alluvial deposits
Calcareous	Calcareous
Siliceous	Siliceous
Acid volcanic	Acid volcanic
Basic volcanic	Basic volcanic
Mean annual rainfall[3]	Mean annual rainfall[3]
Precipitation seasonality[3]	Precipitation seasonality[3]
Mean annual temperature[3]	Precipitation in the wettest month[3]
Altitude (Average, Maximum and SD)[4]	Precipitation in the driest month[3]
Slope (Average and SD)[4]	Mean annual temperature[3]
FootPrint[5]	Annual temperature range
Population density	Temperature in the warmest month[3]
Drainage area	Temperature in the coldest month[3]
	Temperature seasonality[3]
	Isothermality[3]
	Mean annual evapotranspiration[3]
	Altitude (Average, Maximum and SD)[4]
	Slope (Average and SD)[4]
	Soil Quality Index[6]
	Vegetation Quality index[6]
	Distance to headwater
	Downstream distance to the nearest reservoir
	Distance to Guadiana
	FootPrint[5]
	Population density[6]

Data sources:

1 CORINE Land-Cover 1:100.000. Confederación Hidrográfica del Guadiana.
2 Mapa geológico de España 1:1.000.000. Instituto Geológico y Minero de España.
3 WORLDCLIM, Version 1.4. The data is described in Hijmans et al., 2005.
4 SRTM 90 m Digital elevation model from Jarvis et al., 2006.
5. Human footprint. Center for International Earth Science Information Network (CIESIN) at Columbia University (www.ciesin.columbia.edu/wild_areas/)
6. European Environmental Agency. (www.eea.europa.eu).

The model was fitted using a special code provided by Elith and Leathwick (2007) for the mda library within the free statistical software R, Version 2.1.1 (R Development Core TEAM, 2004). The common function provided in R for MARS uses least squares which works appropriately for data with normally distributed errors. With binomial data this results in the range of predicted values being expanded beyond their normal range (0-1), what could result in predicted probabilities lower/higher than 0/1 (Leathwick et al., 2006). To solve this problem the cited code fits a MARS model using the standard R code, extracts the basis functions, and computes a Generalized Linear Model (GLM) which uses the basis functions as predictors of each species´ presence-absence. This procedure ensures 0-1 constrained predictions. We allowed first order interaction between predictors in the models, since previous analysis showed a significant improvement in model performance when they were included. For more statistical details see Leathwick et al. (2005).

Table 2. Principal Component Analysis carried out in the environmental data matrix to select the most representative and independent predictors within the study area. The variance gathered in each Principal Component (PC) is showed in addition to their respective eigenvalues. The variable with the highest loading within each PC was selected as representative of each of them and used as predictors. * Denotes sub-catchment measured variables in opposition to catchment variables.

	Variance explained	Environmental variable	Factor loading
PC1	24.9 (12.9)	Coldest temperature*	-0.95
PC2	16.2 (8.4)	Average slope*	0.83
PC3	8.5 (4.4)	Average Evapotranspiration*	-0.65
PC4	7.6 (3.9)	Altitude (SD)	-0.79
PC5	6.3 (3.3)	Warmest temperature*	-0.65
PC6	4.2 (2.2)	Area	0.53
PC7	3.5 (1.8)	Siliceous	-0.56
PC8	2.8 (1.4)	Extensive agriculture*	0.41
PC9	2.7 (1.4)	Siliceous*	0.40
Total	76.7 (39.9)		

Model performance for each species was assessed through measures of deviance and the area under the received operating characteristic curve (ROC, Fielding and Bell, 1997). The area under the ROC curve (AUC) was assessed through a K-fold cross validation procedure (Hastie et al., 2001). The data was randomly divided in 10 exclusive sub-sets and model performance was calculated by successively removing each sub-set, re-fitting the model with the remaining data, and predicting the omitted data. The average error when predicting to new sites can then be calculated by averaging the AUC across each of the subsets (Leathwick et al., 2005).

Deviance complements AUC because it expresses the magnitude of the deviations of the fitted values from the observations. According to Elith and Leathwick (2007) the full information given in the predictions (raw probabilities of occurrence) were used in both the AUC and deviance analysis, rather than transforming this data into presence-absence estimates with a threshold.

Prior to model construction a Principal Component Analysis was carried out on the environmental data to extract a reduced number of independent predictors for MARS models. The presence-absence of 10 species in the whole biological data set was used for fitting the models (n=151 planning units).

Reserve design

Best solution and irreplaceability of each planning unit were calculated using a simulated annealing selection algorithm (Possingham et al., 2000) provided in the MARXAN software package (Ball and Possingham, 2000). MARXAN aims to find an optimal reserve network minimizing an objective function where feature penalties, spatial design and cost tradeoffs are considered (Formula 1)

$$Objective\ function = \sum_{planning\ units} Cost + \sum_{features} Feature\ Penalty + \sum boundary length \qquad \text{Equation 1}$$

After allocating a random initial reserve, planning units are added or discarded from the reserve system to find the optimal solution with the lowest value for the objective function. The final aim is to represent adequately a set of targets (species in our case) efficiently by selecting as few places as possible in the most compact possible reserve. In Equation 1, cost represents the cost of preserving each planning unit. Since we lacked objective estimates of the economic cost for the preservation of each planning unit, we assumed a homogeneous cost for all of them.

Feature penalty (FP) is a penalty for not fully representing all the features (fish species) in the final reserve solution at the targeted level. MARXAN considers features as objectives rather than constraints so the final solution might unmet completely a feature if a high cost would be necessary for meeting it.

The spatial design of the reserve is included through a boundary length penalty. We used a boundary length penalty which modifies MARXAN to account for the connected nature of rivers according to Linke et al. (2007). With this penalty non-headwater subcatchments cannot be included in the solution without upstream subcatchments being also included. Thus, an isolated subcatchment is a forbidden configuration if it is not a headwater planning unit (Linke et al., 2007). To temper the effect of this rule, which may influence excessively MARXAN reserve design, we weighted the connection between upstream planning units giving more weight to closer ones. This means that planning units directly adjacent to a selected one is more likely to be selected than any other further upstream. We also included a Boundary Length Modifier (BLM) used to build clustered reserves controlling the importance of minimizing the overall boundary length of the reserve system whilst minimizing its area, and maximizing compactness (Carwardine et al., 2007). Compactness is a measure of the degree of spatial clustering and was assessed as the ratio of the boundary length of the reserve network and the circumference of a circle of the same area (Possingham et al., 2000; Wilson et al., 2005b), since it is the most compact shape for a given area. Values closed to one resemble a circle and a compact solution hence.

Species´ probability of occurrence was used as the measure for the conservation features in each planning unit here. In this way the selection of the reserve network was focus on finding planning units which most contribute to the occurrence of species, and thus to reach the conservation goals. In this context, the target level imposed to be met in the reserve could be seen, in a probability term as the number of planning units within the reserve where each species had an absolute probability of occurrence (100%). Thus, the overall probability of occurrence of each target within the reserve is expected to be at least equal or greater than the pre-specified target level. It is also expected that the higher the target level, the higher the number of planning units will be required to be selected, what might affect the spatial design of the reserve. So, different target levels were tried to ensure the higher probability of occurrence for the species while not disturbing other parameters optimizing the reserve design (e. g., total area or reserve compactness). The same target level was used for all the species in the same run.

We checked the sensitivity of the reserve design outcomes to different BLMs and target levels through ANOVA analysis on compactness values. In this way we could find the best combination for both factors leading to the most clustered solution. Then the reserve´s efficiency was revised through the tradeoffs between mean target held and reserve compactness and total river km included. Finally, irreplaceability was assessed as the selection frequency of each planning unit by running the algorithm 100 times.

RESULTS

Predictive models construction and performance

The environmental variables with the highest loading for the 9 first Principal Components (PC) of the environmental PCA were selected as predictors for the MARS-GLM model (Table

2). These 9 PCs accounted for more than 76% of the original variance and ensured high tolerance values for the variables used as predictors, avoiding redundancies.

Presence-absence of 9 out of the 10 species was successfully modelled with at AUC>0.6 (Fielding and Bell, 1997) and an average explained deviance of 29% (Table 2), in consonance with that showed in previous applications (Leathwick et al., 2005). The model only failed fitting *Cobitis paludica* data. It is a widespread species with high prevalence values which probably led to a random distribution at least in relation to the selected predictors. This model was then used to predict the probability of occurrence of each species in the unsampled planning units.

Table 3. MARS-GLM model performance. The deviance explained indicated the reduction in deviance for each species with respect a null model. The proportion of total deviance accounted for is shown in brackets. The discriminatory power of the model for each species is given through the AUC of the ROC curve (calculated by K-fold re-sampling with its SD in brackets).

Species	Deviance explained	ROC	Prevalence (n=151)
Anaecypris hispanica	33.6 (0.41)	0.72 (0.11)	0.05
Luciobarbus comizo	44.7 (0.27)	0.75 (0.15)	0.24
Luciobarbus microcephalus	46.6 (0.25)	0.70 (0.20)	0.31
Luciobarbus sclateri	34.6 (0.34)	0.79 (0.21)	0.10
Iberochondrostoma lemmingii	42.9 (0.30)	0.73 (0.15)	0.28
Pseudochondrostoma willkommii	53.2 (0.24)	0.70 (0.20)	0.17
Cobitis paludica	32.2 (0.02)	0.57 (0.08)	0.66
Salaria fluviatilis	47.8 (0.42)	0.69 (0.28)	0.13
Iberocypris alburnoides	3.8 (0.18)	0.66 (0.12)	0.66
Squalius pyrenaicus	25.5 (0.42)	0.69 (0.16)	0.13
Average	36.5 (0.26)	0.71 (0.16)	0.16

Reserve design

The BLM and the target level imposed to be held had effects on the spatial design, since clear patterns in both, total area and reserve compactness, were found for increasing values of BLMs and targets (Fig. 2). There was a direct increase in total area and reserve compactness values when the target was raised. However, the effect of the BLM on reserve area and compactness followed an asymptotic response (Fig. 2), pointing out optimum values as it has been reported in previous studies.

The most compact solutions, within all the combinations between target levels and BLMs, were found when setting the target level at 5 (Mean ± SE, 1.01 ± 0.04 for different BLMs) (Fig. 2). The mean target held in this set of solutions was 12.2 and the total river length included in the reserve was near 300 km (Fig. 3). Actually, the most clustered reserve found within all the BLM-target combinations (BLM=3 and target=5 whit a compactness=0.98) included 298 km of river and held a mean target of 16.5. Reserve compactness decreased linearly as the km of river increased. Then a reserve with more than 300 km was far from achieving the compactness goal. The difference between the target level set a priori and the total ammount held in the reserve was related to difficulties to fully include a couple of species (Luciobarbus comizo and Salaria fluviatilis) which appeared with high probabilities predominantly at low reaches. Due to the spatial rule we used, this forced the selection of more planning units that necessary for the remaining species. A higher SPF level would have fixed this problem, but given the reduced number of conservation features we gave more weight to holding the whole community.

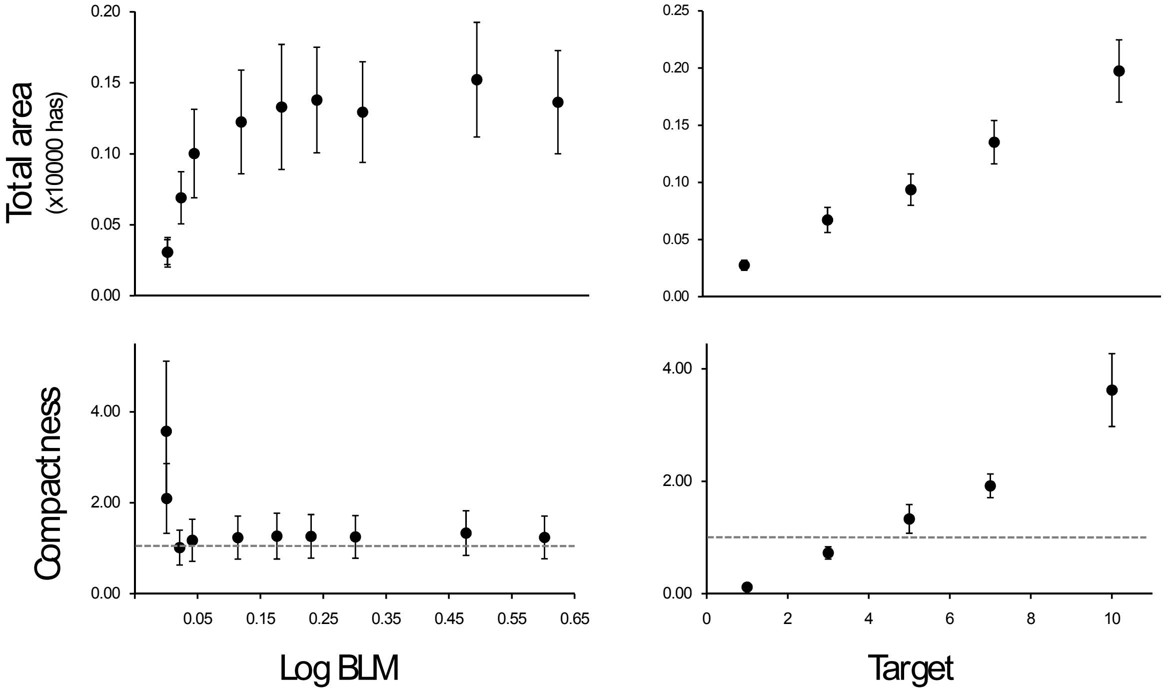

Fig 2. Total area and compactness values (Mean ± SE) for different Boundary Length Modifier (BLM) and target levels (n=49 different combinations of BLM and targets). Dotted line stands out the best compactness value (1), comparable to that of a circle with the same area.

The effect of the BLM was also clear helping the hydrologic connectivity rule to work properly since only headwater or whole upstream sub-catchments were included in the best solution (with the lower value for the objective function) when a BLM was used, whilst isolated planning units even located in the main river channel were selected when the BLM was set at 0 (Fig. 4). The effect of target level on spatial design was mainly related to the increasing need of including planning units to fulfill the representation of all the species (Fig. 4). Irreplaceability values followed a similar pattern with high values for isolated planning units when using no BLM and more clustered solutions with the highest values when setting a BLM (Fig. 4).

The highest irreplaceable planning units were mainly centred in a single medium sized sub-basin (Chanza River). This seems to be a key sub-basin within the study area for the conservation of native fish fauna, since it showed increasing irreplaceability values when the target level was raised (Fig. 4). It is wholly included even as a higher irreplaceable area when using a higher target levels.

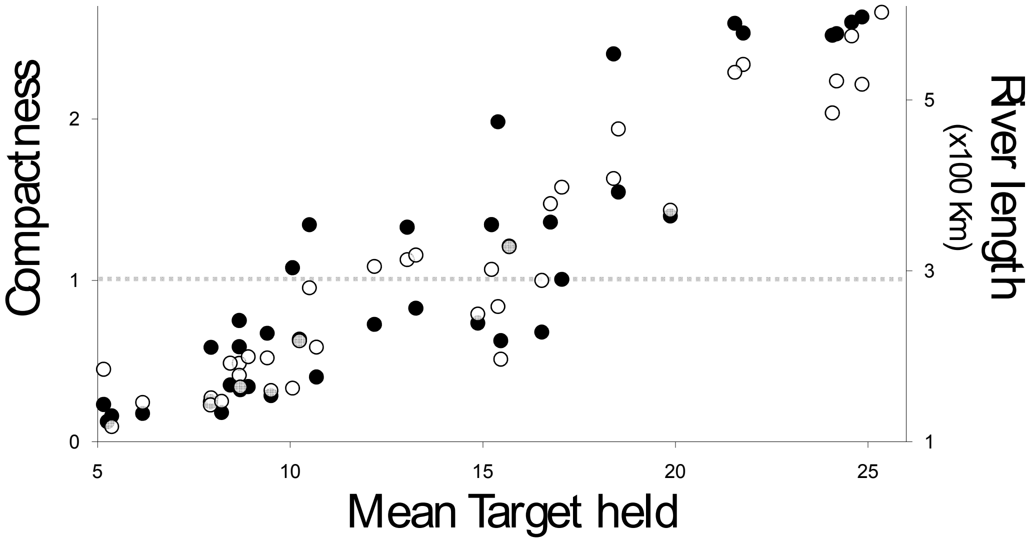

Fig 3. Relationship between mean target held for all the species in the best solution for different combinations of BLM-target levels and reserve compactness and total river length. The best compactness value is marked with a dotted line. It can be seen as the most compact solution includes around 300 river km.

DISCUSSION

Systematic conservation planning aims the selection of a set of areas to ensure the preservation of a targeted biodiversity separating it from processes that threaten their existence (Margules and Pressey, 2000). To fulfill this goal a reserve has to ensure the representation of the whole targeted biodiversity and its long-term persistence (Margules and Pressey, 2000). Attending to this global aim a few other aspects have to be considered to make the process more effective and realistic as flexibility when selecting and managing reserves or accounting for costs. Marxan is provided with tools to face all these questions making systematic conservation planning more efficient (see Ball and Possigham, 2000 for more details on Marxan software) as scientific literature has proved elsewhere (Wilson et al., 2005b; Oetting et al., 2006; Carwardine et al., 2007). However some adaptations were being needed to account for the special connected nature of rivers in freshwater systematic conservation planning.

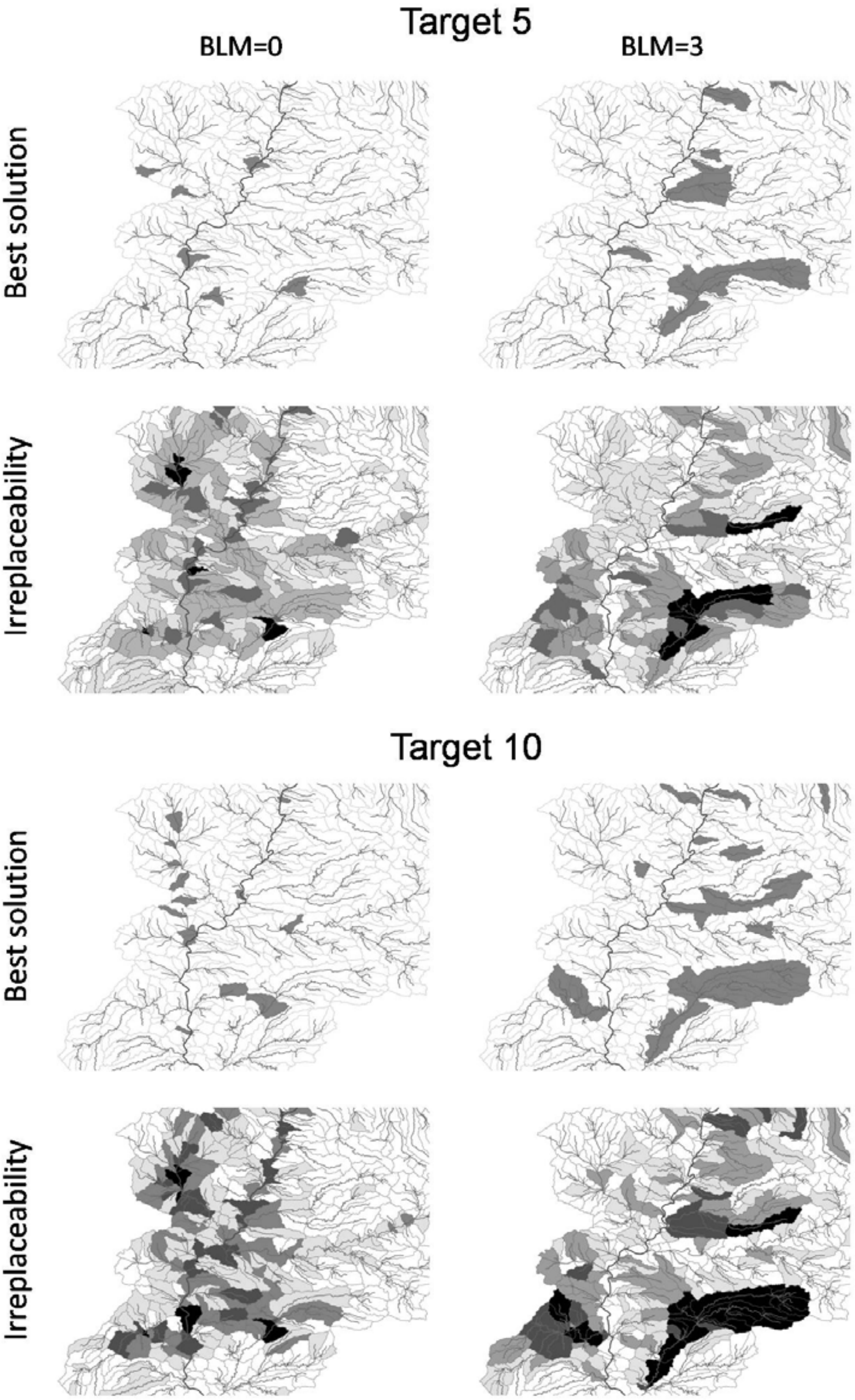

Fig 4. Effect of BLM and target level on best solutions and irreplaceability values. Planning units included in the best solution are pointer out in grey. Higher irreplaceability values are drawn in darker colours. It is only represented a portion of the basin, where the best solutions and irreplaceability values were centred.

Spatial connectivity is generally an issue of major concern in systematic conservation planning (Cabeza, 2003) and especially important in freshwater applications. In riverine environments, connectivity is not a mere conservation topic but an essential factor structuring healthy systems (Poff et al., 1997). Rivers can be seen as interactive pathways along the landscape they drain what makes them especially sensitive to human disturbances through their whole course. Pringle (2001) refers to four main patterns which have important implications for the location and management of freshwater reserves: (i) deterioration of lower watersheds, (ii) deterioration and loss of riverine floodplains, (iii) deterioration of irrigated lands and connecting surface waters and (iv) isolation of upper watersheds. For this reason the consideration of connectivity issues in freshwater systematic conservation planning is a key topic. With the introduction of the spatial rule proposed by Linke et al. (2007) we avoided the selection of unconnected planning units going beyond the optimization of size, shape and other spatial issues of traditional conservation planning practices and providing Marxan software with a solution for connectivity problems. This rule ensures the selection of hydrologically connected reserves which mitigates the drawbacks of present freshwater reservation highlighted by Pringle (2001) and Oetting et al. (2006).

A common problem in conservation planning when using species as conservation features is that the information of the distribution of species of often incomplete. Instead, predicted species distributions can be used (Wilson et al., 2005b; Linke et al., 2007). Some reserve selection methods based on presence-absence data may fail to consider persistence of targets in reserve selection (Araújo and Williams, 2000; Teeffelen et al., 2006), but it can be addressed through different approaches, as (i) at the spatial reserve design, favouring clustered reserves which minimize negative edge effects (Possingham et al., 2000; Cabeza et al., 2004; Carwardine et al., 2007), and (ii) using probabilities of occurrence rather than presence-absence only data (Cabeza, 2003; Cabeza et al., 2004). This probability of occurrence indicates the likelihood of a species to be present in a planning unit considering different species-dependent factors such as habitat requirements or vulnerability to threats (Araújo and Williams, 2000). The first goal was addressed through the use of the BLM and species penalties dealing with the compactness of the reserve system (Possingham et al., 2000). We found that different BLM levels had no significant effects on the spatial design although a BLM had to be used to get a clustered reserve. The BLM has been shown to affect the reserve configuration and extend (usually high irreplaceability values are given to sites with common species) (Carwardine et al., 2007), but there is a general agreement in the benefits for persistence derived from the gain in compactness. In this case, the target level showed to be more influential in the spatial design, probably related to the use of the hydrologic connectivity rule. Both (BLM and hydrologic connectivity) introduced spatial constrains in the selection process, which reduced fragmentation and could make the species´ persistence more stable (Cabeza et al., 2004). The second issue was faced when ensuring the highest probabilities of occurrence for each species in the reserve, setting the target at the highest possible level. We used present probabilities of occurrence instead of potential probabilities which focus the efforts in identifying the more suitable areas for attaining persistence in reserves at the moment. Natural distributions can be highly perturbed due to human impairment (Kouamélan et al., 2003; Light and Marchetti, 2007) and some species may have been relegated to marginal areas within their original distribution or displaced to new areas. In this sense, Araújo et al. (2002) proved how the probability of extinction of a set of species can be reduced if reserve areas are selected to maximize the probability of occurrence at the present time. When using present probabilities we maximize the probability of occurrence if general environmental conditions remain stable. A high probability of occurrence implies that these conditions did not exceed species´ tolerances, so in this way we optimize the use of the reduced resources intended for conservation issues (Knight et al., 2007) focusing our efforts in the areas with the highest probabilities of persistence for the targeted biodiversity.

Alternative approaches to using direct probabilities of occurrence have been tested and used in previous studies, as the transformation of these probabilities into presence-absence data (Polasky et al., 2000; Wilson et al., 2005b). Despite this supposes a low risk approach, given that it normally ensures a higher certainty of occurrence of conservation features within planning units, it also entails a net loss of information on species distribution data. Moreover the threshold used in the transformation influences not only the predicted distribution area for the conservation features, but also the outputs of conservation planning process and has to be carefully set to ensure a suitable use (Wilson et al., 2005b). An advantage of using direct probabilities of occurrence is the ability to better portray the continuous nature of rivers (Vannote et al., 1980) in the conservation process. It also softens the interpretation of the reserve in terms of river length for a given target. Planning units included 10.7 km of river length as average in the most clustered reserve. Since the mean target held for each species in that solution was 16.5, we can assert that in this reserve we are ensuring a total probability of occurrence (100%) in 177 river km or, alternatively in a longer amount of habitat km at a lower probability. This could be related to the spatial needs of each species to develop healthy populations and their probability of persistence hence. A feedback process, where this kind of basic ecological information led the reserve selection, would have beneficial results for the conservation planning task. In this sense a reserve could be spatially designed to portray a determinate km of river habitat, which needed to hold a persistent population. However, as this information is still lacking we have to trust the river habitat length included in the present best reserve to be enough for preserving all the targeted species.

A whole basin approach has been followed in this study. Previous works in the same area were only focused on the Portuguese (Filipe et al., 2004) or Spanish (Hermoso et al, submitted) portions of the basin while global conservation management seems a more effective practice. The most irreplaceable area and the best solution were especially focused on a single sub-basin (Chanza River). This solution highlights the importance of tributaries for conserving freshwater biodiversity as Nel et al. (2007) pointed out. The same area had previously been included in a set of priority areas for conservation through an alternative method based in the reference condition approach (Hermoso et al., submitted). However this is the first work where complementarity has been applied to this basin in particular. Algorithms which incorporate complementarity procedures ensure representativeness which is the other major goal followed in systematic conservation planning, in addition to persistence (Margules and Pressey, 2000; Margules et al., 2002). This guaranties the adequate representation of each species within the reserve overcoming uncovered deficiencies in other reserve selection methods based in scoring and ranking approaches (Williams et al., 1996; Margules et al., 2002).

Flexibility, which is a key issue in conservation planning providing the solutions with a practical sense, is also considered in this study in two different ways, (i) in the reserve selection through the use of irreplaceability measures which reports the relative importance of each planning unit for the conservation goal and (ii) and in the reserve management considering present species´ distributions. This second approach to flexibility mainly refers to the management of the set of selected priority areas dealing with mixed protection-land uses schemes and complementing the traditional approach. High irreplaceability values were given to planning units where the species were predicted with a high probability according to present land uses-management. The highly irreplaceable area stood out in this study contains both official protected areas (a portion of a Natural Park and a Natural Reserve) in conjunction with other human transformed landscapes (forestry and agriculture lands). This multi-use landscape scheme could be maintained for future biodiversity conservation plans, attending to the biodiversity it still holds. However, this should not mean the lack of active management regimes neither within the already reserved areas nor officially unprotected priority areas (Lindermayer et al., 2000; Cowling et al., 2003). The Knowledge of ultimate and proximate threats is crucial in identifying the activities needed to complement and support reserves (Willson et al., 2005a). For this reason the deliberate study of vulnerability should not be avoided for future works as a complement to the conservation planning practice we propose here.

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CONCLUSIONES

CONCLUSIONES

1. Las comunidades de peces de agua dulce del área de estudio, constituida por la cuenca del río Guadiana, estuvo compuesta por un total de 28 especies, de las cuales 16 (57%) fueron nativas y 12 (43%) exóticas. Se ha constatado un incremento significativo en el área de distribución de las especies exóticas, así como en el número de éstas con dos nuevas citas para la cuenca respecto a trabajos previos. Por el contrario se registró un retroceso generalizado en la distribución de las especies nativas.
2. Las clasificaciones derivadas de la aplicación de la Directiva Marco del Agua en el área de estudio, basadas en criterios meramente ambientales, no garantizan la correcta definición de condiciones de referencia, base del proceso de evaluación del estado ecológico. Éste hecho puede tener graves consecuencias sobre las evaluaciones y, por tanto, se recomienda comprobar la consistencia biológica de dichas clasificaciones en futuros trabajos o evitar su uso.
3. Las metodologías predictivas que evitaron las clasificaciones de cualquier tipo (ambientales o biológicas), incorporaron más fielmente la naturaleza continua de los medios fluviales, fueron más eficaces y, consecuentemente, se recomienda su uso. Por ello a la hora de abordar el desarrollo del índice de calidad se decidió emplear una metodología basada en predicciones específicas por sitio, en lugar de las tradicionales metodologías específicas por tipo utilizadas en la Península Ibérica.
4. La presencia-ausencia de las especies nativas más comunes mostró respuestas claras a diferentes tipos de perturbación, tanto de origen humano, como de origen biótico. Ello garantiza su valor potencial como indicadoras y su uso dentro de índices de calidad.
5. Es necesario controlar los efectos de la variación natural, y las interacciones entre diferentes agentes de perturbación, sobre los valores de sensibilidad-tolerancia de las especies. En caso contrario, dichas evaluaciones pueden ser erróneas o insuficientes.
6. La simplificación de la información requerida para la elaboración de los índices de calidad, a través del uso de las presencias-ausencias y evitando las abundancias, no sólo avala la solidez del estos índices (salvando las incorrecciones derivadas de la estima y variación temporal de la abundancia), sino que además reduce los posibles costes de su aplicación.
7. El índice de calidad diseñado, recogiendo las recomendaciones comentadas, responde significativamente ante cambios en la calidad general del hábitat y las perturbaciones biológicas. Ello garantiza la evaluación del estado ecológico en todos sus términos y el diagnóstico de las principales causas del estado encontrado, facilitando la propuesta de medidas correctoras eficientes orientadas a la consecución de los objetivos medioambientales marcados en la Directiva Marco del Agua.

8. Un reducido número de ríos localizados en la porción central-final de la cuenca del Guadiana destacaron por la salud de las poblaciones de peces que contuvieron, medida a través de la pérdida de biodiversidad potencial que han sufrido y la presencia de rarezas.
9. La consideración de la continuidad en la planificación sistemática para la conservación abordó problemas tradicionalmente relacionados con la conservación en ambientes fluviales. Adicionalmente, el uso de distribuciones reales, frente a las potenciales usadas tradicionalmente, aportan solidez al proceso de selección de áreas prioritarias para la conservación.
10. El mantenimiento de las comunidades de peces nativos de la cuenca del río Chanza, junto a un reducido número de pequeños arroyos cercanos, aseguran la persistencia de gran parte de la biodiversidad de la cuenca. Tan sólo aquellas especies consideradas como muy raras debido al escaso número de presencias (que en algunas ocasiones tan sólo fue una) quedaron fuera de este proceso. Éstas, dada su crítica situación, deberían ser objeto de planes más específicos de conservación en la cuenca.

ANEXO I

SPATIAL DISTRIBUTION OF NATIVE FISH SPECIES IN THE GUADIANA RIVER BASIN (SW SPAIN)

Distribución espacial de las especies de peces nativos de la cuenca del río Guadiana (SW España)

(En revisión en Limnetica)

Spatial distribution of native fish species in the Guadiana River basin (SW Spain)

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ABSTRACT

Freshwater biodiversity is under severe threats which in many cases cast doubt on its future persistence. The Guadiana River basin stands out at the circunmediterranean context for its high freshwater fish biodiversity and its threat status. Many efforts are required to offset this situation, and as an initial step the spatial distribution of freshwater native fish species in the Guadiana River is updated through recent surveys (data from 2005-06). Altogether 261 UTM 10x10 Km squares were prospected over the whole basin through the sampling of 241 river stretches and 64 sampling points located in 37 reservoirs and lakes. A total of 16 native fish species were found, which supposes all the previously cited species in the basin except *Alosa fallax* and *Petromyzon marinus*. A pressing fall in the total number of 10x10 UTMs occupied has been reported for all the species except for *Luciobarbus sclateri* and *Salaria fluviatilis*. Moreover all the species appeared predominantly in lotic systems disappearing or infra-using lentic ones. The pronounced decrease in the distribution of most of species linked to their endemic character (Iberian or even exclusive to the Guadiana basin) makes essential the implementation of conservation strategies.

Keywords: Freshwater fish, Iberian endemics, Mediterrenean, threats, biodiversity

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Native freshwater fish biodiversity to the Guadiana River basin stands out, at the circunmediterranean context, for its high species richness level according to Smith & Darwall (2006), only comparable to Po River basin in northern Italy and the lower Orontes River in south west Turkey. It is also remarkable the high proportion of threatened species, since almost the 90% of its native fishes are included in some of the IUCN threats categories (Doadrio, 2002). This is a consequence of many synergic interacting factors as species introductions and translocations, impoundment of rivers and water abstraction, water quality deterioration (pollution or eutrophication), habitat degradation and fragmentation, and overexploitation (Prenda et al., 2006; Collares-Pereira & Cowx, 2004). However little efforts are being carried out to face this complex problem (Abell, 2002).

A periodic monitoring of the status of native freshwater fish populations seems to be essential to assess their evolution under all these pressures. With this aim, the spatial distribution of native fishes in the Guadiana River basin is updated in this work.

During 2005-06´s springs, an extensive survey in the Spanish sector of the Guadiana River basin, excluding transitional waters, was carried out. A total of 241 river stretches and 64 localities in 37 different reservoirs and lakes were sampled, covering 261 10x10 km UTM squares. This comprises most of the potentially sampled UTMs in the basin (those with at least a water mass within it) (Fig. 1). The fieldwork was carried out through electrofishing in rivers stretches (lotic systems hereafter) and a combination of different passive capture methods (trammel-nets, fyke–nets and minnow-traps) in reservoirs and lakes (lentic systems hereafter).

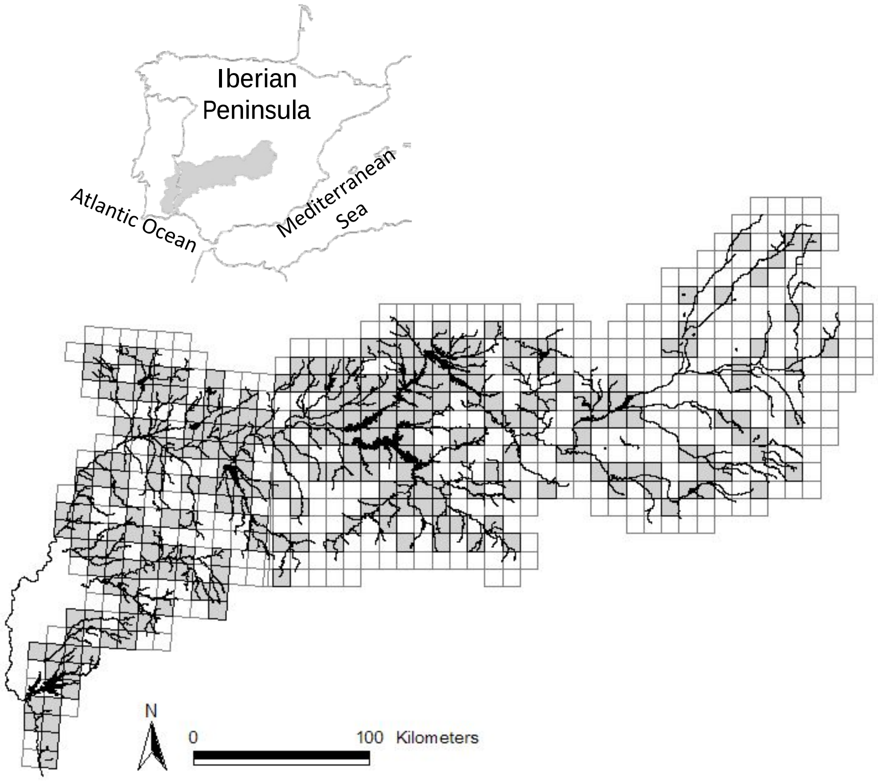


Fig. 1. Study area within the Guadiana River basin. All the UTM 10x10 Km squares included in the Spanish portion of the basin are represented and the prospected ones are filled in grey (n=261).

A total of 16 native fish species were found during the present survey (Table 1). This supposes all the previously native cited species in the basin (Doadrio, 2002), except *Petromyzon marinus* and *Alosa fallax*. These absences may have different causes, since for *A. fallax* it may be due to an inefficient survey of its potential distribution area (lower reaches of the Guadiana main river channel), while the absence of *P. marinus* seems to be an evidence of its apparent local extinction since many of the previously cites 10X10 UTMs with presences in Doadrio (2002) were sampled in this occasion.

Table 1. Native freshwater fish species in the Guadiana River basin (found in this study or previously cited but not found). The threat category for each species in Spain according to Doadrio (2002) is showed. A comparison between the number of 10x10 UTMs reported in Doadrio (202) and this study, with the change in % between brackets, is also included. Finally the number of positive localities in lotic and lentic systems is referred.

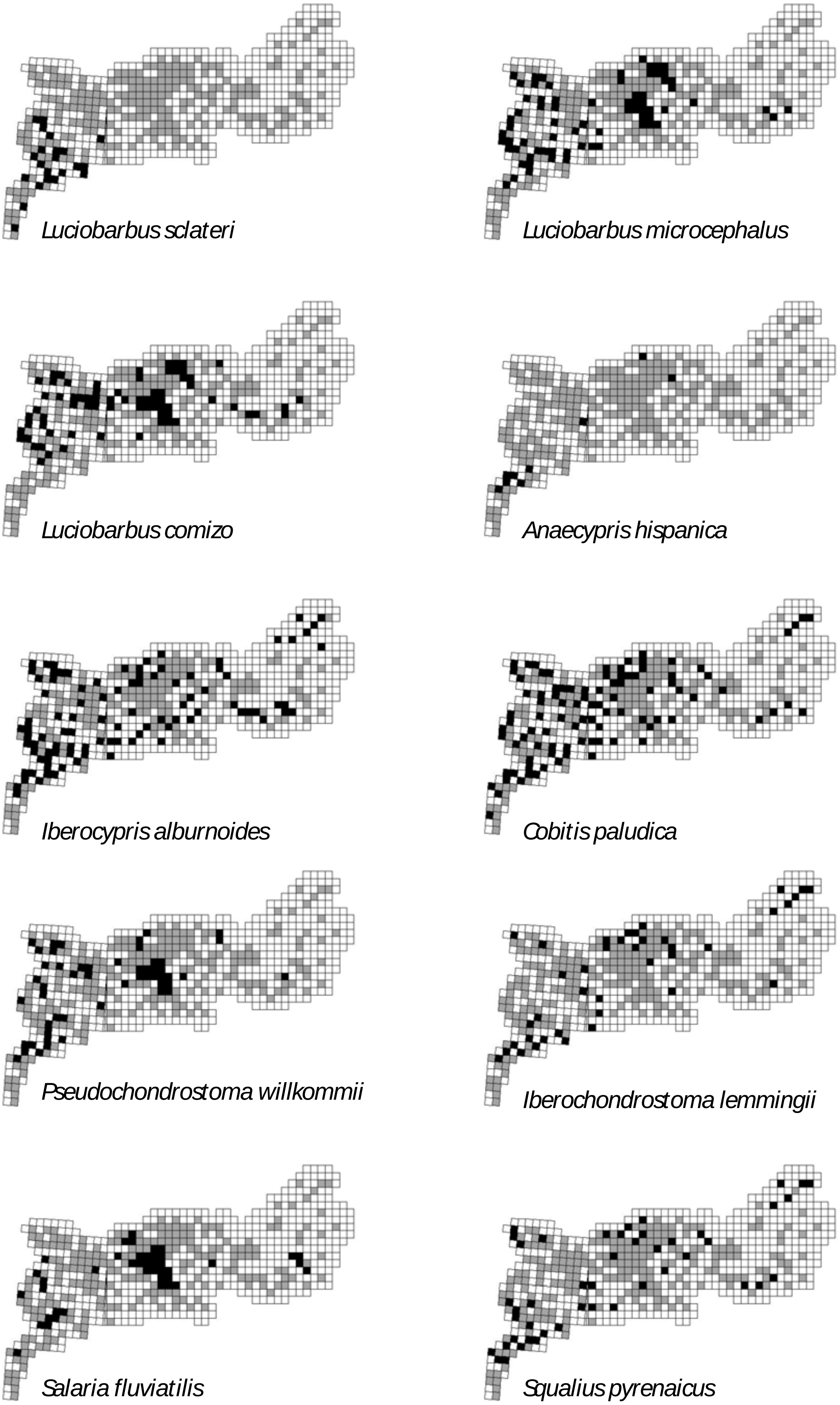
Species	IUCN threat category	Presences in 10x10 UTM according to Doadrio (2002)/Total in this study	Number of presences in rivers (n=241 sites)	Number of presences in reservoirs and lakes (n=64 sites)
Iberocypris alburnoides	VU	280/96 (-0.7)	108	7
Cobitis paludica	VU	271/96 (-0.6)	106	5
Luciobarbus microcephalus	VU	142/72 (-0.5)	51	10
Luciobarbus comizo	VU	131/72 (-0.5)	38	25
Pseudochondrostoma willkommii	VU	145/52 (-0.6)	29	20
Squalius pyrenaicus	VU	149/46 (-0.7)	52	1
Salaria fluviatilis	EN	23/42 (0.8)	21	9
Iberochondrostoma lemmingii	VU	211/40 (-0.8)	44	0
Luciobarbus sclateri	LC	24/37 (0.5)	18	8
Tinca tinca	NT	20/12 (-0.4)	0	4
Anaecypris hispanica	EN	54/9 (-0.8)	10	0
Gobio lozanoi	VU	16/5 (-0.7)	5	0
Luciobarbus guiraonis	VU	10/3 (-0.7)	3	0
Anguilla anguilla	VU	6/2 (-0.7)	2	0
Alosa alosa	VU	25/1 (-1.0)	1	0
Atherina boyeri	VU	0/1 (1.0)	1	0
Alosa fallax	VU	10/0 (-1.0)	-	-
Petromizon marinus	VU	22/0 (-1.0)	-	-
Mean		85.5/32.5 (-0.6)	30.6	5.5

Although the sampling effort is not comparable between previous references in Doadrio (2002) and the present work, a falling tendency has been detected in almost all the species (Table 1). This fall was especially significant for *Alosa alosa*, which appear to be near the extinction with only a presence within the basin (Table 1, Fig. 2), and *Anaecypris hispanica* and *Iberochondrostoma lemmingii* with a reduction of more than 80% of their previous distribution area Other species as *Gobio lozanoi*, *Luciobarbus guiraonis* and *Anguilla Anguilla* were rare species, since they were present in less than 5 UTMs as a consequence of a drastic reduction too (Table 1, Fig. 2). This pattern was also followed by other common species as *Luciobarbus microcephalus*, *Luciobarbus comizo*, *Squalius pyrenaicus* or *Iberocypris alburnoides,* with reductions over 50-70% (Table 1, Fig. 2). Only *Luciobarbus sclateri* and *Salaria fluviatilis* showed a raise, higher in the former species (Table 1, Fig. 2). This situation is specially worrying given that 10 of the species are Iberian endemims (*A. hispanica*, *C. paludica*, *I. alburnoides*, *I. lemmingii*, *L. comizo*, *L. guiraonis*, *L. microcephalus*, *L. sclateri*, *P. willkommii* and *S. pyrenaicus*) and two of them endemics to the Guadiana River basin (*A. hispanica* and *L. microcephalus*).

Most of the species appeared predominantly in lotic systems, being almost absent from lentic ones. This is just the opposite pattern showed by exotic fish species in the same area (Hermoso et al., 2008), which occupied preferentially lentic systems (mainly reservoirs). So, reservoirs are not only an important focus of introduction and dispersion of exotic fish species (Clavero et al., 2004), which are known to be one of the major threats to the conservation of native fish communities (Godinho and Ferreira, 2000; Clavero et al., 2005), but also a harsh environment for native species from which they tend to disappear. Moreover, migratory species (*A. alosa*, *A. fallax* or *A. anguilla*) are known to be highly affected by river regulation (Prenda et al, 2006).Their distribution areas have been dramatically reduced or even disappeared from the Guadiana basin, and this may be related to the difficulties they find to complete their life cycles in regulated rivers. This is a major issue in the Guadiana basin, with 87 large reservoirs (>1 Hm^3) and more than 200 small ones (<1 Hm^3).

The pernicious effect of the remaining previously cited threat factors on native fish communities should not be ruled out given the rising demand of water supply for agriculture and urban uses and the accelerated rate of intervention and modification of habitat quality.

In the view of such a complex situation, specific efforts should be focused on the assessment of native fish community health and the identification and preservation of the most significant areas to warranty the persistence of the important freshwater fish biodiversity that the Guadiana River holds.



Luciobarbus sclateri

Luciobarbus microcephalus

Luciobarbus comizo

Anaecypris hispanica

Iberocypris alburnoides

Cobitis paludica

Pseudochondrostoma willkommii

Iberochondrostoma lemmingii

Salaria fluviatilis

Squalius pyrenaicus

Fig. 2. Distribution maps of the native fish species found in this study in the Guadiana River basin. Positive UTMs are pointed out in black.

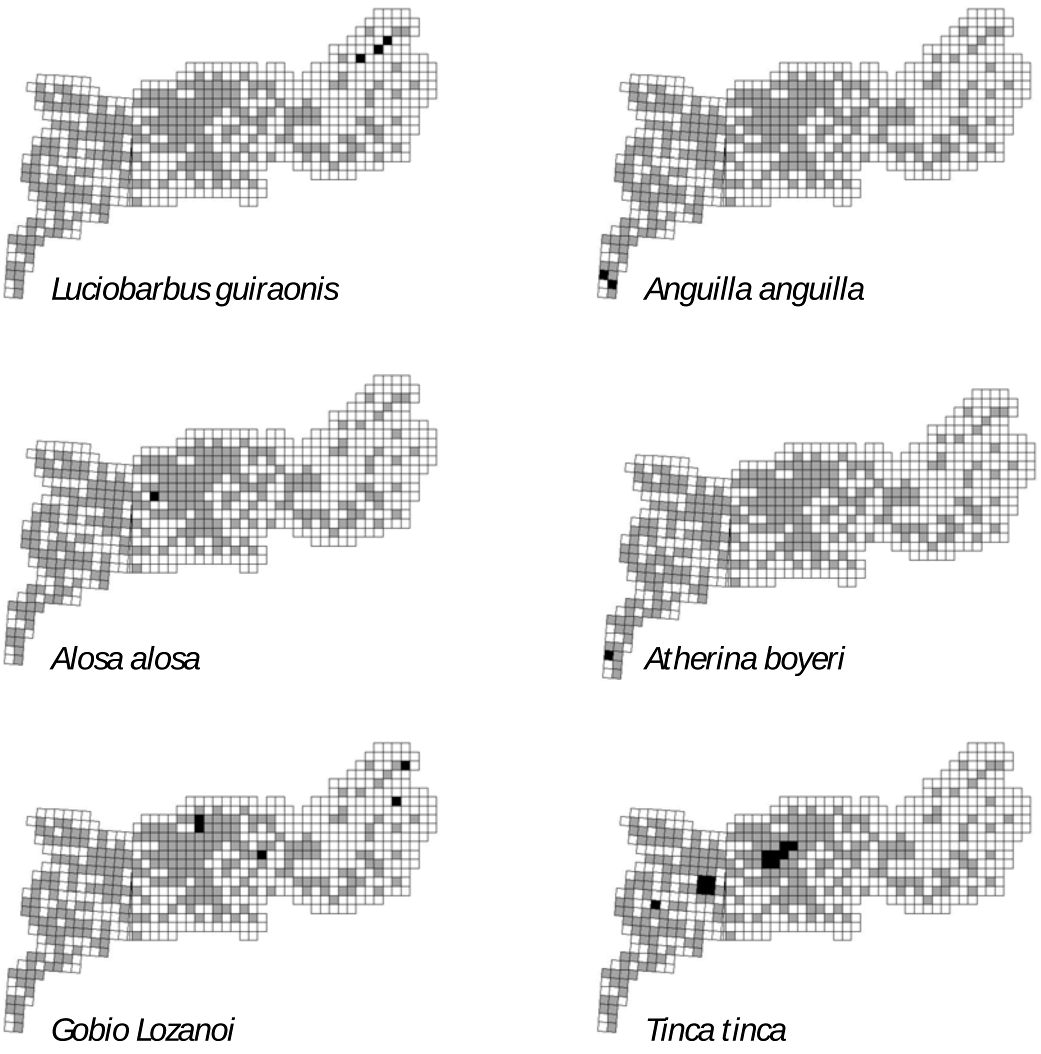


Fig. 2. *(Cont.)*

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ANEXO II

SPATIAL DISTRIBUTION OF EXOTIC FISH SPECIES IN THE GUADIANA RIVER BASIN, WITH TWO NEW RECORDS

Distribución espacial de las especies de peces exóticos en la cuenca del río Guadiana, con dos nuevas citas

(Aceptado para su publicación en Limnetica, 27: 189-194)

Spatial distribution of exotic fish species in the Guadiana River basin, with two new records

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This work updates the spatial distribution of the exotic fish species in the Guadiana river basin. Altogether 261 UTM 10x10 Km squares were prospected through the sampling of 241 river stretches and 37 reservoirs and lakes. A total of 12 exotic species were found, including two new species that had not been previously cited in the area, the channel cat-fish (*Ictalurus punctatus*) and the roach (*Rutilus rutilus*). These two species were usually related to lentic systems and presented a restricted distribution within the basin, probably as a consequence of its recent introduction. The expansion of the remaining exotic species was also confirmed. Some species as the pumpkinseed sunfish (*Lepomis gibbosus*), the mosquitofish (*Gambusia holbrooki)* or the largemouth bass (*Micropterus salmoides)* have become common components of the fish community, not only in reservoirs but also in lotic systems.

KEYWORDS: Exotic species, freshwater fish, Guadiana River, Ictalurus punctatus, Rutilus rutilus, Spain.

Exotic fish species are involved in one of the most irreversible human-induced global changes now under way: the homogenization of Earth's biota (Mooney and Hobbs, 2000; Rahel, 2000). At least 35 alien fish species have been introduced in the Iberian Península in the last century and although not all of them prospered (Elvira and Almodovar, 2001; Ribeiro et al, 2007) most are now widespread in this area, specially linked to degraded environments such as reservoirs (Clavero et al; 2004). Paradoxically these environments may also suppose a barrier to their dispersion (Rahel, 2007). The establishment and expansion of such species has been reported as an important source of local extinction and strong declines in native fish species populations (García-Berthou and Moreno-Amich, 2000; Clavero, et al; 2004; Clavero and García-Berthou, 2005; Prenda et al, 2006; Blanco-Garrido, 2006). According to previous cites (Doadrio, 2002; Gante and Santos, 2002; Pérez-Bote et al, 2004; Ribeiro et al, 2006), a total of

12 exotic fish species inhabit the Spanish part of the Guadiana river basin, which represent the 48.0% of the total freshwater fish species richness of this basin (25 species).

During 2005-06´s spring an extensive survey in the Spanish sector of the Guadiana River basin excluding transitional waters was carried out. A total of 241 river stretches and 37 reservoirs and lakes were sampled, which covered 261 UTM 10x10 Km squares. This comprises most of the potentially sampled UTMs in the basin (those with at least a water course or a water mass in it). The fieldwork was carried out by electrofishing in rivers stretches (lotic systems hereafter) and passive capture methods (trammel-nets, fyke–nets and minnow-traps) in reservoirs (lentic systems hereafter) (Fig. 1A).

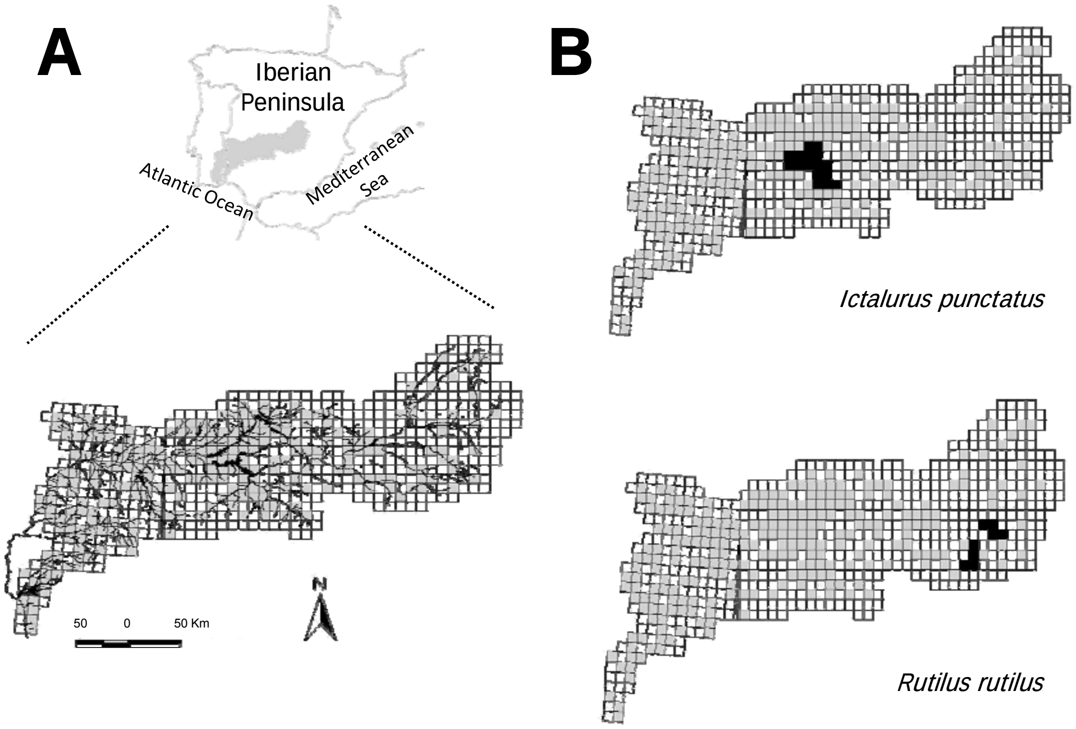

Fig. 1. A) Location of the Guadiana River basin within the Iberian . All the UTM 10x10 Km squares included in the Spanish portion of the basin are represented and the prospected ones are filled in grey (n=261). B) Distribution of the two new species in the basin: the roach (*R. rutilus*) and the channel catfish (*I. punctatus*). Positive squares are pointed out in black.

A total of 11 exotic fish species were captured in this work (Table 1), including two species never reported for the Guadiana River: the channel catfish (Ictalurus punctatus Rafinesque, 1818) and the roach (Rutilus rutilus Linnaeus, 1758). In addition three individuals of common trout (Salmo trutta Linnaeus, 1758) were captured in the Gevora River within an angling reserve. Although this species is considered native to the Iberian Peninsula and it presence in the Guadiana River has been recently confirmed (A. Menor, unpublished data) we consider this population as reintroduced just for angling purposes.

On the other hand, neither the rudd (Scardinius erythrophthalmus Linnaeus, 1758), nor the mummichog (Fundulus heteroclitus Linnaeus, 1766), both previously cited in Doadrio (2002), were found here (Table 1). It could be possible that the previous cites of the rudd would corresponded to roach individuals, given their morphological similarity. The mummichog was not captured as it only inhabits estuarine environments, not prospected in this work.

The distribution extent of the different species varied between the pumpkinseed (Lepomis gibosus, Linnaeus, 1758), the mosquitofish (Gambusia holbrookii Girard, 1859) or the largemouth bass (Micropterus salmoides Lacepède, 1802) each covering more than 35% of the total lotic screened squares and more than a half of the lentic ones, and the common trout (S. trutta), found only in one lotic locality (Table 1). On average the exotic species tended to occupy more frequently lentic systems than lotic ones (Table 1). Thus reservoirs seem to be an optimal environment for the development of exotic communities.

Table 1. Exotic fish species present in the Spanish portion of the Guadiana River basin. The occupation of these species is referred as the total number of UTM 10x10 Km squares observed in this study (separately for lotic and lentic systems). The % of occupation in each environment is also showed in brackets. and in Doadrio (2002). The total number of squares in Doadrio, 2002 and in the present study is compared.

Species	Presences in UTM 10x10 Km squares		Presences in UTM 10x10 Km squares according to Doadrio (2002)/Total in this study
	Lotic (n=160)	Lentic (n=81)	
Lepomis gibbosus	65 (40.6)	73 (90.1)	56/138
Gambusia holbrooki	50 (31.3)	49 (60.5)	59/99
Micropterus salmoides	27 (16.9)	71 (87.7)	60/98
Cyprinus carpio	11 (6.9)	47 (58.0)	50/58
Esox lucius	5 (3.1)	30 (37.0)	31/35
Carassius auratus	10 (6.3)	25 (30.9)	15/35
Alburnus alburnus$	4 (2.5)	19 (23.5)	0/23
Ictalurus punctatus	0 (0.0)	16 (19.8)	0/16
Ameiurus melas¶	8 (5.0)	5 (6.2)	0/13
Rutilus rutilus	1 (0.6)	7 (8.6)	0/8
Australoheros facetus	3 (1.9)	1 (1.2)	3/4
*Salmo trutta**	1 (0.6)	0 (0.0)	1/1
Scardinius erythropthalmus†	-	-	1/0
Fundulus heteroclitus†	-	-	1/0
MEAN	**15.4 (9.6)**	**28.6 (35.3)**	**27.7/44.0**

† Species previously cited in the basin (Doadrio, 2002) but not captured in the present study
**This species comes from fish stocking within angling reserves and can be considered as "reintroduced" in the basin*
¶ Species presence cited in Gante & Santos, 2002
$ Species presence cited in Pérez-Bote et al, 2004

Comparing our data with those published in Doadrio (2002) it seems that almost all exotic species previously cited have increased their distribution range within the basin (Table 1). The pumpkinseed sunfish (L. gibbosus) showed the greatest raising, with 82 UTMs more than the previous survey (Doadrio, 2002) (Table 1). It was followed by the mosquitofish (G. hoolbrooki) and the largemouth bass (M. salmoides) with 40 and 38 UTMs more respectively (Table 1). In contrast, the chameleon cichlid (Australoheros facetus Jenyns, 1842) with a restricted distribution range within the basin (Chanza River and Lagunas de Ruidera) did not show any increase in its distribution extent. This species seems to be at a standstill at the moment, from a spatial point of view. Moreover, there is a significant increase in the number of squares for the species not cited in Doadrio, 2002, but in other studies as the bleak (Alburnus alburnus Linnaeus, 1758) (Pérez-Bote et al, 2004) or the black bullhead (Ameiurus melas Rafinesque, 1820) (Gante and Santos, 2002) (Table 1). Of course these results must be carefully considered as they may be related to the difference in sampling effort between the present study and Doadrio, 2002.

The channel catfish is an ictalurid native to North America (Central drainages of the United States to southern Canada and northern Mexico). It was previously cited only in Delta del Ebro within the Iberian Peninsula (Doadrio, 2002), although its occurrence is confirmed in almost all Europe (Froese and Pauly, 2006). The presence of the channel catfish in the Guadiana basin was restricted to La Serena reservoir (fishes captured in four localities sampled there in August-06). We captured a total of 151 fishes by trammel-nets and fyke-nets, always placed closed to shallow shores at no more than 2 m depth. Fish length, which ranged between 64-617 mm of total length (250.8 ± 82.8 mm, Mean ± SD), showed almost no juveniles, in spite of they could be potentially captured since small individuals of other species appeared in minnow-traps. This species was supposed to be present in the basin (Zujar and Orellana reservoirs, Pérez-Bote, 2006) since early 90s, although these data had not been formally confirmed until this study. We contributed here with 16 new UTM 10x10 Km squares where the species is present in the basin (Fig. 1B).

The roach is a cyprinid native to central and Eastern Europe that could be introduced for angling purposes. The presence of this species within the Iberian Peninsula was restricted to the Llobregat River (Doadrio, 2002). This species was more widespread than the channel catfish: although with local abundances asymmetrically distributed, always in the calcareous section of the basin. It was present both in lentic and lotic systems, being collected in three reservoirs (Peñarroya, Cabezuelas and Vallehermoso, September-06) and two lakes (located within the Ruidera Natural Park: Cueva Morenilla and Colgada lakes, September-06), and in running waters in the Guadiana river within the Ruidera Natural Park (38º 97´ 87´´ N, 2º 89´19´´ W, June-06). All these localities supposed the presence of the species in a total of 8 new UTM

10x10 Km squares (Fig. 1B). In contrast to the channel catfish, young of year were captured in the lotic systems. These individuals ranged 12-265 mm of total length (119.6 ± 38.6 mm, Mean ± SD). All the fishes captured in reservoirs were longer than those, ranging 145-221 mm (188.2 ± 31.0 mm, Mean ± SD). These two new records represent the southern limit of both species distribution within Europe.

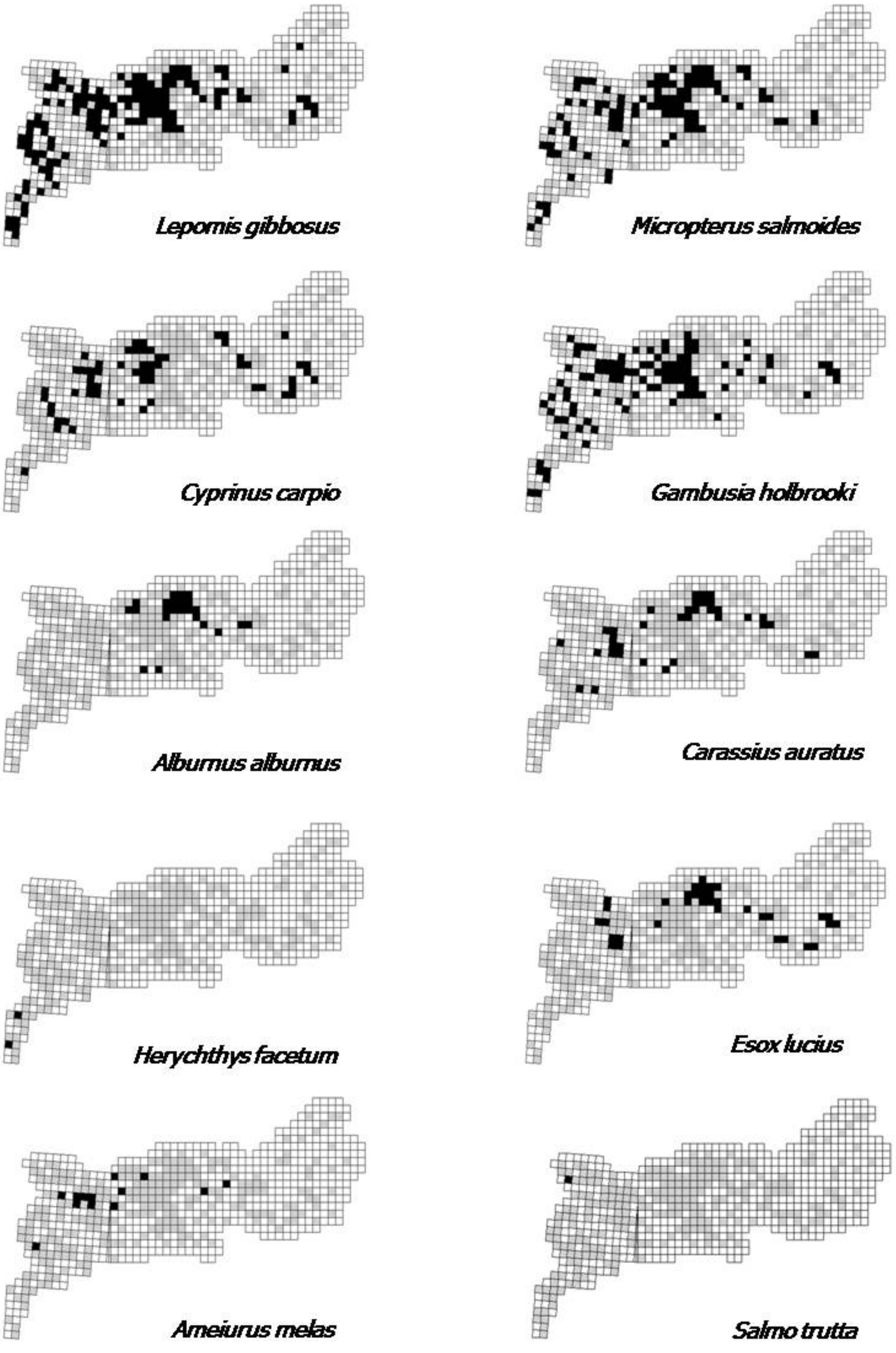

Figure 2. Distribution maps of other exotic species that inhabits the Guadiana River basin. *S. trutta* has been included, although it could be considered a reintroduced species

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