PROYECTO

Riesgos y gestión de las invasiones biológicas vinculadas al comercio de animales de compañía

Diciembre 2017

INFORME FINAL

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1. Breve reseña del marco del proyecto y sus objetivos

Uno de los efectos más visibles de la globalización es la introducción de especies exóticas. Especies transportadas voluntaria e involuntariamente por el hombre desde sus hábitats de origen a áreas geográficas donde históricamente nunca estuvieron presentes. Este movimiento de especies puede no causar problema alguno en los ecosistemas de acogida, pero hay muchos ejemplos de consecuencias catastróficas tanto para la conservación de la biodiversidad nativa (ej. conejos introducidos en Australia) como para la economía de un país (ej. mejillón cebra) o el propio bienestar humano (ej. mosquito tigre) y es en todos estos casos cuando hablamos de especies exóticas invasoras. En las últimas décadas, la introducción accidental o deliberada de especies exóticas vinculadas al comercio de animales de compañía ha destacado por ser una vía principal de entrada de especies invasoras. Sin embargo, pocos estudios han abordado esta cuestión haciendo un análisis detallado de los riesgos sanitarios de estas especies y llevando a cabo medidas eficientes de gestión. Este estudio analizó en detalle la importancia del comercio de peces, anfibios y reptiles exóticos como vía de introducción de especies invasoras utilizando uno de los registros más completos hasta la fecha que incluye mayoristas, comercios de venta al detalle y on-line y de las especies ya establecidas. Asimismo, examina el estado sanitario de las especies ofrecidas a la venta al público y de las mascotas encontradas en los estanques de las zonas urbanas y periurbanas de Barcelona a fin de evaluar el peligro potencial que suponen para la fauna silvestre y para la salud pública. Estos resultados también permitirán proponer, en base a las características de las especies encontradas, una nueva legislación que no sólo reduzca el riesgo de suelta de mascotas no deseadas si no que promueva el bienestar animal en el comercio de animales exóticos. Finalmente, la extracción de la fauna exótica de zonas naturales o semi-naturales para realizar las evaluaciones de estado sanitario también nos ofrece una oportunidad excelente para estudiar los beneficios que supone esta práctica para la conservación de las especies nativas.

2. Situación actual, trabajos resultantes y divulgación

El proyecto de la Beca PRIC avanza a buen ritmo y ya tenemos un artículo publicado, uno en fase de revisión, varias presentaciones en congresos y charlas divulgativas, así como otros artículos en preparación.

2.1. Publicaciones científicas

La carpa (*Cyprinus carpio*) es una de las peores especies invasoras del mundo de acuerdo con el grupo de especialistas en invasiones biológicas de la Unión Internacional para la Conservación de la Naturaleza. En la actualidad esta especie tiene un área de distribución amplia fruto de las translocaciones realizadas desde la época de los romanos tanto con fines de acuicultura tanto ornamental como para consumo humano. De hecho, la carpa koi es una variedad ornamental de *C. carpio* muy apreciada en el comercio de acuarios cuyos ejemplares pueden llegar a tener precios muy elevados, especialmente en el mercado japonés. Si bien estas variedades de colores llamativos tienen un bajo riesgo de invasión por su vulnerabilidad a los depredadores no es el caso de las variedades de coloración silvestre. En el **PRIMER TRABAJO** de este proyecto analizamos los efectos en cascada de la invasión por carpa en dos lagunas de tipo mediterráneo y documentamos su impacto sobre los tapices de plantas sumergidas y la comunidad de aves acuáticas, incluyendo a dos especies de patos mundialmente amenazadas. Asimismo el presente trabajo describe los beneficios de los programas de erradicación de carpas y muestra como éstos pueden verse a los pocos meses de haberse producido la eliminación de la especie invasora. Junto con contribuir a la concienciación sobre el problema de las especies invasoras, incluyendo la carpa que no por llevar mucho tiempo entre nosotros debe considerarse carente de impacto ecológico, el presente trabajo pretende promover medidas legislativas que controlen la expansión de las especies invasoras. A continuación se copia el resumen en inglés del artículo científico publicado en la revista *Biological Conservation* y se cita la referencia completa al mismo.

Maceda-Veiga, A., López, R., & Green, A. J. (2017). Dramatic impact of alien carp *Cyprinus carpio* on globally threatened diving ducks and other waterbirds in Mediterranean shallow lakes. *Biological Conservation*, 212, 74-85.

“Mediterranean shallow lakes support high biodiversity but suffer many anthropogenic threats, including introductions of alien fish. We studied the impact of introduction of common carp (*Cyprinus carpio*) to Medina and Zoñar lakes in SW Spain. Both lakes were protected as Ramsar sites because of their importance for waterbirds, particularly the globally threatened white-headed duck (*Oxyura leucocephala* IUCN Endangered) and common pochard (*Aythya ferina* IUCN Vulnerable). Two carp introduction events in Medina lake, with total eradication of carp in between, provided a unique opportunity to study the impacts of carp on the waterbird community (counted monthly from 2001 to 2013, with up to 69 species) and submerged macrophyte cover (quantified with satellite images). A comparison of waterbird abundance before and after carp eradication in the smaller Zoñar lake supported the results from Medina lake. Carp consistently led to the destruction of macrophyte beds and a radical change in the waterbird community. After controlling for the influence of depth fluctuations, the numbers and species richness of diving ducks were significantly reduced by carp, whilst the opposite effect was observed for piscivores such as herons. Negative impacts on *O. leucocephala*, *A. ferina*, red-crested pochard (*Netta rufina*) and herbivorous coots (*Fulica* spp.) were particularly pronounced. A significant negative impact of carp was also recorded on greater flamingos (*Phoenicopterus ruber*), black-necked grebes (*Podiceps nigricollis*), little grebes (*Tachybaptus ruficollis*) and gadwall (*Anas strepera*). In contrast, carp presence had a positive impact on grey herons (*Ardea cinerea*). The ongoing expansion of alien cyprinids in the Mediterranean region constitutes a major threat for waterbirds and particularly for sedentary, threatened taxa such as the white-headed duck and red-knobbed coot (*F. cristata*). Of 22 key sites for the isolated Iberian population of white-headed duck identified in a European action plan in 1996, at least 14 have since suffered carp invasions. Further development of successful control methods for carp populations is urgently required to support the conservation of waterbirds in the Mediterranean region”.

Extraído de: http://www.sciencedirect.com/science/article/pii/S0006320716309776

El **SEGUNDO TRABAJO** resultante de la beca PRIC es un trabajo que está siendo objeto de revisión por pares en la revista científica *Global Change Biology*. Por este motivo agradeceríamos que no se hiciera difusión de estos resultados hasta que esté publicado en ésta u otra revista. En este trabajo el hilo argumental sigue siendo la carpa pero esta vez estudiamos un problema sanitario de su introducción para los peces nativos: el copépodo parásito *Lernaea cyprinacea*. Este parásito de origen asiático ha sido introducido en medio mundo a través de los stocks de carpa e infecta anfibios y peces, si bien elevada mortalidad ha sido detectada exclusivamente en los últimos. Junto con resaltar el problema sanitario que supone la introducción de peces exóticos para nuestros ecosistemas, el presente trabajo examina de qué manera modificando las condiciones ambientales se puede frenar la expansión de una epizootia como la del gusano ancla *L. cyprinacea*. Es bien sabido que cuando una enfermedad se expande por una red hidrográfica de la extensión de la cuenca del Ebro su erradicación es cuanto menos una utopía y que sólo podemos esperar intentar controlar su expansión. Como hicimos en el trabajo anterior, presentamos a continuación la referencia completa de dicho trabajo y su resumen en inglés en la versión que hemos enviado a la revista donde está siendo considerado para ser publicado.

Maceda-Veiga, A., Mac Nally, R., Green, A. J., Poulin, R. & de Sostoa, A. (2017). Global environmental stressors and the transmission of a widespread alien parasite among riverine fish: additive vs interactive effects (2ª revisión)

"The increased rate of outbreaks of infectious diseases in ecosystems is among the most dramatic consequences of global change, particularly when it affects highly imperilled taxa such as freshwater fish. However, the links between disease-inducing epizootics and widespread environmental stressors, including nutrient pollution and salinization, in freshwater organisms are largely unexplored. Ours is the first study to examine additive and interactive effects of globally pervasive environmental stressors on a riverine host-parasite system. We explored the host and environmental factors that singly, or in combination, are likely to influence the spread of a worldwide invasive parasite, the anchor worm Lernaea cyprinacea, throughout an extensive region of north-eastern Spain (99,700 km2, 15 river catchments, N = 530 sites). Smaller fish experienced higher rates of infection, and lower altitudes, river salinization and nutrient pollution (nitrate and phosphates) promoted infection rates of L. cyprinacea in 19 endemic and widely distributed fish species. We found no evidence that interactive effects among major riverine stressors were related to the spread of L. cyprinacea among fish, despite the current emphasis on the importance of accounting for interactions among stressors in biodiversity management; our results suggest that management can be effective on a stressor-by-stressor basis. Given that nutrient pollution and salinization were two of the major factors contributing to the increasing infection rates of L. cyprinacea, the improvement of wastewater treatment, optimization of agricultural practices, and the restoration of riparian areas would probably reduce the spread of this widespread parasite among native fish."

Además de los resultados científicos la beca PRIC nos ha permitido en este trabajo consolidar nuestra colaboración con el investigador nuevo-zelandés Prof Robert Poulin y el investigador australiano Prof Ralph Mac Nally.

El **TERCER TRABAJO** de la beca PRIC está enviado a la revista *Diseases of Aquatic Organisms* y en él analizamos cuáles son las especies de peces de acuario que más frecuentemente enferman tanto en los comercios del ramo como en las casas de los aficionados (**Figura 1**).

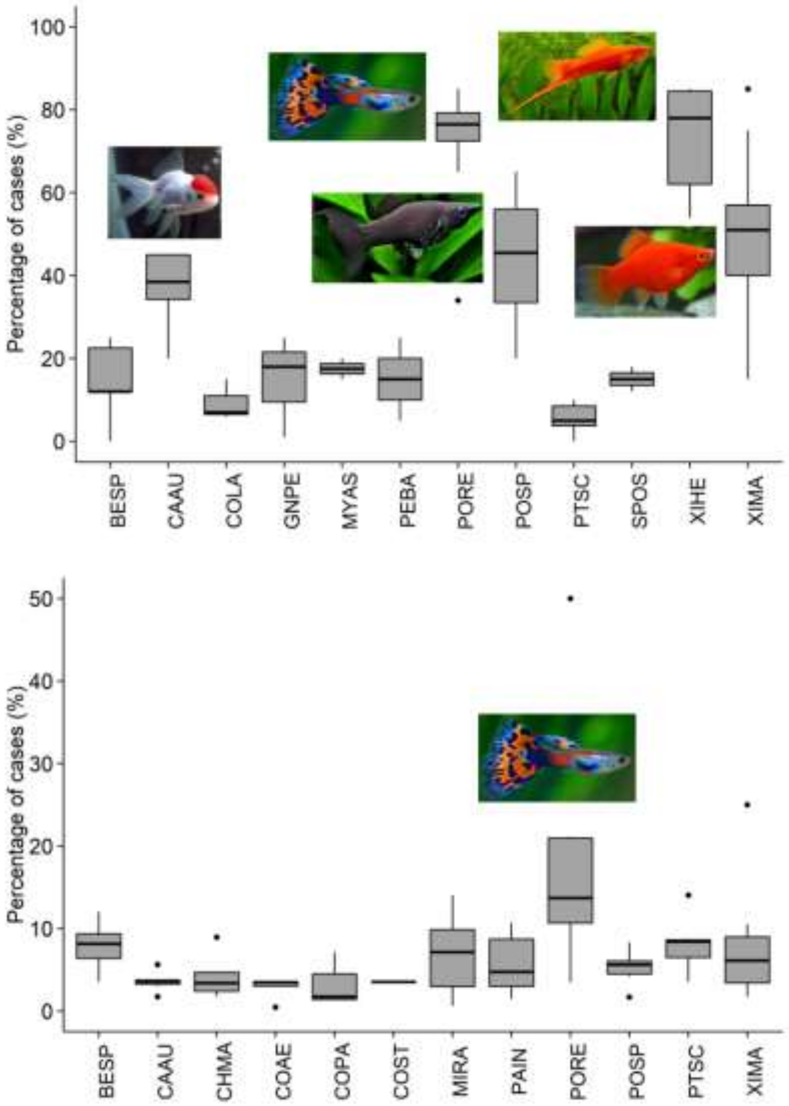


Figura 1. El gráfico de cajas superior representa el número de veces (%) en las que se han encontrado peces enfermos de las especies listadas en 12 tiendas especializadas y no especializadas en acuarios visitadas cada mes durante un año en Barcelona ciudad y provincia (N=12 visitas por tienda). El gráfico inferior muestra el porcentaje de mensajes de los foros dedicados a la afición de los acuarios en los que cada una de las especies listadas mostraba síntomas de enfermedad (N=1200 mensajes en total). Los acrónimos de las especies son BESP: *Betta splendens*; CAAU: *Carassius auratus*, CHMA: *Chromobotia macracanthus*, COAE: *Corydoras aeneus*, COST: *C. sterbai*, COLA: *Colisa lalia*, GNPE: *Gnathonemus petersii*, MIRA: *Mikrogeophagus ramirezi*, MYAS: *Myxocyprinus asiaticus*, PAIN: *Paracheirodon innesi*, PEBA: *Periophthalmus barbarus*, PORE: *Poecilia reticulata*, POSP: *P. sphenops*, PTSC: *Pterophyllum scalare*, SPOS: *Sphaerichthys osphromenoides*, XIHE: *Xiphophorus hellerii* y XIMA: *X. maculatus*. Aquellas con más registros se destacan con una ilustración de la especie en cuestión.

El objetivo de este artículo es doble. Por un lado testamos si existe alguna relación entre la popularidad de estas especies entre los aficionados (frecuencia en los comercios) y su riesgo para contraer enfermedades, lo cual podría sugerir la situación poco ética que las ventas del comercio se sustenten en la mortalidad que sufren estos peces en la casa de sus propietarios. Por otro lado, pretendemos examinar la relación entre la susceptibilidad a enfermar de estas especies de acuario y su riesgo de establecerse en la naturaleza a partir de variables comúnmente utilizadas en la ciencia de invasiones (popularidad entre los aficionados, rango de tolerancia climática e historial invasor). Los resultados de este trabajo muestran que las especies que enferman con más frecuencia son efectivamente aquellas más populares entre los aficionados, pero que existe muy poca relación con su tolerancia térmica y el número de países donde se tiene conocimiento que han establecido poblaciones invasoras. De nuestros resultados también se deriva que la enfermedad del punto blanco causada por el ciliado *Ichthyophthirius multifiliis* (Ich) junto con las enfermedades bacteriana oportunistas son las patologías que con más frecuencia se detectan en los peces de acuario. No obstante, no todas las especies de peces son susceptibles a los mismos patógenos. Por ejemplo, la locha payaso (*Chromobotia macracanthus*) es particularmente susceptible al Ich y las variedades de pez rojo (*Carassius auratus*) son especialmente vulnerables a las infecciones por los gusanos *Gyrodactylus*. Vaya por adelantado que la legislación vigente prohíbe la venta de peces enfermos, si bien la realidad parece ser otra especialmente en los comercios no especializados en peces de acuario (**Figura 1**). No es nuestro deseo criminalizar al sector pero sí hacer énfasis en la necesidad de mejorar la formación de los vendedores y realizar más inspecciones que garanticen el cumplimiento de la legislación vigente.

Los **TRABAJOS RESTANTES** todavía están en fase de elaboración de la base de datos definitiva y/o en fase de análisis preliminares.

El **CUARTO TRABAJO** que está permitiendo la formación de un estudiante del máster de Bioestadística de la UB a cargo del Dr Francesc Carmona. El objetivo general es determinar la eficacia de la ley contra la introducción de especies exóticas que entró en vigor en 2011. Para ello se han seleccionado de la base de datos del CRARC todos los reptiles y anfibios exóticos ingresados en este centro desde 2009 hasta la actualidad. Se trata de 11.549 animales pertenecientes a 144 especies. La idea es aplicar un estudio estadístico que permita ver si hay un efecto de la legislación en los abandonos o ingresos y si éste guarda relación con el hecho que la especie esté o no catalogada como invasora en la legislación de 2011. Los primeros resultados muestran que la tendencia en la entrada de individuos de determinadas especies ha cambiado después de la entrada en vigor de la ley de 2011, si bien la entrada de éstos no se ha reducido por completo. A modo de ejemplo, ponemos el gráfico de la tortuga de “orejas amarillas” (*Trachemys scripta scripta*) donde puede verse una tendencia creciente en la entrada de ejemplares a los estanques urbanos de Barcelona que se invierte una vez entra en vigor la ley de 2011 (**Figura 2**). Ahora estamos trabajando en una manera de automatizar este análisis y poder testar todas las especies de nuestra extensa base de datos de una forma ágil.

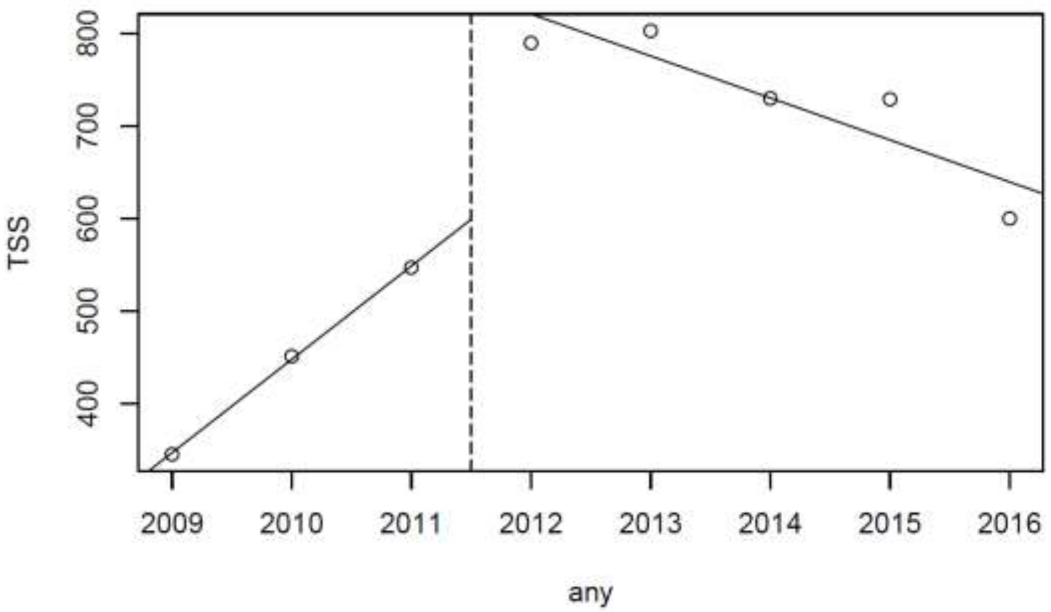


Figura 2. Tendencia en la entrada de tortugas de orejas amarillas (*Trachemys scripta scripta*, TSS) en los estanques urbanos de Barcelona durante el periodo 2009-2016. La línea discontinua muestra el momento en cual entró en vigor la legislación contra especies invasoras y en la cual las tortugas del género *Trachemys* aparecen listadas. Como se observa en la figura, si bien las tortugas se siguen liberando, parece haber una tendencia a la reducción.

Uno de los beneficios de disponer de series temporales largas, como la de nuestro trabajo, es poder analizar las respuestas retardadas que típicamente tienen lugar con la entrada en vigor de nuevas legislaciones como también ocurre con el impacto de las propias especies introducidas sobre el ecosistema. Por ejemplo, puede verse que en el año 2012 el número de tortugas introducidas se incrementa en gran medida en comparación con el año 2011 y otros años pre-legislativos. De hecho, el número de sueltas es significativamente más elevado durante el periodo 2013 a 2016 que durante el periodo 2009 a 2011. Esto, lejos de indicar la inefectividad de la legislación que se comprueba con la pendiente negativa de la recta después de 2011, lo que parece indicar es que los propietarios -quizá por miedo- empezaron a deshacerse de sus ejemplares ahora “ilegales”. Nuestros resultados, por tanto, hacen énfasis en la necesidad de una campaña pedagógica, junto con la entrada en vigor de nuevas medidas legislativas, con el fin que los propietarios de animales que cambian de estatus legal sepan cual es el procedimiento para tenerlos de acuerdo con la legislación vigente y reducir la alarma social.

El **QUINTO TRABAJO** se trata del más laborioso. Su objetivo principal es promover una nueva legislación basada en las características que tienen los individuos que se devuelven como mascotas no deseadas. De este modo, no sólo se pretende reducir el número de especies invasoras si no también evitar la venta libre de especies que pueden causar problemas a sus propietarios por su gran tamaño o comportamiento. Recientes conversaciones con especialistas en el campo del análisis de rasgos han aconsejado incrementar el número de entradas en nuestra base de datos que consta con más de 5000 registros sólo de peces y los registros antes citados del CRARC respecto a reptiles y anfibios. Dado que esta información debe entrarse de forma manual la tarea avanza lenta pero a ritmo constante. Contaremos con la inestimable colaboración del Dr Jörg Freyhof de la Universidad de Berlín para la realización del presente trabajo. Asimismo, pretendemos relacionar, como hemos hecho para el caso de los peces, el riesgo de invasión de las especies de tortugas con el hecho de ser portadoras de patógenos. Este trabajo complementará los resultados del **ARTÍCULO 3**.

El **SEXTO ARTÍCULO** es un artículo que pretende romper algunos paradigmas en la conservación de anfibios y peces continentales. Tradicionalmente, y en especial en los ambientes mediterráneos, se ha pensado en pozas y charcas como los hábitats principales a conservar para la conservación de los anfibios. Si bien el papel de éstos está claro, se desconoce qué papel podrían jugar otros ecosistemas acuáticos en su conservación, tales como los ríos mediterráneos que en la diversidad de hábitats que presentan existe evidencia observacional que bien podrían albergar poblaciones de anfibios. No obstante, esto puede parecer contra-intuitivo si pensamos que en la mayoría de los ríos habitan peces y éstos son considerados los enemigos principales de los anfibios. Precisamente testamos esta dicotomía en este artículo y los resultados que hemos obtenido abogan porque la convivencia entre peces y anfibios es posible, especialmente para el caso de los peces nativos omnívoros y bajo determinadas condiciones ambientales que todavía estamos analizando en detalle.

Para acabar, el presente proyecto contempla un **SÉPTIMO ARTÍCULO** en el que se pretende contribuir al conocimiento de la parasitofauna de las tortugas liberadas en los estanques urbanos, así como disponer de datos sobre el riesgo que suponen para los usuarios que visitan estos estanques. Los resultados preliminares, aun en vías de redacción y publicación muestran ya la presencia habitual de *Salmonella* en los estudios llevados a cabo en parques del área metropolitana de Barcelona.

2.2. Presentaciones en congresos

Miembros del equipo investigador del presente proyecto han participado en tres congresos/jornadas técnicas para hacer difusión de los resultados obtenidos en el presente proyecto agradeciendo explícitamente la financiación recibida. En todas las presentaciones se hace especial mención a la ayuda aportada por las becas PRIC tanto durante la presentación como en los agradecimientos escritos en las actas finales. Las referencias completas de las presentaciones realizadas son:

Maceda-Veiga, A., Colin, N., Cano-Rocabayera, O., Vargas-Amengual, S. & de Sostoa, A. (2016). ‘Impacte de la contaminació i les espècies exòtiques sobre els ecosistemes aqüàtics continentals: dels individus a l’ecosistema’ (In Catalan). Poster. Jornada IRBio: La Biodiversitat enfront el Canvi Global. Barcelona (Spain).

Bayón, A., **Maceda-Veiga, A.**, Tella, JL., Escribano-Alacid, J., Vargas-Amengual, S., Carrete, M. & Vilà, M. (2016). Alien vertebrate pests and ornamental outdoor and aquarium plants with commercial value in the Spanish Peninsular Territory as potential invasive species. Poster. Neobiota 9th International Conference on Biological Invasions. Viaden (Luxemburg).

Martínez-Silvestre, A., Soler, J., **Maceda-Veiga, A.**, Garcia, S., Pascual, G., Llimona, F., Cahill, S., y Cabañeros, Ll. (2017). Eliminación de especies invasoras y conservación de quelonios autóctonos en el Pantano de Vallvidrera (Barcelona). Presentación oral. Aceptada. “EEI 2017” 5º CONGRESO NACIONAL SOBRE ESPECIES EXÓTICAS INVASORAS. Girona, 16-18 octubre 2017.

2.3. Divulgación y labor pedagógica

La **difusión de nuestros resultados** se canaliza a través de las páginas web corporativas de la Universidad de Barcelona (UB), de l’*Institut de Recerca de la Biodiversitat* (IRBio), del *Centre de Recuperació d’Amfibis i Rèptils de Catalunya* (CRARC) y de nuestras cuentas particulares de twitter (ej. @AMacedaVeiga). A su vez, el gabinete de prensa de la UB hace difusión a los principales medios de comunicación en los cuales, si bien no se nos ha permitido agradecer explícitamente a la Fundación Barcelona Zoo, sí que lo hemos hecho nosotros en las publicaciones a las que hacen referencia. Estas labores de divulgación se complementan con una activa labor pedagógica realizada por el Dr Albert Martínez-Silvestre desde el CRARC y por el Dr Alberto Maceda-Veiga desde IrBio. En particular, el CRARC ha atendido a más de 150 escuelas este año a cuyos estudiantes se les ha explicado el problema de las especies invasoras y del riesgo que tiene para la conservación de la fauna autóctona el abandono de reptiles y anfibios que se adquieren como mascotas. Durante el presente año el Dr Martínez-Silvestre también ha sido docente en un curso de la asociación Animal Latitude en el cual se ha agradecido la financiación recibida por la beca PRIC y se ha hecho mención explícitamente al problema de las invasiones biológicas. Sobre esta misma temática ha hablado también el Dr Martínez-Silvestre en la protectora de animales de la ciudad de Mataró.

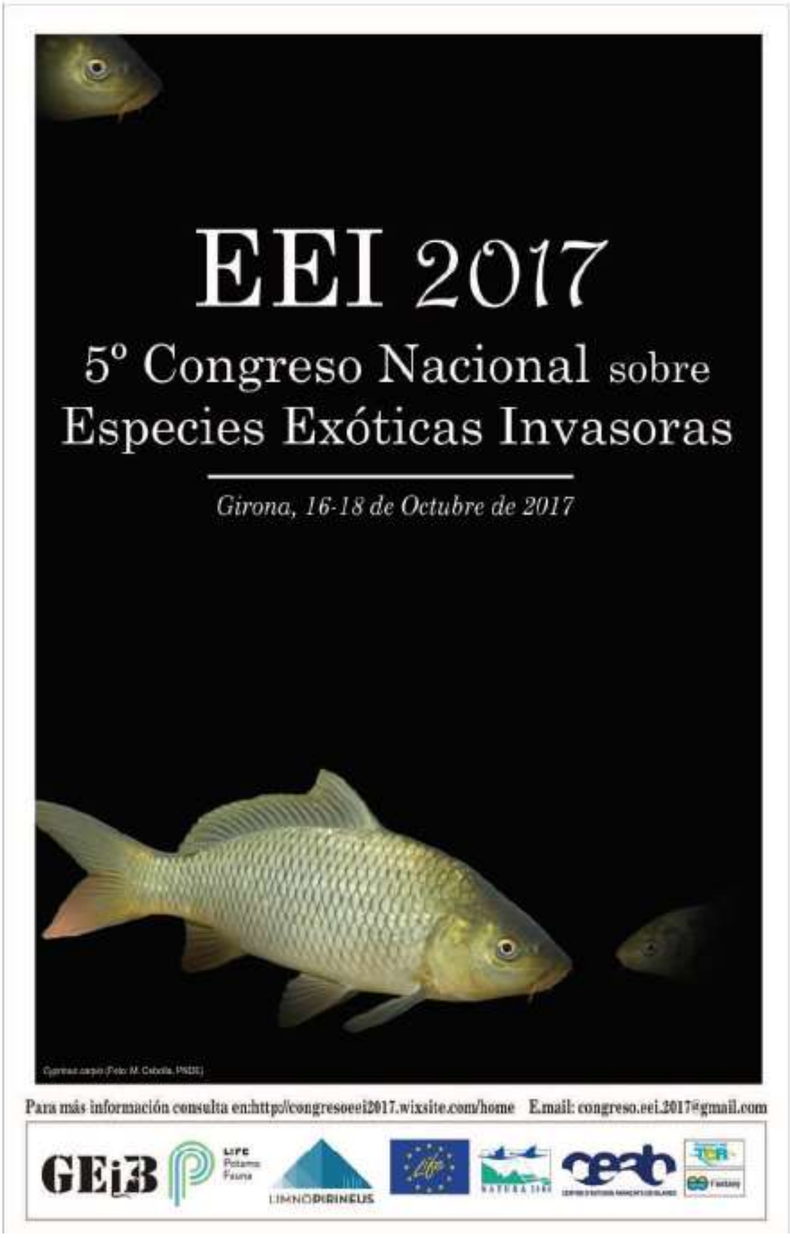
La labor divulgativa del CRARC se complementa con los dos cursos que organiza anualmente el Dr Maceda-Veiga sobre enfermedades de peces de acuario y el riesgo que pueden suponer para la fauna silvestre su liberación al medio. Además ha participado en un reportaje para Barcelona Televisó en enero de 2017 sobre el comercio de peces de acuario (Ver: http://beteve.cat/clip/reportatge-laquariofilia-aficio-als-aquaris/).

2.4. Artículos publicados en formato PDF o en fase de revisión en cuyo caso seguimos el formato de la revista científica en la cual se encuentra en fase de revisión.

Seguidamente se incluyen la ponencia que ha sido publicada durante este año 2017 mostrando los resultados del presente proyecto. Además incluimos todos los artículos en los que la Fundación Barcelona Zoo aparece en agradecimientos, incluyendo algunos artículos en fase de revisión. Para los que están en fase de análisis, rogamos sirva la breve reseña incluida en el texto principal de la presente memoria como justificante del progreso. En todos los casos se ha acordado con los co-autores del trabajo la cuantía de información a mostrar previa a la publicación de los manuscritos en curso. Rogamos entiendan la confidencialidad.

CONGRESO INVASORAS GIRONA. Libro resúmenes (pendiente publicación final con agradecimientos donde consta la beca PRIC del ZOO de Barcelona). Una vez sea publicada se hará llegar a los responsables de la Beca.

LIBRO DE RESÚMENES



EEI 2017

5º Congreso Nacional sobre Especies Exóticas Invasoras

Girona, 16-18 de Octubre de 2017

Cyprinus carpio (Foto M. Cebolla, PNDE)

Para más información consulta en:http://congresoeei2017.wixsite.com/home E.mail: congreso.eei.2017@gmail.com

ELIMINACIÓN DE ESPECIES INVASORAS Y CONSERVACIÓN DE QUELONIOS AUTÓCTONOS EN EL PANTANO DE VALLVIDRERA (BARCELONA)

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RESUMEN

El pantano de Vallvidrera, construido en el siglo XIX, entró en desuso y ya en el siglo XXI fue restaurado, entre otros motivos, para la conservación de la población de anfibios. Sin embargo, la presencia de fauna exótica (cangrejos de rio, peces y tortugas) obstaculiza este objetivo. El parque es una zona muy humanizada (25000 visitantes al año) y ello va asociado a la liberación de animales por parte de los usuarios. Durante el periodo 2015 a 2016 se ha procedido a la retirada de todos los animales. En este periodo se han capturado 125 tortugas exóticas de 5 especies (*Trachemys scripta, Graptemys pseudogeographica* , *Pseudemys concinna, Pseudemys nelsoni, Mauremys sinensis*) así como una autóctona (*Mauremys leprosa*). Los peces capturados fueron de 4 especies exóticas (*Misgurnus angillicaudatus, Carassius auratus, Ctenopharyngodon idella y Hypostomus plecostomus*). Se han eliminado también un gran número de cangrejos de rio americano (*Procambarus clarkii*).

Las tortugas exóticas se han incluido en un estudio de patógenos hacia el ecosistema y/o transmisibles al ser humano. Se han detectado infestaciones por parásitos mediterráneos (*Serpinema microcephalus*), asi como infecciones digestivas por bacterias Gram negativas. La tortuga autóctona es perfectamente compatible con la presencia de anfibios autóctonos. En el plan de conservación, la eliminación de todas las especies introducidas exóticas se complementa con la introducción de la tortuga autóctona, como emblema de valor natural para que los visitantes no sigan utilizando el pantano para abandonar mascotas. La divulgación de este plan mediante señalización y edición en comunicados del parque será parte importante en la lucha contra la introducción de fauna exótica.

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Dramatic impact of alien carp *Cyprinus carpio* on globally threatened diving ducks and other waterbirds in Mediterranean shallow lakes

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ABSTRACT

Mediterranean shallow lakes support high biodiversity but suffer many anthropogenic threats, including introductions of alien fish. We studied the impact of introduction of common carp (*Cyprinus carpio*) to Medina and Zoñar lakes in SW Spain. Both lakes were protected as Ramsar sites because of their importance for waterbirds, particularly the globally threatened white-headed duck (*Oxyura leucocephala* IUCN Endangered) and common pochard (*Aythya ferina* IUCN Vulnerable). Two carp introduction events in Medina lake, with total eradication of carp in between, provided a unique opportunity to study the impacts of carp on the waterbird community (counted monthly from 2001 to 2013, with up to 69 species) and submerged macrophyte cover (quantified with satellite images). A comparison of waterbird abundance before and after carp eradication in the smaller Zoñar lake supported the results from Medina lake. Carp consistently led to the destruction of macrophyte beds and a radical change in the waterbird community. After controlling for the influence of depth fluctuations, the numbers and species richness of diving ducks were significantly reduced by carp, whilst the opposite effect was observed for piscivores such as herons. Negative impacts on *O. leucocephala*, *A. ferina*, red-crested pochard (*Netta rufina*) and herbivorous coots (*Fulica* spp.) were particularly pronounced. A significant negative impact of carp was also recorded on greater flamingos (*Phoenicopterus ruber*), black-necked grebes (*Podiceps nigricollis*), little grebes (*Tachybaptus ruficollis*) and gadwall (*Anas strepera*). In contrast, carp presence had a positive impact on grey herons (*Ardea cinerea*). The ongoing expansion of alien cyprinids in the Mediterranean region constitutes a major threat for waterbirds and particularly for sedentary, threatened taxa such as the white-headed duck and red-knobbed coot (*F. cristata*). Of 22 key sites for the isolated Iberian population of white-headed duck identified in a European action plan in 1996, at least 14 have since suffered carp invasions. Further development of successful control methods for carp populations is urgently required to support the conservation of waterbirds in the Mediterranean region.

1. Introduction

Aquatic ecosystems have often been protected because of their importance for waterbirds, which can act as flagship species for conservation, and also provide important ecosystem services for humans (Green and Elmberg, 2014). The Ramsar Convention has been highly influential in Europe and elsewhere in the protection of wetlands, especially those holding important concentrations of waterbirds (Tomankova et al., 2013; Guareschi et al., 2015). Although it is generally assumed that waterbirds act as bioindicators of aquatic fauna and flora in general, their value as bioindicators can be limited for several reasons (Amat and Green, 2010; Tomankova et al., 2013; Guareschi et al., 2015). For example, because they breathe air and are highly mobile, they can resist deterioration in water quality better than many invertebrates or plants. On the other hand, their numbers at a given locality may vary because of changes elsewhere along their migration routes. Hence it may not always be possible to relate bird abundance in a lake to changes within the lake's food web, such as those caused by biological invasions.

Some of the world's worst invasive organisms are aquatic taxa, including crayfish, mussel and fish species such as the common carp (*Cyprinus carpio*) (Lowe et al., 2001). These invasive species act as ecosystem engineers and cause profound trophic and non-trophic alterations in ecosystems (Crooks, 2002; Gherardi, 2007; Sousa et al., 2009). Many studies have related an invasion by these taxa to dramatic alterations in the underwater community and the aquatic environment

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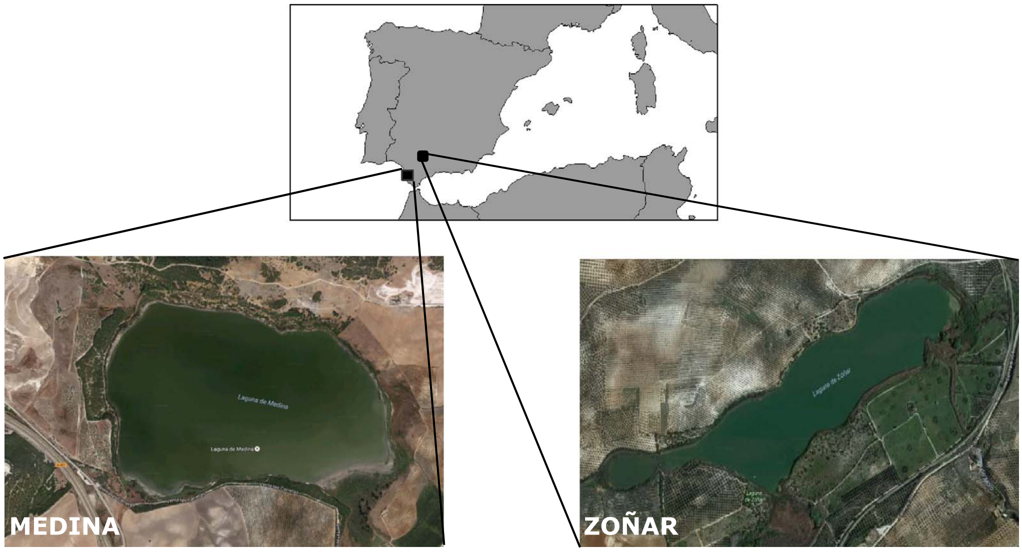

Fig. 1. Geographic location of Medina (Cadiz) and Zoñar (Córdoba) lakes (SW Spain).

(Parkos et al., 2003; Maceda-Veiga et al., 2013; Mathers et al., 2016). However, their cascading impacts on iconic vertebrate taxa (birds and mammals) are less well understood, although they can be expected to be generally positive for carnivores and negative for herbivores (Prigioni et al., 2006; Tablado et al., 2010; Laguna et al., 2016). Research into the impacts of invasive species typically shows a single snapshot of time (e.g. Parkos et al., 2003; Ilarri et al., 2014; Monroy et al., 2014; Maceda-Veiga et al., 2016), and time-lagged responses in invaded areas may be overlooked (Parker et al., 1999; Strayer et al., 2006). Therefore, there is a need for long-term studies examining the effects of non-native aquatic species on waterbird communities.

Shallow lakes and other Mediterranean wetlands provide habitat and food to many resident and migratory waterbirds, including herbivores, omnivores and piscivores (Rendon et al., 2008; Galewski et al., 2011). Different bird guilds are expected to be affected in different ways by the introduction of invasive fish such as the common carp (*Cyprinus carpio*), which has become widespread in the Mediterranean region in recent decades (Vilizzi, 2012). Waterbirds that are highly dependent on submerged macrophytes such as the Eurasian coot (*Fulica atra*) and the red-knobbed coot (*Fulica cristata*) (Varo and Amat, 2008) are likely to be negatively affected, since carp destroy macrophyte beds (Crivelli, 1983; Zambrano and Hinojosa, 1999; Shin-ichiro et al., 2009). *F. cristata* is IUCN Endangered at the European level (BirdLife International, 2015). Carp also reduce the density of benthic invertebrates and may impact diving ducks that feed on such invertebrates, including the globally threatened white-headed duck (*Oxyura leucocephala* IUCN Endangered) and common pochard (*Aythya ferina* IUCN Vulnerable) (Winfield and Winfield, 1994; Sánchez et al., 2000). In semi-arid regions such as southern Spain where wetlands are scarce, fish introductions might be particularly harmful for relatively sedentary birds such as *O. leucocephala* and *F. cristata* with specialised habitat requirements (Green et al., 2002; Sebastián-González et al., 2013). On the other hand, the effects might be reduced for omnivorous dabbling ducks (*Anas* spp.) which have large migratory populations, and can obtain food from the shoreline and from terrestrial habitats including croplands (Dessborn et al., 2011, Soons et al., 2016). Finally, piscivorous birds such as herons, grebes or cormorants might benefit from fish introductions (Laguna et al., 2016, but see Kloskowski, 2012).

Various fish have been shown to have a negative impact on waterbirds. In boreal lakes, pike (*Esox lucius*) predate ducklings whereas perch cause important competition for food (Vaananen et al., 2012). Diving ducks compete with cyprinids and other fish that eat benthic chironomid larvae and molluscs in temperate lakes (Giles et al., 1990; Winfield and Winfield, 1994). Studies in central and northern Europe suggest carp have a negative impact on the breeding success of diving and dabbling ducks (Musil, 2006; Broyer and Calenge, 2010). Such impacts might be expected to be stronger in the Mediterranean region where migratory waterfowl concentrate in winter (Rendon et al., 2008), and high water temperatures all year round increase the activity and food intake of non-native fish such as *C. carpio* (Weber et al., 2015).

In 2003, the cyprinid *C. carpio* was introduced from an adjacent farm pond into Medina lake in SW Spain, a major breeding and wintering area of waterbirds. In order to restore the value of this Ramsar site for birds, the regional government used the piscicide rotenone to eradicate carp in 2007, but a reinvasion occurred when record flooding in the winter of 2010–2011 connected the lake with the nearby River Guadalete. This effectively constitutes a replicated whole lake experiment, and here we examine the effects of *C. carpio* invasion, eradication and re-invasion on the macrophyte cover and waterbird community, including four dominant guilds (herbivorous coots, diving and dabbling ducks, and piscivores). To confirm that the observed impact of carp invasion on waterbirds is similar in other Mediterranean lakes, we use data from a second lake in SW Spain (Zoñar lake) where carp were eliminated with rotenone in 2006 after an introduction in 1985 (Torres-Esquivias et al., 2009).

Rotenone is highly toxic and can have harmful effects on invertebrates, amphibians and humans (Chandler and Marking, 1982; Rayner and Creese, 2006), but the removal of carp was expected to increase the abundance of submerged macrophytes and of herbivorous and omnivorous waterbirds, as observed with other biomanipulation tools elsewhere (Sondergaard et al., 2008; Vilizzi et al., 2015). To assess the importance of carp invasions at a broader scale, we assess how many of the key sites previously identified for *O. leucocephala* (Green and Hughes, 1996) have been affected by carp invasion.

2. Methods

2.1. Study areas

Located at 30 m above sea level in Cádiz province (36°37′18″ N, 6°02′48″ W), Medina lake (120 ha) is the third largest natural lake in Andalusia, SW Spain with a catchment area of 1900 ha (Fig. 1). It is a permanent, shallow, closed-basin lake with mean water temperature of 21 °C during the annual cycle (Florian et al., 2016). This mesohaline lake has a maximum water depth of 3.5 m due to an artificial overflow, and only dries out in case of extreme droughts, such as in 1990 and 2000 (Rodríguez-Rodríguez et al., 2012). Situated 150 km to the northeast in Córdoba province (37°28′59″N, 4°41′23″W), Zoñar lake is a smaller (40 ha), permanent, deeper lake (maximum water depth of 13 m) with a catchment area of 876 ha and a relatively stable water level (± 1 m). Both lakes were declared as hunting refuges by the regional government in 1982. Medina and Zoñar lakes have since been declared as Natural Reserves, wetlands of international importance under the Ramsar Convention, and Special Protection Areas and Sites of European Community Importance due to their value for *Fulica* spp. and *Anatidae*, including *O. leucocephala* and *A. ferina*. Although these species breed there, both lakes are particularly important for waterbirds in the post-breeding and wintering periods (Amat, 1984). The lakes receive birds from nearby temporary or semi-permanent wetlands that dry out in the summer, including the Doñana marshes (Rendon et al., 2008), and Medina lake has often held > 20,000 ducks and coots. *Cyprinus carpio* invasion extirpated the submerged vegetation of both lakes, which was dominated by *Potamogeton pectinatus*, *Zannichellia* spp. and *Chara* spp.

In the absence of carp, these lakes are mesotrophic (e.g. de Vicente et al., 2012), but carp activity makes them eutrophic with high nutrient and chlorophyll concentrations (Fernández-Delgado, 2007; Florian et al., 2016). The piscicide rotenone was applied to Medina lake in September 2007 (PW Rotenone®, at 4 ml per 10^3 l) and to Zoñar lake in August 2006 (CFT Legumine®, at 1.2 ml per 10^3 l), killing all carp. Biomass removed was 13,416 kg in Medina lake (111.8 kg/ha) (Junta de Andalucía, 2008) and 13,000 kg in Zoñar lake (325 kg/ha) (Fernández-Delgado, 2007). The regional government (Consejería de Medio Ambiente, Junta de Andalucía) decided to apply this treatment owing to clear evidence of general degradation in biodiversity at these and other lakes after entry of carp (Torres-Esquivias et al., 2009).

With the exception of a small number of birds in Morocco, Spain supports the entire biogeographical population of *O. leucocephala* in the Western Mediterranean (http://wpe.wetlands.org/). From a European action plan for the species (Green and Hughes, 1996), we identified the 22 wetlands in Spain that were formerly considered Key Sites of particular importance for *O. leucocephala*. We identified which of these sites have since suffered carp invasions by consulting with local managers and scientists in the three autonomous communities affected (Andalusia, Valencia, and Castilla la Mancha).

2.2. Bird censuses and water depth

For lake Medina, waterbird data were obtained from monthly surveys conducted between 2001 and 2013 with a spotting scope from a series of fixed points around the lake edge, by ornithologists from the Regional Government of Andalusia, as part of their monitoring program for all protected wetlands. For our analyses, the two similar coot species *Fulica* spp. were combined because only small numbers of *F. cristata* were recorded and their identification at a distance is problematic. Among other abundant waterbird species, we identified three dominant guilds including diving ducks, dabbling ducks and piscivores. Smaller Podicipedae that feed largely on invertebrates as well as small fish (e.g. Varo et al., 2011) were not included in the piscivore guild, since carp are likely to have negative effects on them through reducing invertebrate abundance, and our objective was to look for positive effects of carp invasion on true piscivores. Water depth was measured at the time of surveys and on other visits by reading a depth gauge placed in the deepest part of lake Medina. To investigate the effect of carp invasion on birds in the deeper lake Zoñar, we extracted bird data from Fernández-Delgado (2007) to compare the abundance of coots, *A. ferina* and *O. leucocephala* before (2001–2006) and after (2006–2009) extirpation of carp in Zoñar.

2.3. Macrophyte cover

As Medina is a shallow lake, when carp were absent the submerged vegetation filled the water column during spring and summer and so was visible at the surface. To assess submerged macrophyte vegetation cover at the water surface, we used Landsat L5TM data between 2001 and 2013. Satellite images were taken on approximately a monthly basis by the TM and ETM + sensors on board the Landsat 5 and Landsat 7 satellites, obtained from the United States Geological Survey. We selected those images free of cloud cover and applied the Normalised Difference Vegetation Index (NDVI, Mather, 1987), which estimates the fraction of active photosynthetic radiation that is intercepted by vegetation based on the reflectance in the red (R) and near infrared (NIR) bands (bands 3 and 4, respectively, for Landsat images, see Alcaraz-Segura et al., 2009 for further details). Using ENVI 4.4 software, one mask was constructed for the total lake surface and a second mask for the cover of terrestrial and aquatic vegetation. These masks were combined to obtain one that contained only aquatic vegetation. The mask area was quantified by the number of pixels (30 m × 30 m each).

2.4. Statistical analyses

Data from Medina and Zoñar lakes were used to independently assess the impact of carp presence on the abundance of coots, *O. leucocephala* and *A. ferina*. Then, we used the replicated whole-lake experiment in Medina to examine the effects of carp invasion in more detail. Using generalised linear models (GLMs with a Quasipoisson error distribution), we analysed the relative contribution of carp invasion and fluctuations in water depth to variation in the total richness and abundance of all waterbird species and of four dominant guilds (herbivorous coots, diving and dabbling ducks, and piscivores). Month was also included as a categorical variable in these models to account for seasonal changes in waterbird abundance. Since the month of carp introduction into Medina lake in 2003 is unknown, we excluded this year from the analyses. Similar analyses were used to explore the impact of *C. carpio* and water depth on the abundance of the most frequently recorded waterbird species (≥ 25% of occurrence among monthly surveys). Changes in the mean abundance (ratio) of waterbirds in periods with and without carp was calculated as a measure of carp effect size.

To assess the effect of carp on aquatic plants, mean macrophyte cover was compared between periods with and without *C. carpio* in Medina lake using a GLM with Gaussian error distribution. Month and water depth were also included in this model to account for differences in phenology and water depth. Then, we used a series of Quasipoisson GLMs to further explore the relative effect of carp invasion over the full set of potentially confounding factors (changes in macrophyte cover, water depth, and season) on the abundance of coots, *O. leucocephala* and *A. ferina*. Owing to the shortage of suitable Landsat images during some months, the inclusion of macrophyte cover reduced the size of our dataset. To specifically appraise the effects of carp invasion on the reproduction of coots, *A. ferina* and *O. leucocephala*, we compared the number of breeding pairs of these birds before and after carp invasion in Medina lake using a Mann-Whitney test.

The significance of explanatory factors in GLMs was tested using F tests. Log-transformation was applied to waterbird abundance and mean macrophyte cover to improve model fitting. Residual diagnostic plots from the models were used to verify the assumptions of normality and homoscedasticity (Züur et al., 2010). The goodness of fit of models (R^2) was calculated as: (null deviance-residual deviance / null deviance) ∗ 100. All statistical analyses were performed in R v.2.15.3 (R Core Team, 2015) using the libraries ‘MASS’ (Venables and Ripley, 2002), and ‘car’ (Fox and Weisberg, 2011). The statistical threshold was $P \leq 0.05$.

3. Results

Up to 32 water bird species were recorded on Medina lake at any one time during the study period (2001 – 2013), with an overall combined total of 69 species. Numbers peaked in the post-breeding and wintering periods. There were four dominant guilds (herbivorous coots,

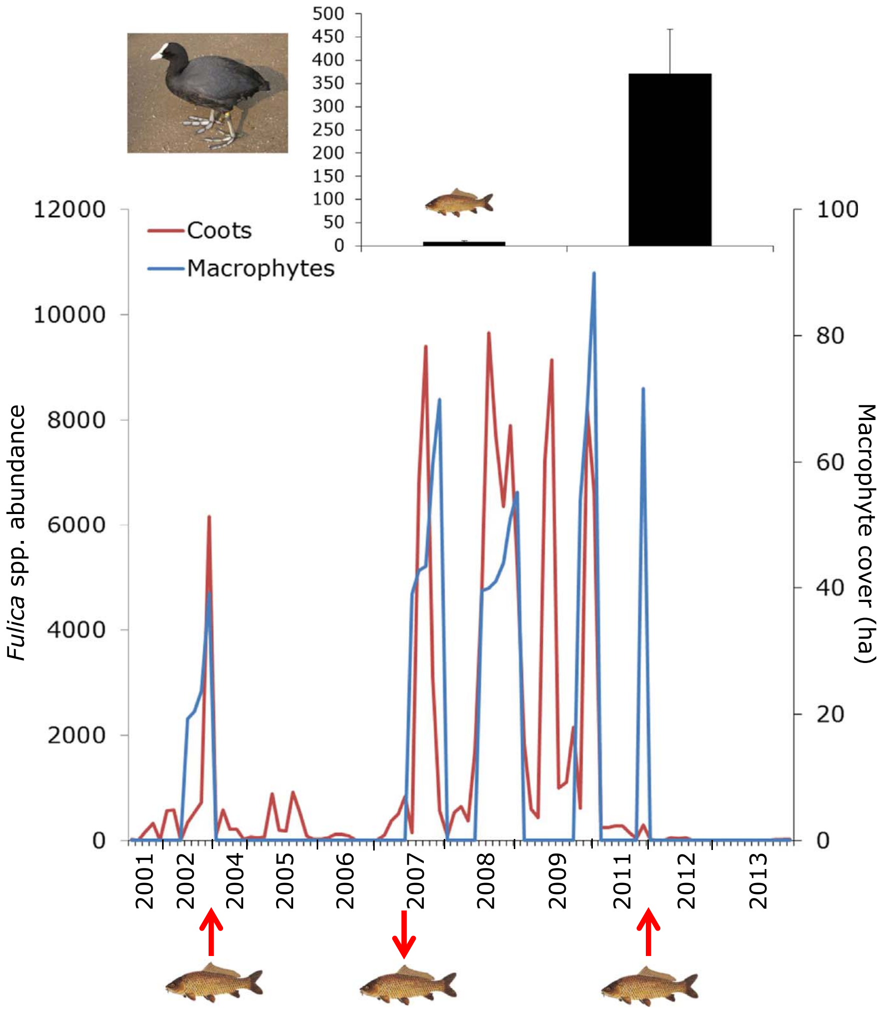

Fig. 2. Simultaneous variation in coot abundance (*Fulica* spp.) and submerged macrophyte cover, as quantified by satellite images, in Medina lake showing the effects of two periods (2004–2007 and 2011–2013) of common carp (*Cyprinus carpio*) invasion. 2003 is excluded because carp was introduced this year but the month of entry is unknown. The boxplot shows changes in coot abundance (*Fulica atra*) before (2001–2006) and after (2006–2009) carp eradication in Zoñar lake.

diving and dabbling ducks, and piscivores), and important concentrations of two globally threatened diving ducks (white headed duck *Oxyura leucocephala* and common pochard *Aythya ferina*) (Table S1). The red-knobbed coot (*Fulica cristata*) was regularly present (Table S1) and up to 7 ferruginous duck *Aythya nyroca* (IUCN near-threatened) were recorded occasionally. Other waterbirds recorded during > 25% of surveys were the greater flamingo (*Phoenicopterus ruber*), the little grebe (*Tachybaptus ruficollis*), the black-necked grebe (*Podiceps nigricollis*), the yellow-legged gull (*Larus michahellis*), the black-necked stilt (*Himantopus himantopus*), the common moorhen (*Gallinula chloropus*) and the purple swamphen (*Porphyrio porphyrio*) (Table S1).

In the absence of alien carp *Cyprinus carpio*, the most abundant waterbirds in Medina lake were coots (*Fulica* spp., up to 12,000 individuals in the post-breeding period) and shoveller (*Anas clypeata*) (up to 2164 individuals during winter) (Fig. 2 and Table S1). Without carp, the lake showed a higher richness and abundance of diving ducks, with up to 800 *O. leucocephala* and 1136 *A. ferina* outside the breeding period (Tables S1, Figs. 3 and 4). A sharp increase in the number of coots ($W = 309$; $P < 0.001$), *O. leucocephala* ($W = 65.5$; $P < 0.001$) and *A. ferina* ($W = 95$; $P < 0.001$) also occurred in Zoñar lake in the absence of carp (Figs. 2 and 4). When *C. carpio* were absent, the Medina lake surface was also covered by a large macrophyte bed during summer and autumn, as quantified by satellite images (Fig. 2).

During the two periods of *C. carpio* invasion in Medina lake, mean values of macrophyte cover, and numbers of coots and diving ducks decreased dramatically in a consistent manner (Tables 1, 2, 3 and S1), as seen also in Zoñar lake. The impact was particularly acute for *O. leucocephala*, which almost disappeared in the presence of carp (Fig. 4). A strong decrease in number was also observed for *A. ferina*, and the loss of macrophytes was reflected by a similar decline of coot numbers (Fig. 2). In fact, variation in macrophyte cover was a major determinant of the monthly variation in coot numbers, as was carp presence (Table 3). Many carp died during the unusually low water levels in summer 2006 (Fig. 5), and the reduction in carp density allowed a recovery of macrophyte cover in 2007 before the rotenone treatment (Fig. 2). Changes in abundance of the diving ducks *O. leucocephala*, *A. ferina* and the red-crested pochard *Netta rufina* were mostly related to carp presence, although macrophytes and depth had significant positive effects on *A. ferina* (Tables 2, 3). The numbers of breeding pairs per year of coots ($W = 45$; $P = 0.003$) and *O. leucocephala* ($W = 36$; $P = 0.02$) were also markedly reduced after carp invasion in Medina lake, although the reduction was not significant for *A. ferina* ($P > 0.05$).

Although carp presence had no significant effect on the overall abundance and species richness of dabbling ducks, there was a marked decline in gadwall (*A. strepera*) numbers during *C. carpio* invasion (Tables 2 and S1). The presence of carp increased the abundance and richness of piscivorous birds, although the effects were not consistent between species and were only significant at the species level for the small population of grey herons (*Ardea cinerea*) (Tables 1 and 2). A significant negative effect of *C. carpio* on abundance was also observed for the small grebes *T. ruficollis* and *P. nigricollis*, and the greater flamingo (Fig. 6). Water depth was influential, and at shallower depths there were increases in abundance of flamingos, herons and stilts as well as decreases in gadwall, grebes and western swamphens (Table 2). However, the partial effect of carp on the abundance of coots, *O. leucocephala*, *A. ferina* and six other species was highly significant when

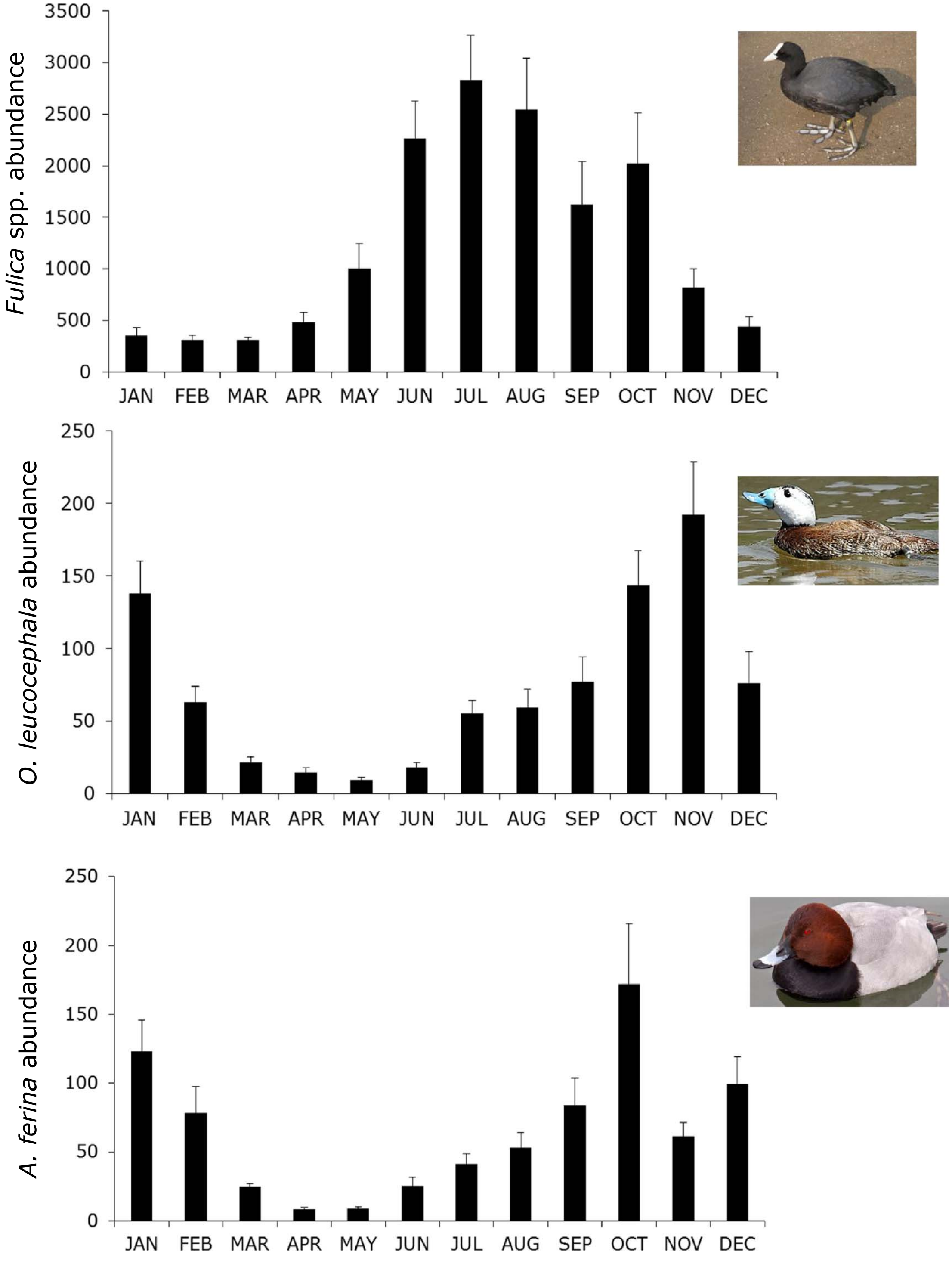

Fig. 3. Monthly variation (mean ± S.E.) in the abundance of coots (*Fulica* spp.), the globally threatened white-headed duck (*Oxyura leucocephala*) and common pochard (*Aythya ferina*) in Medina lake during the study period (2001–2013).

controlling for variation in water level (Tables 1, 2 and 3).

Of 22 key sites for *O. leucocephala* in the Iberian Peninsula identified in 1996, at least 14 (64%) have since been affected by *C. carpio* invasion (Table S2).

4. Discussion

We have shown a consistent, drastic impact of invasion by common carp (*Cyprinus carpio*) on waterbirds in two of the many wetlands recently affected by such invasions in Spain and elsewhere in the Mediterranean region. Particularly striking are the impacts on submerged macrophytes, coots and diving ducks, which crash after carp invasion, affecting threatened species such as the white-headed duck (*Oxyura leucocephala*), common pochard (*Aythya ferina*) and the crested coot (*Fulica cristata*). Similar impacts occurred over two invasion periods in Medina lake, and one in Zoñar lake, supporting the notion that the effects of carp on ecosystems are predictable (Weber and Brown, 2009; Vilizzi et al., 2015).

Unlike two previous studies of carp impact on waterbirds at the whole lake level (Haas et al., 2007; Bajer et al., 2009), ours involved

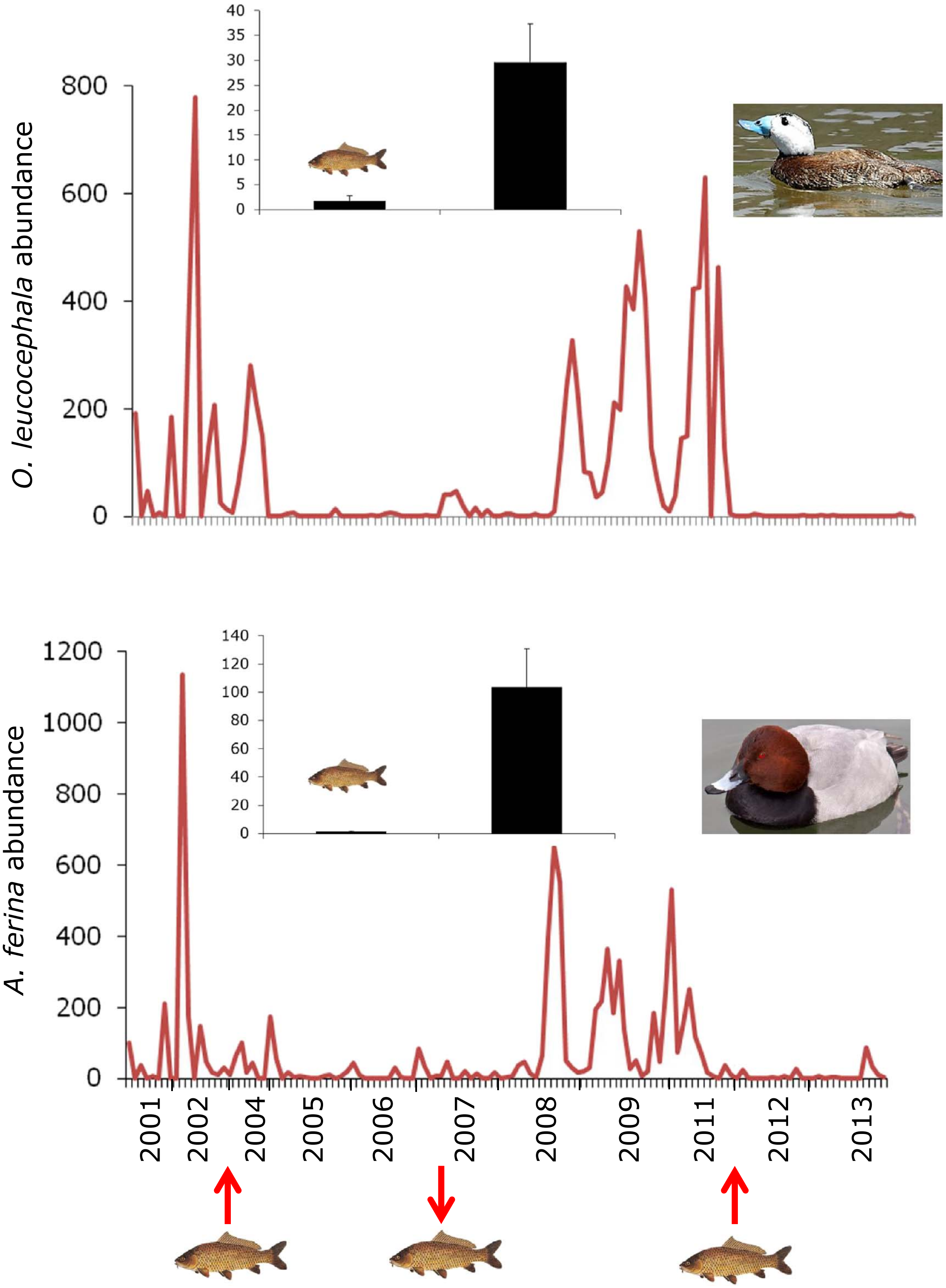

Fig. 4. Fluctuations in the abundance of the globally threatened white-headed duck (*Oxyura leucocephala*) and common pochard (*Aythya ferina*) in Medina lake showing the effects of two periods (2004–2007 and 2011–2013) of common carp (*Cyprinus carpio*) invasion. 2003 is excluded because carp was introduced this year but the month of entry is unknown. Boxplots show changes in the number of these two species before (2001–2006) and after (2006–2009) carp eradication in Zoñar lake.

two whole-lake experiments with an initial carp introduction, an eradication and finally a second introduction in Medina lake, and an eradication in Zoñar lake. The observed effects cannot be explained on the basis of some confounding variable simultaneously influencing the population size of waterbirds, as might be suspected if there was one single ‘before-after’ experiment. Our results are consistent with previous data showing the ecological impact of carp invasion, mostly collected in ponds and experimental enclosures (e.g. Crivelli, 1983; Lammens and Hoogenboezem, 1991; Zambrano and Hinojosa, 1999; Parkos et al., 2003). Little previous information was available from Mediterranean shallow lakes (Torres-Esquivias et al., 2009; Laguna et al., 2016), where low water depth and high winter temperatures probably intensify the impact of carp (Meerhoff et al., 2007; Weber et al., 2015).

Our results demonstrate that carp invasion has negative effects on a range of waterbirds including flamingos and grebes, although coots and

Table 1

Results of GLM models on the effects of the alien carp *Cyprinus carpio*, month and water depth on the abundance and richness of all waterbird species and four dominant guilds (herbivorous coots, diving and dabbling ducks, and piscivores) in Medina lake. Explained deviance is shown as a measure of goodness of fit (R^2) of the models. Regression estimates for carp and depth effects indicate the magnitude and direction of the partial effects of *C. carpio* and water depth fluctuations on bird values in models including month as a predictor. Bold values indicate significance at $P \leq 0.05$.

	C. carpio Test, df	*P* value	Estimates ± S.E. for carp effect	Month Test, df	*P* value	Water depth Test, df	*P* value	Estimates ± S.E. for depth effect	R^2
All bird species									
Abundance	$F_{1,91} = 15.33$	**< 0.001**	− 0.24 ± 0.06	$F_{11,91} = 0.58$	0.84	$F_{1,91} = 0.01$	0.92	− 0.01 ± 0.09	11.95%
Richness	$F_{1,91} = 1.76$	0.19	− 0.08 ± 0.06	$F_{11,91} = 0.59$	0.84	$F_{1,91} = 0.28$	0.59	− 0.05 ± 0.09	4.89%
Herbivorous coots	$F_{1,91} = 38.70$	**< 0.001**	− 0.56 ± 0.09	$F_{11,91} = 0.91$	0.53	$F_{1,91} = 1.75$	0.19	0.20 ± 0.15	22.74%
Diving ducks									
Abundance	$F_{1,91} = 55.17$	**< 0.001**	− 0.73 ± 0.09	$F_{11,91} = 1.02$	0.44	$F_{1,91} = 0.08$	0.78	0.05 ± 0.16	33.26%
Richness	$F_{1,91} = 25.48$	**< 0.001**	− 0.48 ± 0.09	$F_{11,91} = 0.79$	0.64	$F_{1,91} = 0.35$	0.56	0.09 ± 0.16	22.32%
Dabbling ducks									
Abundance	$F_{1,91} = 0.08$	0.78	− 0.02 ± 0.08	$F_{11,91} = 1.35$	0.21	$F_{1,91} = 0.07$	0.79	− 0.03 ± 0.12	10.93%
Richness	$F_{1,91} = 0.49$	0.78	− 0.05 ± 0.07	$F_{11,91} = 3.43$	**< 0.001**	$F_{1,91} = 2.14$	0.14	0.16 ± 0.11	21.21%
Piscivores									
Abundance	$F_{1,91} = 20.79$	**< 0.001**	0.13 ± 0.09	$F_{11,91} = 12.23$	**< 0.001**	$F_{1,91} = 0.35$	0.56	0.26 ± 0.14	68.37%
Richness	$F_{1,91} = 0.18$	0.67	0.06 ± 0.15	$F_{11,91} = 3.72$	**< 0.001**	$F_{1,91} = 0.31$	0.58	− 0.13 ± 0.23	25.33%

diving ducks are particularly badly affected. Given the widespread and ongoing invasion of carp in Spanish wetlands (Table S2), the decrease in waterbird abundance is of major conservation concern, particularly for the globally threatened *A. ferina* and *O. leucocephala* as well as the regionally threatened *F. cristata*. White-headed ducks almost disappeared during carp invasion in Zoñar and Medina lakes which, when carp are absent, can harbour up to one third (800) of the estimated biogeographical population (1600–2500) of this diving duck in Western Europe (Muñoz-Fuentes et al., 2013). Habitat loss is one of the major threats to this species, in addition to others such as hunting, lead poisoning, and hybridisation with the North American Ruddy duck (*Oxyura jamaicensis*) (Green et al., 1996; Taggart et al., 2009; Muñoz-Fuentes et al., 2013). Thus, the loss of an important habitat for waterbirds through carp invasion can have major ramifications at the population level, especially in semi-arid regions such as southern Spain where many lakes were drained in the 20th century (Perennou et al., 2012). The situation is expected to worsen due to climate change (Moss et al., 2009), and because an increasing number of lakes used by *A. ferina*, *F. cristata* and *O. leucocephala* in Spain are being degraded through eutrophication and fish introductions (Fox et al., 2016, Table S2). The size of the *O. leucocephala* population in Spain now appears to be limited by the availability of suitable habitat (Almaraz and Amat, 2004; Sebastián-González et al., 2013), so the loss of suitable habitat by carp invasion is likely to cause an important reduction in population size. Indeed, numbers counted in Spain in recent years have been well below the historical maximum count of 4489 made in 2000 (Torres Esquivias, 2009). This decline is thought to be partly due to the recent abundance of carp in El Hondo wetlands in Alicante, which formerly held most of the Spanish *O. leucocephala* population (JA. Gómez and M. Ferrández pers. comm. April 2017).

The severe impacts of carp invasion on the macrophyte cover and abundance of coots, globally threatened diving ducks and other waterbirds are likely to be explained by several mechanisms that are not mutually exclusive. As a result of its feeding activity, carp reduces macroinvertebrate abundance and macrophyte cover through feeding, uprooting and increasing turbidity (Crivelli, 1983; Kloskowski, 2011; Shin-ichiro et al., 2009; Florian et al., 2016). The loss of macrophytes is likely to cause the observed negative effect on coots, which are herbivorous (Brinkhof, 1997, Varo and Amat, 2008). We found evidence that removal of macrophytes also affects *A. ferina*, which is omnivorous (Kear, 2005). However, the major impact on diving ducks is likely to have been through competition for benthic invertebrates (particularly chironomidae larvae) upon which both carp (García-Berthou, 2001) and diving ducks feed (Winfield and Winfield, 1994, Sánchez et al., 2000). Our results agree with Laguna et al. (2016) who reported a concomitant decrease in the abundance of macrophytes and the diving duck *N. rufina* in enclosures with carp in a Mediterranean wetland. Finally, the reductions in waterbird abundance may have been partly caused by the increased turbidity that impedes visual foraging of diving ducks and grebes (Winfield and Winfield, 1994; Carbone et al., 1996).

Among the dabbling ducks, such alterations in the aquatic environment are most likely to affect gadwalls (*A. strepera*) which feed in submerged vegetation close to the water surface (McKnight, 1998; Kear, 2005), explaining why we found negative effects of carp and increasing depth on this species. We did not find significant changes in the abundance of mallards (*A. platyrhynchos*) which often exploit terrestrial habitats (Soons et al., 2016), or of the largely zooplanktivorous shoveller (*A. clypeata*) (Guillemain et al., 2000), even though the zooplankton community in Medina lake radically changed after carp invasion (Florian et al., 2016). *A. clypeata* may be able to exploit the smaller copepods that dominate zooplankton in the presence of carp (Matsubara et al., 1994, Florian et al., 2016). The observed decrease in the abundance of other invertivores such as the greater flamingo (*Phoenicopterus ruber*) and the black-necked grebe (*Podiceps nigricollis*) is not surprising because they are dependent on larger invertebrates such as chironomid larvae and other insects (Varo et al., 2011, Rodríguez-

Table 2

Results of GLM models on the effects of the alien carp *Cyprinus carpio*, depth and month on the abundance of the most frequently recorded waterbird species in Medina lake (2001–2013). Explained deviance is shown as a measure of goodness of fit (R^2) of the models. Regression estimates for carp and depth effects indicate the magnitude and direction of the partial effect of *C. carpio* and water depth fluctuations on bird numbers in models including month as predictor. Bold values indicate significance at $P \leq 0.05$.

	C. carpio Test, df	*P* value	Estimates ± S.E. for carp effect	Month Test, df	*P* value	Water depth Test, df	*P* value	Estimates ± S.E. for depth effect	R^2
Herbivorous coots									
Fulica spp.	$F_{1,91} = 38.70$	**< 0.001**	− 0.56 ± 0.09	$F_{11,91} = 0.91$	0.53	$F_{1,91} = 1.75$	0.19	0.20 ± 0.15	22.74%
Diving ducks									
O. leucocephala	$F_{1,91} = 77.64$	**< 0.001**	− 1.59 ± 0.19	$F_{11,91} = 0.68$	0.75	$F_{1,91} = 0.73$	0.39	− 0.25 ± 0.29	48.81%
N. rufina	$F_{1,91} = 31.99$	**< 0.001**	− 0.99 ± 0.17	$F_{11,91} = 0.72$	0.72	$F_{1,91} = 1.72$	0.19	0.41 ± 0.32	28.60%
A. ferina	$F_{1,91} = 43.25$	**< 0.001**	− 0.86 ± 0.13	$F_{11,91} = 1.22$	0.29	$F_{1,91} = 3.14$	0.08	0.39 ± 0.22	30.85%
Dabbing ducks									
A. strepera	$F_{1,91} = 22.57$	**< 0.001**	− 0.66 ± 0.13	$F_{11,91} = 0.64$	0.79	$F_{1,91} = 9.37$	**0.003**	0.74 ± 0.25	21.34%
A. platyrhynchos	$F_{1,91} = 0.05$	0.83	0.02 ± 0.09	$F_{11,91} = 1.29$	0.24	$F_{1,91} = 1.50$	0.22	− 0.16 ± 0.13	12.44%
A. clypeata	$F_{1,91} = 2.74$	0.10	− 0.24 ± 0.14	$F_{11,91} = 13.47$	**< 0.001**	$F_{1,91} = 1.34$	0.25	0.25 ± 0.22	60.76%
Piscivores									
P. cristatus	$F_{1,91} = 1.96$	0.164	0.14 ± 0.10	$F_{11,91} = 3.91$	**< 0.001**	$F_{1,91} = 13.98$	**< 0.001**	0.58 ± 0.16	32.77%
A. cinerea	$F_{1,91} = 17.65$	**< 0.001**	20.83 ± 5356.01	$F_{11,91} = 3.72$	**< 0.001**	$F_{1,91} = 68.38$	**< 0.001**	− 0.64 ± 0.10	86.10%
E. garzetta	$F_{1,91} = 3.26$	0.07	0.38 ± 0.23	$F_{11,91} = 3.73$	**< 0.001**	$F_{1,91} = 6.68$	**0.011**	0.71 ± 0.31	29.47%
Other abundant birds									
P. ruber	$F_{1,91} = 26.12$	**< 0.001**	− 1.47 ± 0.30	$F_{11,91} = 2.39$	**0.01**	$F_{1,91} = 20.94$	**< 0.001**	− 1.82 ± 0.40	52.49%
L. michahellis	$F_{1,91} = 1.69$	0.18	0.25 ± 0.20	$F_{11,91} = 3.12$	**0.001**	$F_{1,91} = 0.28$	0.60	− 0.15 ± 0.28	24.88%
T. ruficollis	$F_{1,91} = 53.94$	**< 0.001**	− 1.06 ± 0.15	$F_{11,91} = 2.05$	**0.03**	$F_{1,91} = 13.48$	**< 0.001**	0.93 ± 0.26	37.53%
G. chloropus	$F_{1,91} = 0.67$	0.42	0.13 ± 0.16	$F_{11,91} = 1.41$	0.18	$F_{1,91} = 2.87$	0.09	3.03 ± 0.83	18.50%
P. nigricollis	$F_{1,91} = 41.19$	**< 0.001**	− 0.93 ± 0.14	$F_{11,91} = 0.70$	0.72	$F_{1,91} = 5.88$	**0.02**	0.63 ± 0.26	30.17%
P. porphyrio	$F_{1,91} = 0.95$	0.33	− 0.34 ± 0.34	$F_{11,91} = 1.53$	0.14	$F_{1,91} = 18.45$	**< 0.001**	3.03 ± 0.83	29.30%
H. himantopus	$F_{1,91} = 0.12$	0.73	− 0.09 ± 0.26	$F_{11,91} = 1.15$	0.34	$F_{1,91} = 28.46$	**< 0.001**	− 1.95 ± 0.37	30.29%

Table 3
Results of GLM models on the effects of the alien carp *Cyprinus carpio,* month and water depth on mean macrophyte cover, and for the effects of these four predictors on the abundance of coots (*Fulica* spp.) and the two threatened diving ducks (*Oxyura leucocephala* and *Aythya ferina*). Explained deviance is shown as a measure of goodness of fit (R^2) of the models. Regression estimates for the carp effect indicate the magnitude and direction of the partial effect of *C. carpio* on macrophytes and bird numbers in models including all the predictors stated in the table. Bold values indicate significance at $P \leq 0.05$.

Dependent variable	Predictors	Test, df	*P* value	Estimates ± S.E. for the carp effect	R^2
Macrophyte cover	*C. carpio*	$F_{1,66} = 15.68$	< **0.001**	− 1.35 ± 0.34	37.90%
	Month	$F_{11,66} = 2.51$	**0.011**		
	Water depth	$F_{1,66} = 1.01$	0.32		
Fulica spp.	*C. carpio*	$F_{1,65} = 18.96$	< **0.001**	− 0.49 ± 0.11	39.13%
	Macrophyte cover	$F_{1,65} = 8.19$	**0.005**		
	Month	$F_{11,65} = 0.54$	0.87		
	Water depth	$F_{1,65} = 1.92$	0.17		
O. leucocephala	*C. carpio*	$F_{1,65} = 42.03$	< **0.001**	− 0.49 ± 0.11	51.72%
	Macrophyte cover	$F_{1,65} = 0.05$	0.81		
	Month	$F_{11,65} = 0.88$	0.56		
	Water depth	$F_{1,65} = 0.49$	0.48		
A. ferina	*C. carpio*	$F_{1,65} = 26.35$	< **0.001**	− 0.89 ± 0.17	43.99%
	Macrophyte cover	$F_{1,65} = 5.36$	**0.02**		
	Month	$F_{11,65} = 1.46$	0.17		
	Water depth	$F_{1,65} = 5.78$	**0.02**		

Pérez and Green, 2012). The negative impact of carp on macroinvertebrates has been demonstrated in Zoñar lake (Ferreras-Romero et al., 2016).

Although breeding numbers of waterbirds were reduced by carp, as reported elsewhere in Europe (Musil, 2006), our study particularly shows the impacts of carp in the post-breeding and wintering periods when bird numbers are highest and when carp remain active because of the high temperatures in southern Spain. Some comparisons of impacts on birds can be made with previous studies, but are hindered by the lack of data elsewhere on diving ducks. The reduction in total abundance of diving and dabbling ducks combined was only slightly higher in Medina lake (2.25 fold) than values (1.84–2.10) reported in a USA temperate lake at similar carp densities to Medina lake (112 vs 110 kg/ha) from September to December by Bajer et al. (2009). The decrease in the total combined abundance of particular species common to our study and a previous one (*Fulica atra*, *Anas clypeata*, *A. querquedula*, *A. crecca*, and *Gallinula chloropus* summed together) was lower in ours (4.47) than the range (5–15) reported in temperate experimental lakes in Germany at 100–120 carp/ha from May to September (see the omnivory guild in Haas et al., 2007). However, our results suggest that of this group of 5 species only coots seem particularly sensitive to carp. Furthermore, the impacts of carp we observed on ducks in the winter period have not been reported from temperate regions, where carp would be much less active. In addition, our figures include periods relatively soon after carp invasion when the abundance of larger carp having most impact (Kloskowski, 2011) was probably still low. The census data suggest that more than two years after carp enter the lake, the reduction in waterbird numbers at Medina Lake is considerably stronger than our analyses suggest. Unfortunately, our study is not sufficient to provide a safety threshold of carp abundance. The rotenone treatment allowed us to demonstrate ecological impact with a carp biomass of 112 and 325 kg/ha, a range consistent with Bajer et al. (2009) who suggested that 100 kg carp/ha may already cause a dramatic decline in macrophyte and waterbird abundance. Nonetheless, regional differences in water temperature and other traits such as the size structure of carp populations also influence their ecological impact (Goolish and Adelman, 1984; Kloskowski, 2011; Weber et al., 2015).

Rotenone application reversed the severe impacts of carp on macrophytes and waterbirds in Medina and Zoñar lakes. Despite its efficacy, rotenone is now banned in Spain due to its strong side effects on other taxa (Chandler and Marking, 1982; Rayner and Creese, 2006). Repeated seining and physical barriers can be alternative, more environmentally

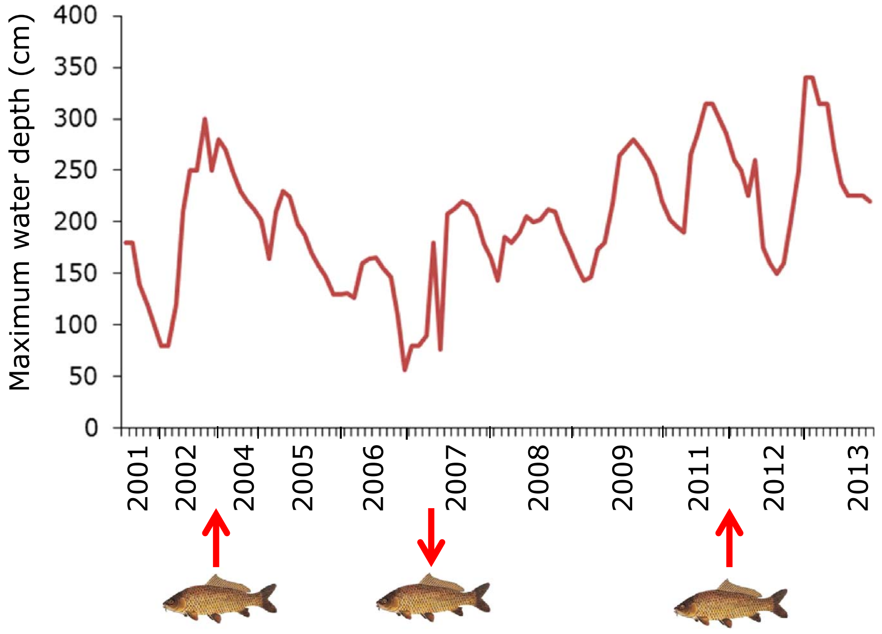

Fig. 5. Changes in water depth in Medina lake during the study period (2001–2013).

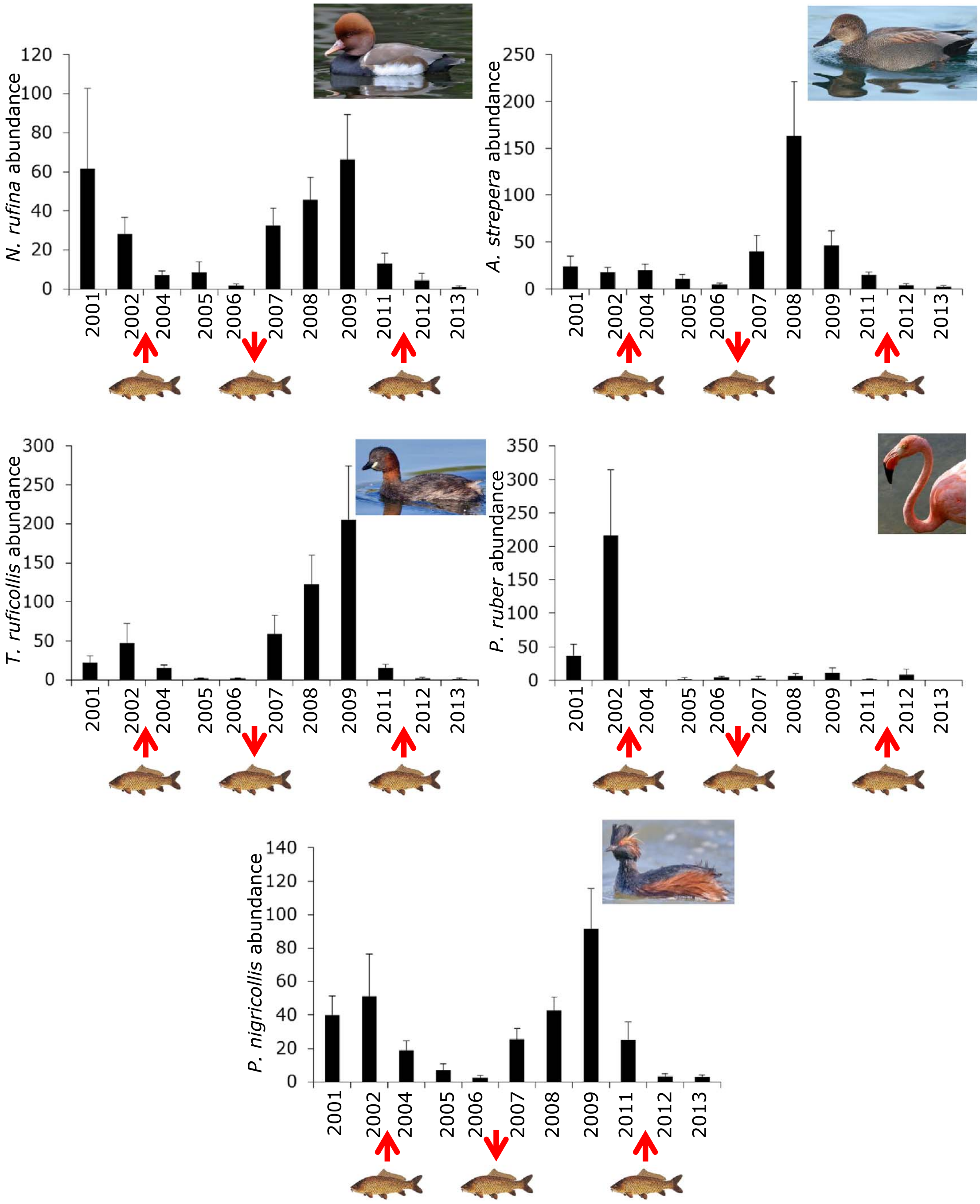

Fig. 6. Variation in the abundance (mean ± S.E.) of *Netta rufina*, *Anas strepera*, *Tachybaptus ruficollis*, *Phoenicopterus ruber* and *Podiceps nigricollis* (from top left to bottom right) showing the effects of two periods (2004–2007 and 2011–2013) of common carp (*Cyprinus carpio*) invasion in Medina lake. 2003 is excluded because carp was introduced this year but the month of entry is unknown.

safe methods for carp control (Stuart et al., 2006; Bajer et al., 2011), and further research is required to develop effective methods that can be applied to Mediterranean lakes. Our study demonstrates the negative effects of carp on globally threatened bird species, and it is very important to take measures preventing the spread of carp into those natural and artificial wetlands that currently remain free of this invasive species. The ongoing expansion of alien cyprinids in the Mediterranean region, including *C. carpio* (Vilizzi, 2012; Maceda-Veiga et al., 2017) and the *Carassius* species complex (Wouters et al., 2012; Ribeiro et al., 2015), can be expected to have a major negative impact on the conservation of waterbirds and other aquatic fauna. Owing to interests for sport fishing and human consumption in some parts of Europe, there are attempts to exclude *C. carpio* from regional lists of invasive species in Europe (e.g. it is excluded from the Spanish decree RD 630/2013 implementing measures against invasive species). Studies like ours are necessary to improve our ability to predict and manage impacts caused by alien fish stocks.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.biocon.2017.06.002.

References

Alcaraz-Segura, D., Cabello, J., Paruelo, J., 2009. Baseline characterization of major Iberian vegetation types based on the NDVI dynamics. Plant Ecol. 202, 13–29.

Almaraz, P., Amat, J.A., 2004. Multi-annual spatial and numeric dynamics of the white-headed duck *Oxyura leucocephala* in southern Europe: seasonality, density dependence and climatic variability. J. Anim. Ecol. 73, 1013–1023.

Amat, J.A., 1984. Las poblaciones de aves acuáticas en las lagunas andaluzas: Composición y diversidad durante un ciclo anual. Ardeola 31, 61–79.

Amat, J., Green, A.J., 2010. Waterbirds as bioindicators of environmental conditions. In: Hurford, C., Schneider, M., Cowx, Y.I. (Eds.), Conservation Monitoring in Freshwater Habitats: A Practical Guide and Case Studies. Springer, Dordrecht, pp. 45–52.

Bajer, P.G., Sullivan, G., Sorensen, P.W., 2009. Effects of a rapidly increasing population of common carp on vegetative cover and waterfowl in a recently restored Midwestern shallow lake. Hydrobiologia 632, 235–245.

Bajer, P.G., Chizinski, C.J., Sorensen, P.W., 2011. Using the Judas technique to locate and remove wintertime aggregations of invasive common carp. Fish. Manag. Ecol. 18, 497–505.

BirdLife International, 2015. *Fulica cristata*. The IUCN Red List of Threatened Species 2015: e.T22692907A60022797. Downloaded on 12 April 2017.

Brinkhof, M.W., 1997. Seasonal variation in food supply and breeding success in European Coots *Fulica atra*. In: Ardea-Wageningen. 85. pp. 51–66.

Broyer, J., Calenge, C., 2010. Influence of fish-farming management on duck breeding in French fish pond systems. Hydrobiologia 637, 173–185.

Carbone, C., de Leeuw, J.J., Houston, A.I., 1996. Adjustments in the diving time budgets of tufted duck and pochard: is there evidence for a mix of metabolic pathways. Anim. Behav. 51, 1257–1268.

Chandler Jr., J.H., Marking, L.L., 1982. Toxicity of rotenone to selected aquatic invertebrates and frog larvae. Prog. Fish Cult. 44, 78–80.

Crivelli, A.J., 1983. The destruction of aquatic vegetation by carp. Hydrobiologia 106, 37–41.

Crooks, J.A., 2002. Characterizing ecosystem-level consequences of biological invasions: the role of ecosystem engineers. Oikos 97, 153–166.

Dessborn, L., Brochet, A.L., Elmberg, J., Legagneux, P., Gauthier-Clerc, M., Guillemain, M., 2011. Geographical and temporal patterns in the diet of pintail *Anas acuta*, wigeon *Anas penelope*, mallard *Anas platyrhynchos* and teal *Anas crecca* in the Western Palearctic. Eur. J. Wildl. Res. 57, 1119–1129.

Fernández-Delgado, C., 2007. Eradication of carp (Cyprinus carpio) in Zoñar lagoon. Available at: http://www.uco.es/aphanius/includes/descargapub.php?id = 4 (In Spanish).

Ferreras-Romero, M., Marquez-Rodriguez, J., Fernandez-Delgado, C., 2016. Long-time effect of an invasive fish on the Odonata assemblage in a Mediterranean lake and early response after rotenone treatment. Odonatologica 45, 7–21.

Florian, N., Lopez-Luque, R., Ospina-Alvarez, N., Hufnagel, L., Green, A.J., 2016. Influence of a carp invasion on the zooplankton community in Laguna Medina, a Mediterranean shallow lake. Limnetica.

Fox, J., Weisberg, S., 2011. An {R} Companion to Applied Regression, Second Edition. Thousand Oaks CA, Sage. http://socserv.socsci.mcmaster.ca/jfox/Books/Companion.

Fox, A.D., Caizergues, A., Banik, M., Devos, K., Dvorak, M., Ellermaa, M., et al., 2016. Recent changes in the abundance of breeding Common Pochard *Aythya ferina* in Europe. Wild 66, 22–40.

Galewski, T., Collen, B., Mcrae, L., Loh, J., Grillas, P., Gauthier-Clerc, M., Devictor, V., 2011. Long-term trends in the abundance of Mediterranean wetland vertebrates: from global recovery to localized declines. Biol. Conserv. 144, 1392–1399.

García-Berthou, E., 2001. Size-and depth-dependent variation in habitat and diet of the common carp (*Cyprinus carpio*). Aquat. Sci. 63, 466–476.

Gherardi, F., 2007. Understanding the impact of invasive crayfish. In: Biological Invaders in Inland Waters: Profiles, Distribution, and Threats. Springer, Netherlands, pp. 507–542.

Giles, N., Street, M., Wright, R.M., 1990. Diet composition and prey preference of tench, *Tinca tinca* (L.), common bream, *Abramis brama* (L.), perch, Perca fluviatilis L. and roach, *Rutilus rutilus* (L.) in two contrasting gravel pit lakes: potential trophic overlap with waterfowl. J. Fish Biol. 37, 945–957.

Goolish, E.M., Adelman, I.R., 1984. Effects of ration size and temperature on the growth of juvenile common carp (*Cyprinus carpio* L.). Aquaculture 36 (1–2), 27–35.

Green, A.J., Elmberg, J., 2014. Ecosystem services provided by waterbirds. Biol. Rev. 89, 105–122.

Green, A.J., Hughes, B., 1996. Action plan for the white-headed duck *Oxyura leucocephala* in Europe. In: Heredia, B., Rose, L., Painter, M. (Eds.), Globally Threatened Birds in Europe. Action Plans. Council of Europe Publishing, Strasbourg, pp. 119–145.

Green, A.J., Fox, A.D., Hilton, G.M., Hughes, B., Yarar, M., Salathé, T., 1996. Threats to Burdur Lake ecosystem, Turkey and its waterbirds, particularly the White-headed Duck *Oxyura leucocephala*. Biol. Conserv. 76, 241–252.

Green, A.J., El Hamzaoui, M., El Agbani, M.A., Franchimont, J., 2002. The conservation status of Moroccan wetlands with particular reference to waterbirds and to changes since 1978. Biol. Conserv. 104, 71–82.

Guareschi, S., Abellan, P., Laini, A., Green, A.J., Sanchez-Zapata, J.A., Velasco, J., Millan, A., 2015. Cross-taxon congruence in wetlands: assessing the value of waterbirds as surrogates of macroinvertebrate biodiversity in Mediterranean Ramsar sites. Ecol. Indic. 49, 204–215.

Guillemain, M., Fritz, H., Guillon, N., 2000. Foraging behavior and habitat choice of wintering Northern Shoveler in a major wintering quarter in France. Waterbirds 23, 353–363.

Haas, K., Kohler, U., Diehl, S., Kohler, P., Dietrich, S., Holler, S., Jaensch, A., Niedermaier, M., Vilsmeier, J., 2007. Influence of fish on habitat choice of water birds: a whole system experiment. Ecology 88, 2915–2925.

Ilarri, M.I., Souza, A.T., Antunes, C., Guilhermino, L., Sousa, R., 2014. Influence of the invasive Asian clam *Corbicula fluminea* (Bivalvia: Corbiculidae) on estuarine epibenthic assemblages. Estuar. Coast. Mar. Sci. 143, 2e19.

Junta de Andalucía, 2008. Proyecto de erradicación de la carpa (*Cyprinus carpio*) en la reserva natural de Laguna de Medina. Memoria Final. Enero 2008. Consejería de Medio Ambiente. Junta de Andalucía.

Kear, J. (Ed.), 2005. Ducks, Geese and Swans. Volume 2. Oxford University Press, Oxford.

Kloskowski, J., 2011. Differential effects of age-structured common carp (*Cyprinus carpio*) stocks on pond invertebrate communities: implications for recreational and wildlife use of farm ponds. Aquac. Int. 19, 1151–1164.

Kloskowski, J., 2012. Fish stocking creates an ecological trap for an avian predator via effects on prey availability. Oikos 121, 1567–1576.

Laguna, C., López-Perea, J.J., Viñuela, J., Florín, M., Feliu, J., Chicote, Á., et al., 2016. Effects of invasive fish and quality of water and sediment on macrophytes biomass, and their consequences for the waterbird community of a Mediterranean floodplain. Sci. Total Environ. 551, 513–521.

Lammens, E.H.R.R., Hoogenboezem, W., 1991. Diets and feeding behaviour. In: Cyprinid Fishes. Springer, Netherlands, pp. 353–376.

Lowe, S., Browne, M., Boudjelas, S., 2001. 100 of the world's worst invasive alien species. In: A Selection from the Global Invasive Species Database. Auck-land, IUCN-ISSG.

Maceda-Veiga, A., De Sostoa, A., Sánchez-Espada, S., 2013. Factors affecting the establishment of the invasive crayfish *Procambarus clarkii* (Crustacea, Decapoda) in the Mediterranean rivers of the northeastern Iberian Peninsula. Hydrobiologia 703, 33–45.

Maceda-Veiga, A., Basas, H., Lanzaco, G., Sala, M., de Sostoa, A., Serra, A., 2016. Impacts of the invader giant reed (*Arundo donax*) on riparian habitats and ground arthropod communities. Biol. Invasions 18, 731–749.

Maceda-Veiga, A., Baselga, A., Sousa, R., Vilà, M., Doadrio, I., Sostoa de, A., 2017. Fine-scale determinants of conservation value of river reaches in a hotspot of native and non-native species diversity. Sci. Total Environ. 574, 455–466.

Mather, J.R., 1987. Vegetation and climate vegetation and climate. In: Climatology. Springer, US, pp. 902–910.

Mathers, K.L., Chadd, R.P., Dunbar, M.J., Extence, C.A., Reeds, J., Rice, S.P., Wood, P.J., 2016. The long-term effects of invasive signal crayfish (*Pacifastacus leniusculus*) on instream macroinvertebrate communities. Sci. Total Environ. 556, 207–218.

Matsubara, T., Sugimori, F., Iwabuchi, K., Aoyama, K., 1994. The relation between the feeding activity of wintering Shovelers (*Anas clypeata*) and the horizontal distribution of zooplankton in Lake Teganuma, Japan. Hydrobiologia 294 (3), 253–261.

McKnight, S.K., 1998. Effects of food abundance and environmental parameters on foraging behavior of gadwalls and American coots in winter. Can. J. Zool. 76, 1993–1998.

Meerhoff, M., Clemente, J.M., De Mello, F.T., Iglesias, C., Pedersen, A.R., et al., 2007. Can warm climate related structure of littoral predator assemblies weaken the clear water state in shallow lakes? Glob. Chang. Biol. 13, 1888–1897.

Monroy, M., Maceda-Veiga, A., Caiola, N., De Sostoa, A., 2014. Trophic interactions between native and introduced fish species in a littoral fish community. J. Fish Biol. 85, 1693–1706.

Moss, B., Hering, D., Green, A.J., et al., 2009. Climate change and the future of freshwater biodiversity in Europe: a primer for policy-makers. Fr. Rev. 2, 103–130.

Muñoz-Fuentes, V., Green, A.J., Negro, J.J., 2013. Genetic studies facilitated management decisions on the invasion of the ruddy duck in Europe. Biol. Invasions 15, 723–728.

Musil, P., 2006. A review of the effects of intensive fish production on waterbird breeding populations. In: Boere, G.C., Galbraith, C.A., Stroud, D.A. (Eds.), Waterbirds Around the World. The Stationery Office, Edinburgh, UK, pp. 520–521.

Parker, I.M., Simberloff, D., Lonsdale, W.M., Goodell, K., Wonham, M., Kareiva, P.M., et al., 1999. Impact: toward a framework for understanding the ecological effects of invaders. Biol. Invasions 1, 3–19.

Parkos, J.J., Santucci Jr., V.J., Wahl, D.H., 2003. Effects of adult common carp (*Cyprinus carpio*) on multiple trophic levels in shallow mesocosms. Can. J. Fish. Aquat. Sci. 60, 182–192.

Perennou, C., Beltrame, C.A.G., Tomas Vives, P., Caessteker, P., 2012. Existing areas and past changes of wetland extent in the Mediterranean region: an overview. Econ. Med. Anim. 38, 53–66.

Prigioni, C., Balestrieri, A., Remonti, L., Gargaro, A., Priore, G., 2006. Diet of the Eurasian otter (*Lutra lutra*) in relation to freshwater habitats and alien fish species in southern Italy. Ethol. Ecol. Evol. 18, 307–320.

R Core Team, 2015. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org.

Rayner, T.S., Creese, R.G., 2006. A review of rotenone use for the control of non-indigenous fish in Australian fresh waters, and an attempted eradication of the noxious fish, *Phalloceros caudimaculatus*. N. Z. J. Mar. Freshw. Res. 40, 477–486.

Rendon, M.A., Green, A.J., Aquilera, E., Almaraz, P., 2008. Status, distribution and long-term changes in the waterbird community wintering in Donana, South-West Spain. Biol. Conserv. 141, 1371–1388.

Ribeiro, F., Rylková, K., Moreno-Valcárcel, R., Carrapato, C., Kalous, L., 2015. Prussian carp *Carassius gibelio*: a silent invader arriving to the Iberian Peninsula. Aquat. Ecol. 49, 99–104.

Rodríguez-Pérez, H., Green, A.J., 2012. Strong seasonal effects of waterbirds on benthic communities in shallow lakes. Fresh. Sci. 31, 1273–1288.

Rodríguez-Rodríguez, M., Green, A.J., López, R., Martos-Rosillo, S., 2012. Changes in water level, land use and hydrological budget in a semi-permanent playa lake, Southwest Spain. Environ. Monit. Assess. 184, 797–810.

Sánchez, M.I., Green, A.J., Dolz, J.C., 2000. The diets of the White-headed Duck *Oxyura leucocephala*, Ruddy Duck *O. jamaicensis* and their hybrids from Spain. Bird Study 47, 275–284.

Sebastián-González, E., Fuentes, C., Ferrández, M., Echevarrías, J.L., Green, A.J., 2013. Habitat selection of Marbled Teal and White-headed Duck during the breeding and wintering seasons in south-eastern Spain. Bird Conserv. Int. 23, 344–359.

Shin-ichiro, S.M., Usio, N., Takamura, N., Washitani, I., 2009. Contrasting impacts of invasive engineers on freshwater ecosystems: an experiment and meta-analysis. Oecologia 158, 673–686.

Sondergaard, M., Liboriussen, L., Pedersen, A.R., Jeppesen, E., 2008. Lake restoration by fish removal: short- and long-term effects in 36 Danish lakes. Ecosystems 11, 1291–1305.

Soons, M.B., Brochet, A.L., Kleyheeg, E., Green, A.J., 2016. Seed dispersal by dabbling ducks: an overlooked dispersal pathway for a broad spectrum of plant species. J. Ecol. 104, 443–455.

Sousa, R., Gutiérrez, J.L., Aldridge, D.C., 2009. Non-indigenous invasive bivalves as ecosystem engineers. Biol. Invasions 11, 2367–2385.

Strayer, D.L., Eviner, V.T., Jeschke, J.M., Pace, M.L., 2006. Understanding the long-term effects of species invasions. Trends Ecol. Evol. 21, 645–651.

Stuart, I.G., Williams, A., McKenzie, J., Holt, T., 2006. Managing a migratory pest species: a selective trap for common carp. N. Am. J. Fish Manag. 26, 888–893.

Tablado, Z., Tella, J.L., Sánchez-Zapata, J.A., Hiraldo, F., 2010. The paradox of the long-term positive effects of a north American crayfish on a European community of predators. Conserv. Biol. 24, 1230–1238.

Taggart, M.A., Green, A.J., Mateo, R., Svanberg, F., Hillström, L., Meharg, A.A., 2009. Metal levels in the bones and livers of globally threatened marbled teal and white-headed duck from El Hondo, Spain. Ecotoxicol. Environ. Saf. 72, 1–9.

Tomankova, I., Boland, H., Reid, N., Fox, A.D., 2013. Assessing the extent to which temporal changes in waterbird community composition are driven by either local, regional or global factors. Aquat. Conserv. 23, 343–355.

Torres Esquivias, J.A., 2009. La Malvasía cabeciblanca (*Oxyura leucocephala*) durante los primeros años del siglo XXI. In: Oxyura: Revista sobre las zonas húmedas. 12. pp. 87–116.

Torres-Esquivias, J.A., Arenas González, R.M., Fernández Delgado, C., 2009. La malvasía cabeciblanca (*Oxyura leucocephala*) de nuevo en la Laguna de Zóñar. In: Oxyura: Revista sobre las Zonas Húmedas. 12. pp. 41–48.

Vaananen, V.M., Nummi, P., Poysa, H., Rask, M., Nyberg, K., 2012. Fish-duck interactions in boreal lakes in Finland as reflected by abundance correlations. Hydrobiologia 697, 85–93.

Varo, N., Amat, J.A., 2008. Differences in foraging behaviour of sympatric coots with different conservation status. Wildl. Res. 35, 612–616.

Varo, N., Green, A.J., Sanchez, M.I., Ramo, C., Gomez, J., Amat, J.A., 2011. Behavioural and population responses to changing availability of Artemia prey by moulting black-necked grebes, *Podiceps nigricollis*. Hydrobiologia 664, 163–171.

Venables, W.N., Ripley, B.D., 2002. Modern Applied Statistics with S, Fourth Edition. Springer, New York ISBN 0-387-95457-0.

de Vicente, I., López, R., Pozo, I., Green, A.J., 2012. Nutrient and sediment dynamics in a Mediterranean shallow lake in southwest Spain. Limnetica 31, 231–250.

Vilizzi, L., 2012. The common carp, *Cyprinus carpio*, in the Mediterranean region: origin, distribution, economic benefits, impacts and management. Fish. Manag. Ecol. 19, 93–110.

Vilizzi, L., Tarkan, A.S., Copp, G.H., 2015. Experimental evidence from causal criteria analysis for the effects of common carp *Cyprinus carpio* on freshwater ecosystems: a global perspective. Rev. Fish. Sci. Aquac. 23, 253–290.

Weber, M.J., Brown, M.L., 2009. Effects of common carp on aquatic ecosystems 80 years after “carp as a dominant”: ecological insights for fisheries management. Rev. Fish. Sci. 17, 524–537.

Weber, M.J., Brown, M.L., Wahl, D.H., Shoup, D.E., 2015. Metabolic theory explains latitudinal variation in common carp populations and predicts responses to climate change. Ecosphere 6 (4), 1–16.

Winfield, I.J., Winfield, D.K., 1994. Feeding ecology of the diving ducks pochard (*Aythya ferina*), tufted duck (*A. fuligula*), scaup (*A. marila*) and goldeneye (*Bucephala clangula*) overwintering on Lough Neagh, Northern Ireland. Freshw. Biol. 32, 467–477.

Wouters, J., Janson, S., Luskova, V., Olsen, G.H., 2012. Molecular identification of hybrids of the invasive gibel carp *Carassius auratus gibelio* and crucian carp *Carassius carassius* in Swedish waters. J. Fish Biol. 80, 2595–2604.

Zambrano, L., Hinojosa, D., 1999. Direct and indirect effects of carp (*Cyprinus carpio* L.) on macrophyte and benthic communities in experimental shallow ponds in central Mexico. In: Shallow Lakes'. 98. Springer, Netherlands, pp. 131–138.

Züur, A.F., Ieno, E.N., Elphick, C.S., 2010. A protocol for data exploration to avoid common statistical problems. Methods Ecol. Evol. 1 (1), 3–14.

Environmental stressors and the transmission of a globally invasive parasite in riverine fish: additive vs interactive effects

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Abstract

The increased rate of outbreaks of infectious diseases in ecosystems is among the most dramatic consequences of global change, particularly when outbreaks affect highly threatened, important taxa, such as freshwater fish. However, the links between disease–inducing epizootics and widespread environmental stressors, including nutrient pollution and salinization, in freshwater organisms are largely unexplored. Ours is the first study to examine additive and interactive effects of pervasive environmental stressors on a riverine host–parasite system along large–scale environmental gradients. We explored the host and environmental factors that singly, or in combination, are likely to influence the spread of a worldwide invasive parasite, the anchor worm *Lernaea cyprinacea*, in an extensive region of north–eastern Spain (99,700 km^2, 15 river catchments, $N = 530$ sites). Smaller fish species had higher probabilities of infection, while lower altitudes, river salinity and nutrient pollution were associated with higher probabilities of infection in 19 endemic and widely distributed fish species. We found no evidence that interactive effects among major riverine stressors affected the occurrence of *L. cyprinacea* in fish despite the current emphasis on the importance of accounting for interactions among stressors in biodiversity management; our results suggest that parasite management might be effective on a stressor–by–stressor basis. Given that nutrient pollution and salinization were two of the major factors contributing to increased occurrence of *L. cyprinacea*, the improvement of wastewater treatment and agricultural practices, and the restoration of riparian areas probably would reduce the occurrence of this widespread parasite among native fish.

Keywords: alien species; epizootics; eutrophication; generalized linear mixed models; hierarchical partitioning; host–parasite interactions; multiple stressors; salinization

Introduction

Infectious diseases are emerging at unprecedented rates and scales, raising concerns among conservationists, ecologists, and public health agencies (Jones *et al*., 2008; Smith *et al*., 2014). Although the causes of the emergence of many diseases are uncertain, human actions probably contribute substantially to many epidemics (Budria & Candolin, 2014; Rogalski *et al*., 2017). Human activities, such as intensive agriculture (Ponisio *et al*., 2016), fishing (Kaiser *et al*., 2015), and dam construction (Poff *et al*., 2007), are homogenizing aquatic ecosystems around the world, and disease outbreaks often arise when ecosystems become simplified (Elton, 1958). Ecosystems are altered further by the accumulation of alien species, which continues unabated (Seebens *et al*., 2017). Given that the global human footprint is expected to increase even further (Hoekstra & Wiedmann, 2014), understanding how human impacts affect infection dynamics is a priority for disease ecology under global change.

Global change factors consist of a variety of biotic and abiotic alterations induced by human activities, which can interact in complex ways (see Piggott *et al*., 2015; Côté *et al*., 2016). Whereas 'native' host–parasite interactions have a long co–evolutionary history, this is not the case for novel pathogenic interactions, if say, native host vulnerability to alien parasites is higher than it is to native parasites (Dunn & Hatcher, 2015). Other global change processes, such as aquatic nutrient pollution (Sinha *et al*., 2017), can further increase host vulnerability if such processes increase the virulence of a disease (Vega Thurber *et al*., 2014). However, the impacts of pollution on host-parasite relationships are difficult to predict due to the dependence on context (Marcogliese & Pietrock, 2011; Budria *et al*., 2017). While high concentrations of pollutants, including nutrients, can kill both parasites and hosts (Vidal–Martínez *et al*., 2010; Smallbone *et al*., 2016), nutrients also may improve host body-condition and facilitate hosts to better cope with the effects of pollutants and parasites (Marcogliese & Pietrock, 2011; Budria *et al*., 2017). Nutrient pollution also is expected to promote the transmission of horizontally transmitted parasites (e.g. ectoparasites) because it increases host population density (McKenzie & Townsend, 2007). External parasites are often more sensitive to environmental fluctuations in aquatic systems than are internal parasites (Overstreet, 1993). Riverine ecosystems are among the most dynamic and human–affected ecosystems in the world (Williamson *et al.*, 2008), yet the ways in which multiple natural and anthropogenic factors affect interactions and epidemiology in host–parasite systems remain little studied (Blasco–Costa *et al*., 2013; Hofmann *et al*., 2016).

A globally important parasite is the Asiatic anchor worm *Lernaea cyprinacea* Linnaeus 1758, which is a parasitic copepod introduced widely into temperate and tropical regions with carp *Cyprinus carpio* Linnaeus, 1758 (Hoffman, 1999; Hassan *et al*., 2008; Welicky *et al*., 2017). The anchor worm is capable of infecting fish and amphibians (Hoffman, 1999; Kupferberg *et al*., 2009), and high mortalities have been reported for fish suffering from anchor worm infections (Noga, 2011; Raissy *et al*., 2013). The anchor worm is expected to spread further with global warming (Bulow *et al*., 1979; Sánchez–Hernández, 2017) and when fish are forced into high concentrations during drought (Medeiros & Maltchik, 1999; but see Welicky *et al*., 2017). Infections by parasitic copepods, including *L. cyprinacea*, are influenced by fish size (Poulin *et al*., 1991), water–quality stressors, such as nutrient enrichment (Palm, 2011) and salinity, which can kill the parasites (Noga, 2011). However, rivers experience other stressors, such as alterations in river morphology and riparian areas, which can reduce body condition in fish hosts (Maceda–Veiga *et al*., 2014), potentially increasing fishes' susceptibility to disease. On the other hand, interactions among stressors may be advantageous to the fish if, say, salinization reduces nutrient toxicity (Sampaio *et al*., 2002; Noga, 2011). Therefore, the effects of combinations of environmental stressors may not be predictable from the established individual effects of stressors; i.e., the effects of stressors may not be additive (Williams et al., 2007; Hiers *et al*., 2016).

Determining the spatial location of disease outbreaks, the individuals that are most vulnerable, and the environmental conditions that promote infection, are central thinking in disease ecology (Stephens *et al*., 2016). A major limitation in understanding parasitic infections is that most surveys are limited spatially (Stephens *et al*., 2016), which hinders the identification of clear relationships between disease and large–scale environmental gradients. While the effects of multiple factors on parasite transmission are testable experimentally (e.g. Lenihan *et al*., 1999; Studer & Poulin, 2013), manipulations rarely can be conducted at the spatial and temporal scales at which biota actually experience the stressors (Mac Nally, 1997). Smaller scale experiments may not represent well patterns of spatial and temporal variation in stressors and hence the consequent biotic effects (Englund & Cooper, 2003). Experiments exploring the effects of multiple factors also require many animals as experimental units, which is ethically unacceptable for studies on vertebrates, especially threatened species, under animal welfare legislation (e.g. EU Directive 2010/63/UE5). Therefore, the use of extensive field surveys typically is used to identify the factors that, alone or in combination, are harmful to biota (e.g. Dahm *et al*., 2013; Gieswein *et al*., 2017; Radinger *et al*., 2017), including the causes of parasite transmission.

Here, we explored the host and environmental factors that singly, or in combination, might influence the spread of the alien parasite *L. cyprinacea* in 19 fish species in an extensive area of northeastern Spain (99,700 km^2, 15 catchments). These rivers are subject to major environmental stressors that are common in rivers worldwide (e.g. impeded water flows, riparian removal, nutrient pollution, and salinization) (Tockner *et al*., 2009; Moyle, 2014), and such rivers are highly prone to biological invasions (Leprieur *et al*., 2008), including by *L. cyprinacea* (Sánchez–Hernández, 2017). The wide range of orographic and river conditions in this Spanish region (Sabater *et al*., 2009; Maceda–Veiga *et al*., 2013; 2017a) allowed us to explore how combinations of different stressors might affect a host–parasite system along upstream–downstream gradients. Fish species include endemic taxa (e.g. *Barbus haasi* Mertens 1925, *Parachondrostoma miegii* Steindachner, 1866), widely distributed European native species (e.g. *Anguilla anguilla* Linnaeus, 1758, *Salmo trutta* Linnaeus, 1758), and worldwide invasive fish species (e.g. *Cyprinus carpio* Linnaeus, 1758, *Gambusia holbrooki* Girard, 1859) (Froese & Pauly, 2017). Alien fish often replace native fish in degraded rivers (Maceda–Veiga *et al*., 2017a), but the anchor worm has low host–specificity (Hoffman, 1999; Noga, 2011), and so, can exploit most fish hosts.

Anchor worm infections were expected to be more prevalent in lowland rivers because the copepod is a warm–water species, and infections may be more limited by the riverine environment than by host availability given the parasite’s lack of host specificity. We expected the probability of parasite occurrence to be the highest in degraded rivers because pollution often induces immunosuppression in fish and promotes disease (but see Biagianti–Risbourg *et al*., 2013; Smallbone *et al*., 2016). We explored the occurrence of interactions among some of the major natural and anthropogenic stressors that may govern parasite dynamics. Given that such stressor combinations are present around the world, the identification of these interactions will help to project the distribution of the parasite beyond our study area. Moreover, our work will inform whether the management of river stressors to control diseases can be on a stressor–by–stressor basis or needs to consider their interactive effects on the parasite.

Materials and methods

Study area

We gathered data on river environmental conditions and fish from surveys performed in northeastern Spain from 2002 to 2009 (e.g. Figuerola *et al*., 2012; Maceda–Veiga *et al*., 2017a,b). This data set consisted of 530 sampled sites that involved all Catalonian catchments from the Muga to Riudecanyes basins, and the whole River Ebro and part of the Garonne basin (Fig. 1). We surveyed most river typologies in this region for hydrological alterations, riparian characteristics, geology, water quality, river size and elevation. Most of these rivers are small and have a typical Mediterranean hydrological regime, with droughts in summer and potentially torrential floods in autumn. Large rivers peak in flow in spring from snowmelt. We surveyed in low–flow conditions when fish populations are more stable and can be sampled most effectively using electrofishing (see below). Water temperatures ranged from 9–31ºC, depending on elevation, and both native and alien fish species were present in a wide range of geographic, hydro-morphological, and water conditions (see Maceda-Veiga *et al*., 2017 for further details). The conservation status of riparian areas ranges from relatively well–preserved riparian forests to grasslands, extensive agriculture areas, and weedy areas (Sabater *et al*., 2009), including stands of the globally invasive *Arundo donax* (Maceda–Veiga *et al*., 2016).

Fish surveys

We used an international standardized fish sampling method (CEN standards EN 14962 and EN 14011), in accordance with the European Water Framework Directive (EU Directive 2000/60/EC). Fish were sampled by single–pass electrofishing using a portable unit that generated up to 200V and 3 A pulsed DC in an upstream direction, covering the whole wetted width of the 100–m long reaches at each location (e.g. Maceda–Veiga *et al*., 2017a). We selected the location of each sampling site based on representativeness, and included a variety of habitat types (pools, rifles and runs). The same fishing equipment was used at all sites to avoid potential bias in fish captures. Fish captures were expressed as captures per unit of effort (CPUE, fish caught divided by fishing time in minutes and the area surveyed in m^2). A pilot study in our study area, comparing estimates of richness and abundance between single– and four–pass electrofishing, showed that estimates from single pass were reasonably high with 80–

100 % of the species detected and 60–90 % of the individuals captured (A. Sostoa, *unpublished data*).

Fish were identified to species, counted, and a random set of individuals (≥40 if possible) from each species in each sampling site was measured (total length, mm) and weighed (mg) after being anaesthetized in a buffered MS222® solution (0.02%, Tricaine methane–sulfonate, Sigma®). The entire external surface of measured fish was inspected visually for infections of *L. cyprinacea*. This copepod is easy to detect visually (see Noga, 2011), which facilitates logistics in extensive fish surveys. All fish were allowed to recover in buckets provided with air pumps and released at the site of capture. The procedures used in this study were approved by the University of Barcelona Ethical Committee (C.E.E.A. 510/14) and fish captures were authorized by the Autonomous Government of Catalonia (AP/003).

Taxonomic status of four fish taxa (*Barbatula* spp., *Phoxinus* spp., *Gobio* spp., and *Carassius* spp.) was uncertain due to the description of new species after we completed our surveys and the presence of morphologically cryptic species of the genus *Carassius*. A straightforward change in the nomenclature of the former is complicated because these taxa are subject to inter–basin transfers by anglers (e.g. Doadrio *et al*., 2011). The native status of fish was defined as follows (Table 1): (a) native, if the fish historically occurred in the basin where captured according to regional faunal lists (Doadrio *et al*., 2011), and (b) alien, if the fish species did not historically occur in the basin of capture or elsewhere in the Iberian Peninsula (IP). Each fish species was assigned to a trophic guild (mostly ‘omnivorous’ or ‘invertivorous’), a swimming guild (‘benthic’ or ‘water column’) and a migratory-behaviour guild (‘almost sedentary’ or ‘migratory’) based on regional fish atlases (de Sostoa *et al*., 1990; Doadrio *et al*., 2011), scientific articles (e.g. Benejam *et al*., 2010; Colin *et al*., 2016) and our experience because the ecology of some of these species is still poorly reported in the literature. In our study area, only the European eel (*A. anguilla*) migrates between marine and freshwater habitats, while the other migratory species (e.g. *S. trutta*, *P. miegii*, *Luciobarbus graellsii* Steindachner, 1866) typically perform within-river upstream migrations for spawning (Doadrio *et al*., 2011).

Environmental stressors

We used 12 variables related to geography, water quality and habitat quality to explore which river environmental conditions might be associated with the spread of *L.*

cyprinaea (Table S2). We recorded the basin name and elevation (m.a.s.l.) using Google Earth®. Elevation was used as a surrogate for the position of the sampling site in the river, and to represent natural spatial factors affecting aquatic organisms (e.g. Maceda–Veiga *et al*., 2017a). We calculated the Strahler stream order number (Strahler, 1964) using a map (1:50000) as a measure of river size. Rivers were ranked from small, first–order tributaries to the largest main river based on a hierarchy of tributaries.

Prior to conducting each fish survey, we measured seven water–quality variables *in situ*. A digital multiparametric YSI® probe was used for temperature (ºC), conductivity (µS/cm) and pH, and the colorimetric test kit VISOCOLOR® for ammonium (mg/l), nitrite (mg/l), nitrate (mg/l) and phosphate–P (mg/l) concentrations. These water variables measure nutrient pollution, acidity, and changes in the overall ionic composition of water (i.e. salinity), which are major stressors affecting freshwater organisms directly (e.g. toxic effects) and indirectly (e.g. alterations in disease dynamics, food availability, and biogeochemical cycles) (e.g. Johnson *et al*., 2010; Noga, 2011; Cañedo–Argüelles *et al*., 2016). Many toxicants in sewage from industries and urban and agricultural areas (e.g. heavy metals, pesticides, and drugs) alter nutrients, pH and conductivity in rivers (e.g. textile industry in Colin *et al*., 2016), so that these variables are used as general indicators of chronic water pollution. We estimated silting as the % of the streambed covered by sediment < 1 mm (Townsend *et al*., 2008). Silting is associated with high levels of eutrophication, agricultural runoff, and the release of detritus from sewage treatment plant outflows.

River hydro–morphology and the quality of riparian areas were characterized with the index QBR (Munné *et al*., 2003), which is an integrated measure widely used by water agencies in Spain. These factors can affect host–parasite relationships either directly by altering ecosystem productivity (Acuña & Tockner, 2010), or indirectly by altering a river's ability to degrade pollutants (Gurr & Reinhard, 2006). QBR ranks the total riparian cover (the presence of riparian areas and their connectivity among them and with adjacent woodlands), cover structure (percentage of riparian trees, shrubs and emergent aquatic plants), cover quality (presence of alien riparian species), and river channel naturalness (e.g. presence of weirs, channels, and walls) on a scale from 0 to 100. Last, we calculated the mean current speed (m/s) in each sampling site from three values measured along transects set perpendicular to the water flow at 20–m intervals.

Statistical analyses

Analyses were conducted in R (R Core Team, 2016) using the packages and functions outlined below. We constructed binomial generalized linear mixed models using *L. cyprinacea* presence (0 or 1) on each individual fish as the response variable to determine which environmental and host predictors are important for this infection. There were random factors (collectively referred to as ρ.) for year, species, basin, and site, which accounted for multiple measurements for a unit (e.g. multiple conspecifics for the same site) and potential systematic differences among components of these factors. All predictors (Table S1) were standardized (0 mean, unit variance) so that all predictors were on comparable scales (i.e. each predictor is expressed in units of its standard deviation) after continuous variables were log-transformed to reduce the influence of extreme values. Predictors were collectively referred to as *host* (fish host variables), *geo* (geography), *wv* (water velocity), *wq* (water quality), and *phq* (physical habitat quality) for brevity (Table S1). However, all predictors within each set of predictors were included in the models as individual variables. Temperature was excluded because of its high correlation with elevation (Spearman's $\rho > 0.70$). Nutrient concentrations were aggregated based on their toxic severity (Camargo & Alonso, 2006; Noga, 2011), such as ammonium and nitrite (TN) and nitrate and phosphate–P (NP).

Following Grueber *et al*. (2011), we built a 'global' model using the function *glmer* in the *lme4* package (Bates *et al*., 2015). This model included all nine environmental predictors, five host predictors, and the random factors for year, species, basin and sampling site. We then constructed nested models (i.e. models with and without a given predictor) to explore whether parasite transmission was strongly associated with fish host abundance, fish body size, fish trophic guild, fish migratory behaviour and fish swimming behaviour (Table S2). Host abundance in a given site was the total combined abundance of all fish species recorded as hosts for *L. cyprinacea* in our region (e.g. Sánchez–Hernández, 2017; Maceda–Veiga *et al*., 2017). We used individual length as a measure of fish body size but outcomes were similar using fish body mass (results not shown).

Using the *best* model (see below for a description of how this was determined) with selected host predictors as a baseline, we built nested models to explore the potential additive effects of all four sets of environmental predictors (*geo*, *wv*, *wq*, and *phq*) to the probability (Pr) of *L. cyprinacea* infection (Table S3). We then used the resultant *best* model to test for additive effects (+) of *geo*, *wv*, *wq*, and *phq* compared with five models including interactions (*) among these four sets of stressors. These models explored whether: (1) the effects of poor habitat and water quality interact with elevation ($\mathrm{Pr} = \alpha + host + geo + \mathrm{elevation} * wv + \mathrm{elevation} * wq + \mathrm{elevation} * phq + \rho$).; (2) changes in water velocity interact with water or habitat quality degradation ($\mathrm{Pr} = \alpha + host + geo + wv * wq + wv * phq + \rho$).; (3) water and physical habitat quality interact together ($\mathrm{Pr} = \alpha + host + geo + wv + wq * phq + \rho$).; (4) salinization interacts with nutrient toxicity ($\mathrm{Pr} = \alpha + host + geo + wv + \mathrm{salinity} * \mathrm{TN} + \mathrm{salinity} * \mathrm{NP} + \mathrm{pH} + phq + \rho$).; and (5) pH interacts with nutrient pollution ($\mathrm{Pr} = \alpha + host + geo + wv + \mathrm{pH} + \mathrm{pH} * \mathrm{TN} + \mathrm{pH} * \mathrm{NP} + phq + \rho$). Although the number of stressor combinations is large, we only sought to compare additive and interactive effects among some of most prevalent stressors, which is a crucial first step in multiple-stressors studies (Piggott *et al*., 2015).

Best models were identified using the Akaike Information Criterion (AIC) and the Bayesian Information Criterion (BIC), which penalize model complexity to different degrees and hence are recommended for use in combination (see Fabozzi *et al*., 2014 for further details). Top–ranked models (ΔAIC or ΔBIC ≤ 2.0) were considered the *best* descriptors of the data from the candidate set and the likelihood of non-additive effects was determined by whether the *best* model included just additive or additive *and* interactive terms. For example, if interactive models had greater values of AIC or BIC than the corresponding additive models, then the stressors most probably are operating independently of each other and the likelihood of them having interactive effects on the probability of *L. cyprinacea* occurrence would be low. That is, the added model complexity of interactive models does not provide sufficient improvement in model fit to justify the inference that interactions are important. The absolute adequacy of the *best* models for predicting the presence of *L. cyprinacea* was assessed using the function *auc* (Area Under Curve, designated as 'AUC' in Tables) in the *pROC* package (Robin *et al*., 2011). The *model.avg* function (Burnham & Anderson, 2002) was then used to calculate average parameter estimates from the final *best* models, and indicated the importance of each set of predictors (host attributes, *geo, wv, wq,* or *phq*) on a scale from 0 to 1.

The relative importance of the host and environmental descriptors selected by the AIC-BIC approach in explaining variation in the occurrence of *L. cyprinacea* was compared by using hierarchical partitioning analysis of deviance (HP; function *hier.part* Walsh & Mac Nally, 2002). The function *rand.hp* was used to assess the significance of HP models using a randomization test for hierarchical partitioning analysis based on the upper 0.95 confidence limit. While causality cannot be determined in correlational studies, HP partitions the independent contributions of each predictor to variation in the response variable, and distinguishes these from the joint contribution with other predictors. We note that predictors are always correlated, even when the most highly correlated are excluded (Spearman's $\rho > 0.70$), which can bias regression estimates of GLMMs (see details in Mac Nally, 2002). The strength of GLMMs is that they can handle random effects, which is not possible in HP analysis, so we used averaged fish size per sampling site to avoid pseudo-replication. The importance of the environmental descriptors selected by the AIC-BIC approach was further assessed by comparing data between sites with and without infected fish using Mann-Whitney tests with $\alpha = 0.05$.

Results

A total of 30 932 fish individuals from three orders and seven families was captured in the 15 Mediterranean catchments. Infection was seen in all fish species, apart from the alien *G. holbrooki* and the natives *Cobitis calderoni* Bacescu, 1962 and *Achondrostoma arcasii* Steindachner, 1966 (Table 1). The highest prevalence of infection was in five native cyprinids (*B. haasi*, *Gobio* spp., *L. graellsii*, *P. miegii*, and *Squalius laietanus* Doadrio, Kottelat & de Sostoa, 2007) and in two alien species, the cyprinid *C. carpio* and the centrarchid *Lepomis gibbosus* Linnaeus, 1758 (Table 1).

Among host predictors, generalized linear mixed models (GLMM) revealed that fish length was more influential than total fish abundance or fish species' ecology (trophic guild, swimming and migratory behaviour) in accounting for the presence of *L. cyprinacea*, after having controlled for potential systematic differences among years, species, basins, and sites (see B5 in Table 2). This was an adequate model based on auc, and indicated that smaller fish were more vulnerable to *L. cyprinacea* infection than larger ones (Table 3). However, model averaging showed that water properties were as influential in accounting for infection as fish length, followed by geographical features, physical habitat quality, and water speed (see predictor importance in Table 3). The regression estimates suggested that salinity and nutrient pollution (i.e. nitrate and phosphate–P) made the largest contribution to variation in the probability of *L. cyprinacea* occurrence, with an overall positive effect (Table 3; Fig. 2). The relationship between elevation and the probability of *L. cyprinacea* occurrence elevation was negative (Table 3; Fig. 2).

The hierarchical partitioning analysis of deviance examined the independent contribution (in % of explained deviance) of the predictors identified by the GLMM analyses (fish size, salinity, nutrient pollution and pH) to variation in the occurrence of *L. cyprinacea*. The occurrence of *L. cyprinacea* was significantly associated with fish length (53.3% of independently explained deviance), although the collective effect of the set of environmental predictors was also significant and explained almost as much independent variation (46.7%). Of the latter, variation in *L. cyprinacea* occurrence was about twice as strongly related to salinity (17.3%) than to the other three environmental predictors (elevation 9.2%, pH 9.3% and nutrients 11.0%), but all four environmental predictors were significantly related to the occurrence of *L. cyprinacea* (assessed by the HP randomization test). There were significant differences in elevation (Mann Whitney U $W = 3523$; $P < 0.05$), salinity ($W = 1877$; $P < 0.05$), nutrient pollution ($W = 1922$; $P < 0.05$) but not in pH ($W = 2299$; $P > 0.05$) between sites with infected and non-infected fish. Univariate tests did not include the effects of variation in other predictors of the multivariate models (i.e. generalized linear mixed models and hierarchical partitioning) and large differences detected in the GLMMs or HP may be visually subtle on a stressor-by-stressor basis (Fig. 2).

GLMM showed that the base additive model, including geographical, hydro–morphological features, water properties and fish length, was more parsimonious (i.e. had the lowest AIC and BIC values) than the five interactive models (see AM in Table 4), so that including interactive effects among environmental stressors in the base additive model did not markedly improve its fit.

Discussion

Our study is the first to examine additive and interactive effects of widespread water and physical habitat stressors on a riverine host–parasite system. The presence of a widespread alien parasite, the anchor worm *L. cyprinacea*, was explained mostly by fish–host size, additive combinations of altitude, water degradation and, to a minor degree, hydro–morphological alterations. Smaller fish and fish in lowlands experienced a higher rate of infection. Contrary to our expectations, the presence of the parasite was positively related to salinity, which is a widespread pollutant (Cañedo–Argüelles *et al*., 2016) with anti–parasitic properties (Noga, 2011). We found that nutrient concentrations in rivers passing through extensive agricultural areas, such as in north–eastern Spain, promoted infection rates of *L. cyprinacea*. Given that freshwater fish are in marked decline around the world (Closs *et al.*, 2016), and that this parasite is a host generalist (Hoffman, 1999), our results can contribute information to management actions to reduce the spread of *L. cyprinacea* among riverine fish.

Whereas interactions among stressors are at the core of many discussions of the underlying reasons for the current biodiversity crisis (Paine *et al.,* 1998; Piggott *et al.,* 2015), little is known for freshwater fish (Jackson *et al.,* 2016; Schinegger *et al.,* 2016). We found no evidence that interactive effects among major riverine stressors were related to the occurrence of *L. cyprinacea* in fish. These results support the notion that the risk of interactive effects may have been overstated (Côté *et al*., 2016; Maceda–Veiga *et al*., 2017), and suggest that the individual management of stressors probably is the most appropriate course of action to prevent the expansion of this highly invasive parasite. Although the global decline of freshwater fish is due to many factors (Closs *et al.,* 2016), there is growing concern about the role of infectious diseases (e.g. Gozlan *et al*., 2005). We detected the highest prevalence of *L. cyprinacea* in native fish species (e.g. *P. miegii*, *S. laietanus*) that have experienced significant declines in northeastern Spain (Maceda–Veiga *et al.,* 2010). However, the lowest prevalence was in widespread, alien fish species, such as *G. holbrooki* and *A. alburnus* (Froese & Pauly, 2017), excepting *C. carpio*, which was the probable source of *L. cyprinacea* (Hoffman, 1999; Hassan *et al*., 2008).

After controlling for the inter–specific variability in the presence of *L. cyprinacea*, we found that smaller fish had a higher probability of infection. This outcome differs from the work of Poulin *et al*. (1991), who found, in controlled exposure trials in clean laboratory water, that infection rates by another species of parasitic copepod increased with fish size. The contrasting patterns could be due to pollution–induced immunosuppression in our systems, which may affect juveniles more than adult fish (see Biagianti–Risbourg *et al*., 2013). Differences in the microhabitat used by each life stage also may explain the results; juveniles generally occupy slow–flowing waters (Ribeiro *et al*., 2013), which facilitate the spread of *L. cyprinacea* (Medeiros & Maltchik, 1999). High spatial concentrations of fish were expected to promote the transmission of *L. cyprinacea* (Hoffman, 1999; Noga, 2011), but we did not find evidence for such an effect. Given that there were large differences in prevalence among fish species (Table 1), our host–parasite relationships may be obscured by differences in the taxonomic composition of fish communities depending on the riverine environment (e.g. Maceda–Veiga *et al*., 2017). Ecological traits of the fish hosts (e.g.

migratory behaviour, trophic ecology) also seemed to have played a limited role in explaining the prevalence of *L. cyprinacea*, possibly because we lack detailed information on the ecology of these species, such as differences in diet and habitat use (e.g. home-range), a major determinant of infection rate (Knudsen *et al*., 1996; MacColl, 2009). We found that the occurrence of *L. cyprinacea* was almost equally related to environmental conditions than to fish–host factors. Other potential parasite reservoirs, such as frogs, for which we have no data, may have affected the prevalence of *L. cyprinacea* (see Kupferberg *et al*., 2009).

Our results suggest that the spread of *L. cyprinacea* might be limited by high altitude and hence low water temperatures (Hoffman, 1999; Noga, 2011). However, even species that prefer cold water, such as the strictly freshwater populations of *S. trutta* in the Mediterranean (Doadrio *et al*., 2011), were infected, raising concerns about the spread of *L. cyprinacea* through entire vulnerable faunas from climate warming (Sánchez–Hernández, 2017), although our results suggest that improving water chemistry could mitigate that effect. Our findings indicated that a decrease in nitrate and phosphate concentrations probably would reduce the spread of *L. cyprinacea*, a conclusion that differed from those of Palm (2011), who suggested that nutrients negatively affected crustacean parasites. Sewage discharges from urban areas and intensive farming are the major reason for cultural eutrophication in many aquatic ecosystems (Smith & Schindler, 2009; Rissman & Carpenter, 2015). Given that human pressures on riverine ecosystems continue to grow (Vörösmarty *et al*., 2010; Sinha *et al*., 2017), it is crucial to make these by–products of human activity less environmentally damaging by, say, optimizing agricultural fertilizer application (Basso *et al*., 2016) and by regenerating riparian vegetation that acts as 'green filters' (Naiman & Decamps, 1997).

Improvement in sewage treatment and nutrient retention in riparian areas and reductions in water abstraction would reduce river salinity (e.g. Basin Salinity Management Plant, 2015; Tal, 2017), for which we found a positive association on the presence of *L. cyprinacea*, although aquacultural practices posit that salinity kills freshwater ectoparasites (Noga, 2011). We used conductivity as a proxy for salinity, which is widely done (Cañedo–Argüelles *et al*., 2016), but conductivity is altered by common ions of clean sea water (e.g. chlorides, sodium) and by industrial discharges (e.g. metal pollution, Colin *et al*., 2016). Therefore, conductivity might have been a general indicator of many pollutants in our study rather than a measure of salinity *per* *se*. Our results support the notion that the effects of pollution on host–parasite relationships are context–dependent (Marcogliese & Pietrock, 2011). The probability of *L. cyprinacea* occurrence increased with nitrate and phosphate concentrations, although no infected fish were found in waters with concentrations of between 15–25 mg/l. Although the direct toxicity of nutrients to *L. cyprinacea* remains to be determined, there may be a lethal effect, which was shown experimentally for the impact of nitrate on the guppy–*Gyrodactylus* fish–parasite relationship (Smallbone *et al*., 2016). pH was an important contributing factor to explaining variation in the probability of *L. cyprinacea* occurrence in our models, but it was not significant in Mann-Whitney U tests. Univariate tests do not account for the relative effects of covariates, i.e. pH effects on biota are likely to have been conditioned by the additive effects of nutrients such as ammonia (Emerson *et al*., 1975). However, simple bivariate plots (see Fig. 2) may be more valuable for managers to take action on particular stressors than when the differences are shown as linear combinations of predictors in models.

Hydro–morphological alterations and riparian zone characteristics appeared to be of little importance in explaining the variation in the probability of *L. cyprinacea* occurrence, notwithstanding that such alterations are known stressors for riverine biota (Poff *et al*., 2007; Maceda–Veiga *et al*., 2017). The absence of a signal might be due to rivers being very dynamic and that snapshot samples of biota and abiotic conditions may not necessarily reveal strong relationships (Heino *et al*., 2015; Maceda–Veiga *et al*., 2017). However, water velocity is likely to vary as much as nutrient concentrations and salinity, and these two factors had discernible effects on the occurrence of *L. cyprinacea*. A plausible explanation for the lack of association with river hydro–morphology is that slow water velocity is only critical during the attachment stage of the parasite, and we found mostly mature *L. cyprinacea* individuals with egg clutches (A. M.V., personal observation). Therefore, although infection was not clearly related to water velocity in our study, there may be negative relationships at an early stage of the infection, for which we have no data. Similarly, riparian forests are composed of native tree species with large differences in the decomposition rates of leaves, which influences food availability (Naiman & Decamps, 1997) and hence fish growth and body condition. Given that the index of riparian quality used for the current study (i.e. QBR) did not discriminate well among native tree species, it is likely that detailed data on the taxonomic composition of riparian areas might have provided better discrimination between riparian quality and the probability of *L. cyprinacea* infection.

Despite the control of parasitic infections being highly complex in natural waters, our study provides guidance at scales relevant to resource managers. Nitrate, phosphate and high conductivity are still major widespread stressors to European rivers up to 10 years after the enforcement of the EU's Water Framework Directive (EC, 2000). These results suggest that although more than 70% of the human population in northern and central Europe is connected to a wastewater treatment plant that implements tertiary treatment for removal of nutrients, this is an urgent need for such treatment in southern European countries, where this percentage is just 50% in Spain and as little as 13% in Malta (Environmental Protection Agency, 2013). Although these countries are relatively small territories, the figures are very important at the EU scale considering the Mediterranean area, including Spain, is a global hotspot of biodiversity (Myers *et al*., 2000). As with all correlative study, many factors can affect our results, including secondary infections (e.g. bacteria, viruses, fungi) promoted by *L. cyprinacea* that kill the fish host rapidly due to their short life cycles (Noga, 2011) and so, may have underestimated our environment–parasite relationships. Improving water quality has long been advocated for its multiple benefits in biodiversity conservation, including the advantages rivers provided to society (Hering *et al*., 2015; Liu *et al*., 2017; Zhou *et al*., 2017). Our study shows that another benefit would be the control of the spread of a widespread alien parasite among native fish.

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References

Acuña, V., & Tockner, K. (2010). The effects of alterations in temperature and flow regime on organic carbon dynamics in Mediterranean river networks. *Global Change Biology*, 16, 2638–2650.

Basso, B., Dumont, B., Cammarano, D., Pezzuolo, A., Marinello, F., & Sartori, L. (2016). Environmental and economic benefits of variable rate nitrogen fertilization in a nitrate vulnerable zone. *Science of the Total Environment*, 545, 227-235.

Basin Salinity Management (2015). Basin Salinity Management 2030 BSM2030. Murray–Darling Basin Authority. GPO Box 1801, Canberra ACT 2601

Bates, D., Maechler, M., Bolker, B. & Walker, S. (2015). Fitting Linear Mixed–Effects Models Using lme4. *Journal of Statistical Software*, 67, 1–48.

Benejam, L., Angermeier, P. L., Munne, A., & García-Berthou, E. (2010). Assessing effects of water abstraction on fish assemblages in Mediterranean streams. *Freshwater Biology*, 55(3), 628-642.

Biagianti–Risbourg, S., Paris–Palacios, S., Mouneyrac, C., Amiard–Triquet, C. (2013). Pollution Acclimation, Adaptation, Resistance, and Tolerance in Ecotoxicology. In Encyclopedia of Aquatic Ecotoxicology (pp. 883–892). Springer Netherlands.

Blasco–Costa, I., Koehler, A. V., Martin, A., & Poulin, R. (2013). Upstream–downstream gradient in infection levels by fish parasites: a common river pattern?. *Parasitology*, 140, 266–274.

Budria, A., & Candolin, U. (2014). How does human–induced environmental change influence host–parasite interactions?. *Parasitology*, 141, 462–474.

Budria, A. (2017). Beyond troubled waters: the influence of eutrophication on host–parasite interactions. *Functional Ecology* (In press).

Bulow, F. J., Winningham, J. R., & Hooper, R. C. (1979). Occurrence of the copepod parasite *Lernaea cyprinacaea* in a stream fish population. *Transactions of the American Fisheries Society*, 108, 100–102.

Burnham, K. P., & Anderson, D. R. (2003). Model selection and multimodel inference: a practical information–theoretic approach. Springer Science & Business Media.

Camargo, J. A., & Alonso, Á. (2006). Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: a global assessment. *Environment International*, 32, 831–849.

Cañedo–Argüelles, M., Hawkins, C. P., Kefford, B. J., Schäfer, R. B., Dyack, B. J., Brucet, S. et al. (2016). Saving freshwater from salts. *Science*, 351, 914–916.

Closs, G. P., Krkosek, M. & Olden, J.D. (2016). Conservation of Freshwater Fish. Cambridge.

Colin, N., Maceda–Veiga, A., Flor–Arnau, N., Mora, J., Fortuño, P., Vieira, C. et al. (2016). Ecological impact and recovery of a Mediterranean river after receiving the effluent from a textile dyeing industry. *Ecotoxicology and Environmental Safety*, 132, 295–303.

Colin, N., Porte, C., Fernandes, D., Barata, C., Padrós, F., Carrassón, M. et al. (2016). Ecological relevance of biomarkers in monitoring studies of macro-invertebrates and fish in Mediterranean rivers. *Science of the Total Environment*, 540, 307-323.

Côté, I. M., Darling, E. S., & Brown, C. J. (2016). Interactions among ecosystem stressors and their importance in conservation. *Proceedings of the Royal Society B*, 283, 2015–2592.

Dahm, V., Hering, D., Nemitz, D., Graf, W., Schmidt–Kloiber, A., Leitner, P. et al. (2013). Effects of physico–chemistry, land use and hydromorphology on three riverine organism groups: a comparative analysis with monitoring data from Germany and Austria. *Hydrobiologia*, 704, 389–415.

Doadrio, I. (Ed). Ictiofauna española. Bases para su seguimiento. Ministerio de Agricultura y Pesca (In Spanish). 2011.

Dunn, A. M., & Hatcher, M. J. (2015). Parasites and biological invasions: parallels, interactions, and control. *Trends in Parasitology*, 31, 189–199.

Emerson, K., Russo, R. C., Lund, R. E., & Thurston, R. V. (1975). Aqueous ammonia equilibrium calculations: effect of pH and temperature. *Journal of the Fisheries Board of Canada*, 32(12), 2379-2383.

Englund, G., & Cooper, S. D. (2003). Scale effects and extrapolation in ecological experiments. Advances in Ecological Research, 33, 161–213.

Environmental Protection Agency (2013). Urban waste water treatment (last modified 4 Sep 2015). Available at: https://www.eea.europa.eu/data–and–maps/indicators/urban–waste–water–treatment/urban–waste–water–treatment–assessment–3.

Elton, C. S. (1958). The ecology of invasion by plants and animals. *Methuen, London*, *181*.

Fabozzi, F.J., Focardi, S.M., Rachev, S.T., Arshanapalli, B.G. (2014). Model Selection Criterion: AIC and BIC (Appendix E). The Basics of Financial Econometrics: Tools, Concepts, and Asset of Management Applications. John Wiley & Sons, Inc.

Figuerola, B., Maceda–Veiga, A., & De Sostoa, A. (2012). Assessing the effects of sewage effluents in a Mediterranean creek: fish population features and biotic indices. *Hydrobiologia*, 694, 75–86.

Froese, R. , Pauly, D. Editors. 2017. FishBase. World Wide Web electronic publication. www.fishbase.org.

Gozlan, R. E., St–Hilaire, S., Feist, S. W., Martin, P., & Kent, M. L. (2005). Biodiversity: disease threat to European fish. *Nature*, 435, 1046–1046.

Gieswein, A., Hering, D., & Feld, C. K. (2017). Additive effects prevail: The response of biota to multiple stressors in an intensively monitored watershed. *Science of the Total Environment*, 593, 27–35.

Grueber, C. E., Nakagawa, S., Laws, R. J., & Jamieson, I. G. (2011). Multimodel inference in ecology and evolution: challenges and solutions. *Journal of Evolutionary Biology*, 24(4), 699-711.

Gurr, C.J. & Reinhard, M. (2006). Harnessing natural attenuation. *Environmental Science and Technology*, 1, 2872–2876.

Hassan, M., Beatty, S. J., Morgan, D. L., Doupé, R. G., & Lymbery, A. J. (2008). An introduced parasite, Lernaea cyprinacea L., found on native freshwater fishes in the south west of Western Australia. *Journal of the Royal Society of Western Australia*, *91*, 149–153.

Hering, D., Carvalho, L., Argillier, C., Beklioglu, M., Borja, A., Cardoso, A. C. et al. (2015). Managing aquatic ecosystems and water resources under multiple stress—An introduction to the MARS project. *Science of the Total Environment*, 503, 10–21.

Hiers, J. K., Jackson, S. T., Hobbs, R. J., Bernhardt, E. S., & Valentine, L. E. (2016). The precision problem in conservation and restoration. *Trends in Ecology & Evolution*, 31, 820–830.

Hoekstra, A. Y., & Wiedmann, T. O. (2014). Humanity’s unsustainable environmental footprint. *Science*, 344, 1114–1117.

Hoffman, G. L. (1999). *Parasites of North American freshwater fishes*. Cornell University Press.

Hofmann, H., Blasco–Costa, I., Knudsen, R., Matthaei, C. D., Valois, A., & Lange, K. (2016). Parasite prevalence in an intermediate snail host is subject to multiple anthropogenic stressors in a New Zealand river system. *Ecological Indicators*, *60*, 845–852.

Jackson, M. C., Loewen, C. J., Vinebrooke, R. D., & Chimimba, C. T. (2016). Net effects of multiple stressors in freshwater ecosystems: a meta–analysis. *Global Change Biology*, 22, 180–189.

Johnson, P. T., Townsend, A. R., Cleveland, C. C., Glibert, P. M., Howarth, R. W., McKenzie, V. J. et al. (2010). Linking environmental nutrient enrichment and disease emergence in humans and wildlife. Ecological *Applications*, 20, 16–29.

Jones, K. E., Patel, N. G., Levy, M. A., Storeygard, A., Balk, D., Gittleman, J. L., & Daszak, P. (2008). Global trends in emerging infectious diseases. *Nature*, 451, 990–993.

Kaiser, M. J., Hilborn, R., Jennings, S., Amaroso, R., Andersen, M., Balliet, K., & Boyd, C. (2015). Prioritization of knowledge-needs to achieve best practices for bottom trawling in relation to seabed habitats. *Fish and Fisheries* 22, 175–183.

Knudsen, R., Klemetsen, A., & Staldvik, A. (1996). Parasites as indicators of individual feeding specialization in Arctic charr during winter in northern Norway. *Journal of Fish Biology*, 48, 1256-1265

Kupferberg, S. J., Catenazzi, A., Lunde, K., Lind, A. J., & Palen, W. J. (2009). Parasitic copepod (*Lernaea cyprinacea*) outbreaks in foothill yellow–legged frogs (*Rana boylii*) linked to unusually warm summers and amphibian malformations in Northern California. *Copeia*, 3, 529–537.

Lenihan, H. S., Micheli, F., Shelton, S. W., & Peterson, C. H. (1999). The influence of multiple environmental stressors on susceptibility to parasites: an experimental determination with oysters. *Limnology and Oceanography*, 44, 910–924.

Leprieur, F., Beauchard, O., Blanchet, S., Oberdorff, T., & Brosse, S. (2008). Fish invasions in the world's river systems: when natural processes are blurred by human activities. *PLoS Biol*, *6*(2), e28.

Liu, Y., Engel, B. A., Flanagan, D. C., Gitau, M. W., McMillan, S. K., & Chaubey, I. (2017). A review on effectiveness of best management practices in improving hydrology and water quality: Needs and opportunities. *Science of The Total Environment*, 601, 580–593.

Maceda–Veiga, A., Monleón–Getino, A., Caiola, N., Casals, F. & de Sostoa, A. (2010). Changes in fish assemblages in catchments in north–eastern Spain: biodiversity, conservation status and introduced species. *Freshwater Biology*, 55, 1734–1746.

Maceda–Veiga, A., Green, A. J., & De Sostoa, A. (2014). Scaled body–mass index shows how habitat quality influences the condition of four fish taxa in north–eastern Spain and provides a novel indicator of ecosystem health. *Freshwater Biology,* 59, 1145–1160.

Maceda–Veiga, A., Basas, H., Lanzaco, G., Sala, M., de Sostoa, A., & Serra, A. (2016). Impacts of the invader giant reed (*Arundo donax*) on riparian habitats and ground arthropod communities. *Biological Invasions*, 18, 731–749.

Maceda–Veiga, A., Baselga, A., Sousa, R., Vilà, M., Doadrio, I., & de Sostoa, A. (2017a). Fine–scale determinants of conservation value of river reaches in a hotspot of native and non–native species diversity. *Science of the Total Environment*, 574, 455–466.

Maceda–Veiga, A., Mac Nally, R. & de Sostoa, A. (2017b). The presence of non–native species is not associated with native fish response to water pollution in greatly hydrologically altered rivers. *Science of the Total Environment,* 608, 549–557.

MacColl, A.D.C. (2009). Parasite burdens differ between sympatric three-spined stickleback species. *Ecography*, 32, 153-160

Mac Nally, R. (1997). Scaling artefacts in confinement experiments: a simulation model. *Ecological Modelling*, 99, 229–245.

Mac Nally, R. (2002) Multiple regression and inference in ecology and conservation biology: further comments on identifying important predictor variables. *Biodiversity and Conservation*, 11, 1397–1401.

Marcogliese, D.J. & Pietrock, M. (2011). Combined effects of parasites and contaminants on animal health: parasites do matter. *Trends in Parasitology*, 27, 123–130.

McKenzie, V. J., & Townsend, A. R. (2007). Parasitic and infectious disease responses to changing global nutrient cycles. *EcoHealth*, 4, 384–396.

Medeiros, E. S., & Maltchik, L. (1999). The effects of hydrological disturbance on the intensity of infestation of *Lernaea cyprinacea* in an intermittent stream fish community. *Journal of Arid Environments*, 43, 351–356.

Moyle, P. B. (2014). Novel aquatic ecosystems: the new reality for streams in California and other Mediterranean climate regions. *River Research and Applications*, 30, 1335–1344.

Myers, N., Mittermeier, R.A., Mittermeier, C.G., Da Fonseca, G.A., Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature,* 403, 853–858.

Munné, A., Prat, N., Sola, C., Bonada, N., & Rieradevall, M. (2003). A simple field method for assessing the ecological quality of riparian habitat in rivers and streams: QBR index. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 13, 147–163.

Naiman and, R. J., & Decamps, H. (1997). The ecology of interfaces: riparian zones. *Annual review of Ecology and Systematics*, 28, 621–658.

Noga, E. J. (2011). Fish disease: diagnosis and treatment. John Wiley & Sons.

Overstreet, R. M. (1993). Parasitic diseases of fishes and their relationship with toxicants and other environmental factors. *Pathobiology of Marine and Estuarine Organisms*, 111–156.

Paine, R. T., Tegner, M. J., & Johnson, E. A. (1998). Compounded perturbations yield ecological surprises. *Ecosystems*, 1, 535–545.

Palm, H. W. (2011). Fish parasites as biological indicators in a changing world: can we monitor environmental impact and climate change?. *In Progress in Parasitology* (pp. 223–250). Springer Berlin Heidelberg.

Piggott, J. J., Townsend, C. R., & Matthaei, C. D. (2015). Reconceptualizing synergism and antagonism among multiple stressors. *Ecology and evolution*, 5, 1538–1547.

Poff, N. L., Olden, J. D., Merritt, D. M., & Pepin, D. M. (2007). Homogenization of regional river dynamics by dams and global biodiversity implications. *Proceedings of the National Academy of Sciences*, 104, 5732–5737.

Ponisio, L. C., M'gonigle, L. K., & Kremen, C. (2016). On-farm habitat restoration counters biotic homogenization in intensively managed agriculture. *Global Change Biology*, 22, 704–715.

Poulin, R., Curtis, M. A., & Rau, M. E. (1991). Size, behaviour, and acquisition of ectoparasitic copepods by brook trout, Salvelinus fontinalis. *Oikos*, 169–174.

R Core Team (2016) R: A language and environment for statistical computing (https://www.R–project.org/). R Foundation for Statistical Computing, Vienna, Austria.

Radinger, J., Essl, F., Hölker, F., Horký, P., Slavík, O., & Wolter, C. (2017). The future distribution of river fish: the complex interplay of climate and land use changes, species dispersal and movement barriers. *Global Change Biology* (In press).

Raissy, M., Sohrabi, H. R., Rashedi, M., & Ansari, M. (2013). Investigation of a parasitic outbreak of Lernaea *cyprinacea* Linnaeus (Crustacea: Copepoda) in Cyprinid fish from Choghakhor lagoon. *Iranian Journal of Fisheries Sciences*, 12, 680–688.

Ribeiro, F., Magalhães, M. F., & Collares–Pereira, M. J. (2013). Spatial and temporal variation in assemblage structure of fish larvae in mediterranean–type streams: contrasts between native and non–native species. *Environmental Biology of Fishes*, 96, 467–480.

Robin, X., Turck, N., Hainard, A., Tiberti, N., Lisacek, F., Sanchez, J.C. & Müller, M. (2011). pROC: an open–source package for R and S+ to analyze and compare ROC curves. *BMC Bioinformatics*, 12, p.77.

Rogalski, M. A., Gowler, C. D., Shaw, C. L., Hufbauer, R. A., & Duffy, M. A. (2017). Human drivers of ecological and evolutionary dynamics in emerging and disappearing infectious disease systems. *Philosophical Transactions Royal Society* B, 372, 20160043.

Sánchez–Hernández, J. (2017). *Lernaea cyprinacea* (Crustacea: Copepoda) in the Iberian Peninsula: climate implications on host–parasite interactions. *Knowledge & Management of Aquatic Ecosystems*, (418), 11.

Sabater, S., Feio, M. J., Graça, M. A., Muñoz, I., & Romaní, A. M. (2009). The Iberian Rivers. Rivers of Europe, 113–149.

Sampaio, L. A., Wasielesky, W., & Miranda–Filho, K. C. (2002). Effect of salinity on acute toxicity of ammonia and nitrite to juvenile *Mugil platanus*. *Bulletin of Environmental Contamination and Toxicology*, 68, 668–674.

Schinegger, R., Palt, M., Segurado, P., & Schmutz, S. (2016). Untangling the effects of multiple human stressors and their impacts on fish assemblages in European running waters. *Science of the Total Environment*, 573, 1079–1088.

Seebens, H., Blackburn, T. M., Dyer, E. E., Genovesi, P., Hulme, P. E., Jeschke, J. M. et al. (2017). No saturation in the accumulation of alien species worldwide. *Nature communications*, *8*, 14435.

Sinha, E., Michalak, A. M., & Balaji, V. (2017). Eutrophication will increase during during the 21st century as a result of precipitation changes. *Science*, 357, 405–408.

Smallbone, W., Cable, J., & Maceda–Veiga, A. (2016). Chronic nitrate enrichment decreases severity and induces protection against an infectious disease. *Environment International*, 91, 265–270.

Smith, V. H., & Schindler, D. W. (2009). Eutrophication science: where do we go from here?. *Trends in Ecology & Evolution*, 24, 201–207.

Smith, K. F., Goldberg, M., Rosenthal, S., Carlson, L., Chen, J., Chen, C., & Ramachandran, S. (2014). Global rise in human infectious disease outbreaks. *Journal of The Royal Society Interface*, *11*, 20140950.

Stephens, P. R., Altizer, S., Smith, K. F., Alonso Aguirre, A., Brown, J. H., Budischak, S. A. et al. (2016). The macroecology of infectious diseases: a new perspective on global-scale drivers of pathogen distributions and impacts. *Ecology Letters*, 19, 1159–1171.

Strahler, A.N. (1964). Quantitative geomorphology of drainage basin and channel networks. *Handbook of Applied Hydrology*.

Studer, A., & Poulin, R. (2013). Cercarial survival in an intertidal trematode: a multifactorial experiment with temperature, salinity and ultraviolet radiation. *Parasitology Research*, 112, 243–249.

Tal, A. (2016). Rethinking the sustainability of Israel's irrigation practices in the Drylands. *Water Research*, 90, 387–394.

Tockner, K., Uehlinger, U., & Robinson, C. T. (2009). *Rivers of Europe*. Academic Press.

Townsend, C. R., Uhlmann, S. S., & Matthaei, C. D. (2008). Individual and combined responses of stream ecosystems to multiple stressors. *Journal of Applied Ecology*, 45, 1810–1819.

Vega Thurber, R. L., Burkepile, D. E., Fuchs, C., Shantz, A. A., McMinds, R., & Zaneveld, J. R. (2014). Chronic nutrient enrichment increases prevalence and severity of coral disease and bleaching. *Global Change Biology*, 20, 544-554.

Vidal–Martínez, V. M., Pech, D., Sures, B., Purucker, S. T., & Poulin, R. (2010). Can parasites really reveal environmental impact?. *Trends in Parasitology*, 26, 44–51.

Vörösmarty, C. J., McIntyre, P. B., Gessner, M. O., Dudgeon, D., Prusevich, A., Green, P. et al. (2010). Global threats to human water security and river biodiversity. *Nature*, 467, 555.

Wagenmakers, E. J., & Farrell, S. (2004). AIC model selection using Akaike weights. *Psychonomic Bulletin & Review*, 11(1), 192-196.

Welicky, R. L., De Swardt, J., Gerber, R., Netherlands, E. C., & Smit, N. J. (2017). Drought–associated absence of alien invasive anchorworm, *Lernaea cyprinacea* (Copepoda: Lernaeidae), is related to changes in fish health. *International Journal for Parasitology: Parasites and Wildlife*.

Williams, J. W., & Jackson, S. T. (2007). Novel climates, no-analog communities, and ecological surprises. *Frontiers in Ecology and the Environment*, *5*, 475–482.

Williamson, C.E., Dodds, W., Kratz, T.K. & Palmer, M.A. (2008) Lakes and streams as sentinels of environmental change in terrestrial and atmospheric processes. *Frontiers in Ecology and the Environment*, 6, 247–254.

Zhou, Y., Ma, J., Zhang, Y., Qin, B., Jeppesen, E., Shi, K. et al. (2017). Improving water quality in China: Environmental investment pays dividends. *Water Research*, 118, 152–159.

Figure legends

Fig. 1 Location of the 530 sampling sites surveyed in rivers of north–eastern Spain with protected areas highlighted in green.

Fig. 2 Changes in altitude, salinity, nitrate and phosphate–P concentrations and pH at sites at which the alien parasitic copepod *Lernaea cyprinacea* was found infecting any of the 19 fish species examined (0 = absence, 1 = presence). We only showed the environment–parasite relationships with the strongest correlations from generalized linear mixed models in Table 2 (see full details on river environmental conditions in the presence or absence of *L. cyprinacea* in Table S1). Note that untransformed data are used for these graphs to facilitate interpretation, although log-transformed data and linear combinations of predictors were used in the models.

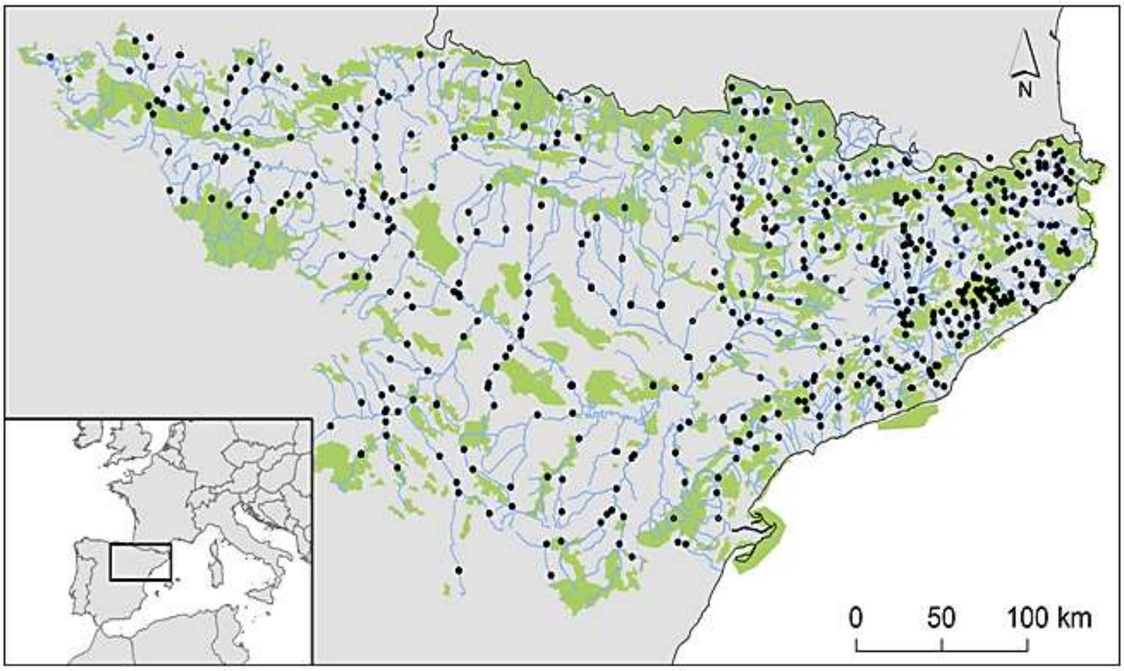

Fig 1.

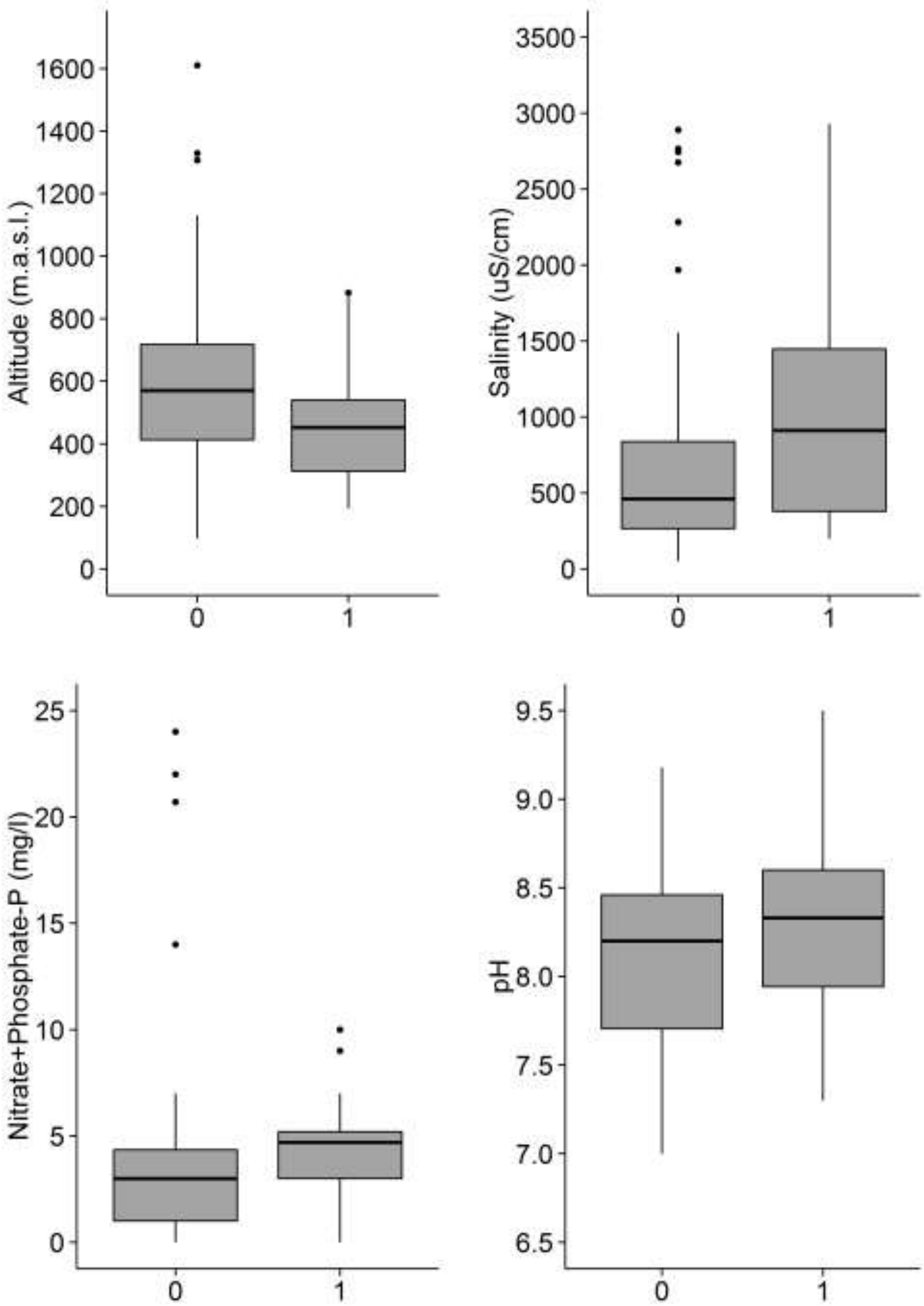
Altitude (m.a.s.l.)
0
200
400
600
800
1000
1200
1400
1600
Salinity (uS/cm)
0
500
1000
1500
2000
2500
3000
3500
Nitrate+Phosphate-P (mg/l)
0
5
10
15
20
25
pH
6.5
7.0
7.5
8.0
8.5
9.0
9.5
0
1

Fig 2.

Table 1 Mean (± S.E.) fish host size, status in Spain, and prevalence of the alien parasitic copepod *Lernaea cyprinacea* and minimum–maximum range (min–max) for each of the frequently captured fish species (≥10 sites) in the 15 catchments of NE Spain (*N* = number of sites where the species occurred, out of 530 sites).

			L. cyprinacea prevalence†		Fish length (mm)	
Scientific name	Status	*N*	Mean ± S.E.	Min–Max	Mean ± S.E.	Min–Max
Achondrostoma arcasii	Native	25	0	0	63 ± 1	8–133
Anguilla anguilla	Native	61	0.10 ± 0.03	0–4	287 ± 11	75–810
Barbatula spp.*	Native	61	0.07 ± 0.03	0–4	65 ± 0.5	16–108
Barbus haasi	Native	141	3.25 ± 0.56	0–100	93 ± 0.8	10–253
Barbus meridionalis	Native	83	3.89 ± 0.45	0–68	74 ± 0.7	20–305
Cobitis calderoni	Native	13	0	0	45 ± 0.5	5–85
Gobio spp.*	Native	100	1.45 ± 0.49	0–100	73 ± 0.5	6–150
Luciobarbus graellsii	Native	149	7.70 ± 0.49	0–100	160 ± 12	6–5297
Parachondrostoma miegii	Native	116	4.04 ± 0.76	0–100	88 ± 0.7	17–453
Phoxinus spp.*	Native	128	0.06 ± 0.02	0–6	54 ± 4	5–153
Salaria fluviatilis	Native	24	0.74 ± 0.18	0–18	72 ± 0.6	9–128
Salmo trutta	Native	182	0.34 ± 0.18	0–50	141 ± 1.1	8–932
Squalius laietanus	Native	86	6.59 ± 0.94	0–100	99 ± 1.5	5–438
Alburnus alburnus	Alien	48	0.55 ± 0.13	0–18	34 ± 1	8–133
Cyprinus carpio	Alien	79	8.82 ± 1.18	0–100	187 ± 6	20–735
Gambusia holbrooki	Alien	13	0	0	30 ± 0.5	10–65
Lepomis gibbosus	Alien	22	5.59 ± 1.02	0–100	6 ± 1	20–156
Oncorhynchus mykiss	Alien	10	1.25 ± 0.19	0–13	299 ± 6	234–377
Scardinius erythrophthalmus	Alien	18	0.45 ± 0.07	0–6	53 ± 4	24–259

*species identity requires genetic confirmation in some catchments; †percentage of infected individuals out of the number of individuals inspected of each fish species in each site

Table 2 Statistics from generalized mixed models for the presence of the alien parasitic copepod *Lernaea cyprinacea* as a function of the fish host predictors (length, *Le*; abundance, *Ab*; swimming behaviour, *Swi*; migration, *Mig*; trophic guild, *Tro*). All random terms and river descriptors (see methods for details) are individually included as predictors in the models, but are collectively referred to here as ρ and *Env* for brevity. The *best* model (defined by the lowest AIC or BIC values) is highlighted in bold and was used as baseline in further analyses of the relative importance of geography and the full set of environmental stressors (see Table S2 and Table 3). Model fit (i.e. AUC) of the best model is also shown. We provide information on the differences in AIC for each model (ΔAIC) and the corresponding probability of support relative to the best model (Prob. = $e^{-\Delta AIC/2}$; Wagenmakers & Farrell, 2004).

Models	Model equations	AIC	ΔAIC	Prob.	BIC	AUC
B1	$\alpha+Le+Ab+Swi+Mig+Tro+Env+\rho$	4265.4	+3.7	0.024	4417.3	
B2	$\alpha+Le+Ab+Swi+Mig+Env+\rho$	4264.1	+2.4	0.091	4408.1	
B3	$\alpha+Le+Ab+Swi+Env+\rho$	4264.7	+3.0	0.049	4399.3	
B4	$\alpha+Le+Ab+Env+\rho$	4264.9	+3.2	0.041	4394.0	
B5	$\boldsymbol{\alpha+Le+Env+\rho}$	**4261.7**	**-**	**-**	**4383.9**	**0.80**
B6	$\alpha+Env+\rho$	4307.0	+45.3	2.12E-20	4421.1	

Table 3 Model–average standardized coefficients, standard error (S.E.), and 95% confidence intervals (CI) of the selected fish host predictors from Table 2 plus the additive effects of the river descriptors retained as having a significant effect for the transmission of the alien parasite *Lernaea cyprinacea* among fish (see the full set of candidate models in Table S2). The relative importance of the four sets of predictors from 0 to 1, and model fit (i.e. AUC) of the best model, are also shown. Note that CIs for stream order, silt, QBR, TN and water speed included zero.

Predictor	Stdz coefficients	S.E.	2.5%CI	95%CI	Predictor importance
Model fit (AUC = 0.80)					
Intercept	–6.75	0.39	–7.52	–5.98	
Host predictor					1
Fish length	–0.33	0.05	–0.43	–0.24	
Geography (geo)					0.99
Elevation	–0.38	0.16	–0.69	–0.06	
Stream order number	0.20	0.16	–0.13	0.52	
Water quality (*wq*)					1
Salinity	0.62	0.17	0.29	0.96	
pH	0.40	0.14	0.12	0.70	
TN	–0.10	0.12	–0.34	0.14	
NP	0.64	0.16	0.33	0.95	
Silt	–0.03	0.14	–0.31	0.25	
Physical habitat quality (*phq*)					0.53
QBR	–0.22	0.15	–0.51	0.07	
River water speed (ws)					0.33
Water velocity	–0.12	0.15	–0.41	0.17	

TN = the sum of ammonium and nitrite concentrations; NP = the sum of nitrate and phosphate–P concentrations; Stream order number is used as proxy of river size; Conductivity is used as proxy of salinity; QBR is an index of the degree of naturalness of river morphology and the quality of riparian areas.

Table 4 Statistics for the comparison of an additive model including fish host predictors and river descriptors (i.e. the *best* model from Table 3 is the Additive Model here) compared to five models including interactive effects for relevant environmental combinations (see methods for model equations and further details) for the transmission of the alien parasitic copepod *Lernaea cyprinacea* among fish. The top–ranked model was defined by the lowest AIC and BIC values is highlighted in bold, and its fit was assessed by using AUC. We provide information on the differences in AIC for each interactive model (ΔAIC) and the corresponding probability of support relative to the Additive Model A (Prob. = $e^{-\Delta AIC/2}$; Wagenmakers & Farrell, 2004).

Models	AIC	ΔAIC	Prob.	BIC	AUC
Additive Model (AM)	**4261.7**	**-**	**-**	**4383.9**	**0.8**
AM with interactive effects for elevation	4274.5	+12.8	0.002	4495.5	
AM with interactive effects for water velocity	4273.3	+11.6	0.003	4485.2	
AM with interactive effects for *wq* and *phq*	4276.3	+14.6	0.001	4480.0	
AM with interactive effects for salinity and nutrients	4267.4	+5.7	0.058	4446.6	
AM with interactive effects for nutrients and pH	4271.0	+9.3	0.01	4450.3	

wq refers to all water quality variables (sum of ammonium and nitrite, sum of nitrate and phosphate–P, pH, silt and salinity); nutrients refers to ammonium, nitrite, nitrate and phosphate–P; and *phq* refers to physical habitat quality as estimated by the index QBR

Supplementary Information

Table S1 Mean and minimum–maximum range (min–max) of geographical and environmental stressors used to explain the spread of the alien parasitic copepod *Lernaea cyprinacea* among fish from northeastern Spain. We show the full range of environmental conditions available and those of river reaches at which infected fish were found. Acronyms (in italics and in brackets) are the codes used in equations to indicate that all predictors within each of these categories were included in the models individually.

	All sampling sites (*N* = 530)		Sites with infected fish (*N* = 60)	
Descriptors	Mean	Min–max	Mean	Min–max
Geographical features (geo)				
Elevation (m.a.s.l.)	552	3–1814	464	193–883
Stream order number	2	1–8	3	1–5
River water speed (ws)				
Water velocity (m/s)	0.38	0–2.5	0.42	0.02–1
Physical habitat quality (phq)				
QBR (score)	25	0–100	18	0–75
Water quality (wq)				
pH	8.2	6.5–9.6	8.2	7.3–9.5
Salinity (µS/cm)	776	20–5220	1043	198–2930
TN (mg/l)	0.14	0–6	0.09	0–0.8
NP (mg/l)	3.68	0–50	4	0–10
Silt (%)	19	1–60	26	1–57

TN = the sum of ammonium and nitrite concentrations; NP = the sum of nitrate and phosphate–P concentrations; Conductivity is used as proxy of river salinity, and Stream order number as proxy of river size.

Table S2. Possible directions of the effect of fish host and environmental predictors on the presence of the parasitic copepod *Lernaea cyprinacea* with a brief description of the reasons behind each effect that complements our explanations in main text

	Parasite	Rationale
Host traits		
Abundance	+	High host abundance facilitates fish-fish infections of ectoparasites
Size	+	The more surface area of the fish host, the higher is the probability that a free-swimming parasite encounters a fish host
Migratory guild	+	Migration facilitates dispersion of parasites within a river
	-	Parasites reach less suitable habitats
Trophic guild	+	Fish hosts prey upon invertebrates that feed on the larvae of the copepod parasite or alter valuable habitat structures for it
	-	Fish hosts feed on the larvae of the copepod parasite or promote the presence of invertebrate predators that feed upon it
Swimming guild	+	Benthic fish hosts are closer to the bottom where contaminants can accumulate and so promote immune-suppression and hence infection
	-	Pelagic fish hosts tend to be more mobile than benthic hosts which make difficult parasite attachment
Environmental factors		
Elevation	+	Low temperatures promote temperate species
	-	High temperatures promote thermophilous species. River drift promotes individuals to move downstream.
Stream order	+	Habitat complexity diversifies in large rivers promoting the abundance of parasites and fish hosts
	-	
Salinity	+	Salinity is an integrate measure of many pollutants which can immune-suppress the fish host promoting infection
	-	These pollutants, including salinity itself can also be lethal to fish host or its parasites
Ammonia+Nitrite	+	An increase in primary production can favour tolerant species to ammonia and nitrite compounds
	-	The benefits of an increased primary production can be offset by the high toxicity of ammonia and nitrite
Nitrate+Phosphates	+	An increase in primary production can favour tolerant species to nitrate and phosphate-P
	-	Fish hosts and their parasites can perish due to the direct toxicity of these compounds at high concentrations or their indirect effects (e.g. toxic algal blooms)
Silt	+	High water turbidity reduces fish movements because it reduces visual detection
	-	A high concentration of suspended particles can make the free-swimming of parasite larvae difficult
Water velocity	-	High water velocity reduces the probability of parasite attachment to the fish host
Riparian tree	+	The release of leaves promote ecosystem productivity and increase habitat complexity promoting biodiversity
	-	High riparian cover reduces light penetration and hence ecosystem productivity

Table S3 Full list of candidate models including the geographical and environmental predictors used (from Table S1; geography, *geo*; water velocity, *wv*; water quality, *wq*; and physical habitat quality, *phq*) to explain the spread of the alien parasitic copepod *Lernaea cyprinacea* among fish. All models included basin, year, sampling site, and fish species as random terms (ρ), and controlled for the effect of host predictors selected in Table 2. The top–ranked models (defined by the lowest AIC and BIC values) are indicated as *best* models and all five were used in the model-averaging step (Table 3).

Model equations	AIC	BIC	Interpretation
$\alpha+host+geo+wv+wq+phq+\rho$	4261.7	4383.9	Best AIC model
$\alpha+host+geo+wv+wq+\rho$	4261.9	4376.0	Best AIC model
$\alpha+host+geo+wv+phq+\rho$	4297.8	4379.3	
$\alpha+host+geo+wq+phq+\rho$	4260.4	4374.5	Best AIC model
$\alpha+host+wv+wq+phq+\rho$	4268.8	4374.7	
$\alpha+host+geo+wq+\rho$	4260.6	4366.5	Best AIC and BIC models
$\alpha+host+geo+phq+\rho$	4300.7	4374.1	
$\alpha+host+wv+wq+\rho$	4269.6	4367.4	
$\alpha+host+wv+phq+\rho$	4309.3	4374.5	
$\alpha+host+phq+\rho$	4309.7	4366.8	
$\alpha+host+wq+\rho$	4270.4	4360.1	
$\alpha+host+wq+phq+\rho$	4266.8	4364.6	Best BIC model
$\alpha+host+\rho$	4322.2	4371.1	
$\alpha+host+geo+\rho$	4308.6	4373.7	
$\alpha+host+geo+wv+\rho$	4306.3	4379.6	