Universidad de Navarra

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ECOLOGÍA DEL CANGREJO SEÑAL Y SUS INTERACCIONES CON LA BIODIVERSIDAD

ECOLOGY OF SIGNAL CRAYFISH AND ITS INTERACTIONS WITH BIODIVERSITY

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Memoria presentada por D. Iván Vedia Jiménez para aspirar al grado de Doctor por la Universidad de Navarra

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Pamplona, 14 de Abril de 2016

Dr. Rafael Miranda Ferreiro Dr. Enrique Baquero Martín

«No sé lo que le podré parecer al mundo, pero a mí me parece como si hubiese sido un niño que juega en la orilla del mar y se divierte de tanto en tanto encontrando un guijarro más pulido o una concha más hermosa de lo común, mientras que el inmenso océano de la verdad se extiende inexplorado frente a mí».

(Albert Einstein 1879-1955).

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Esta tesis doctoral es una colección de manuscritos en diferentes estados de publicación, cada uno de los cuales constituye un capítulo. Los manuscritos se reproducen íntegros y en el idioma en el que fueron publicados o enviados para su publicación, incluyendo siempre un resumen en castellano. Los artículos publicados han sido reproducidos con el permiso de las editoriales.

En cumplimiento de la normativa para la presentación de tesis doctorales en la Facultad de Ciencias de la Universidad de Navarra se incluyen los siguientes apartados en castellano: (1) un Resumen integrador del contenido de la tesis doctoral; (2) una Introducción general que sitúa el trabajo realizado en su contexto teórico, planteando los Objetivos de la tesis doctoral; (3) una Discusión general, y (4) un apartado de Conclusiones generales.

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RESUMEN

ABSTRACT

Resumen

En esta tesis doctoral se abordan las principales relaciones ecológicas existentes entre el cangrejo señal *Pacifastacus leniusculus* (Dana, 1852) y los factores abióticos (parámetros físicoquímicos del agua y características del hábitat) y bióticos (algas, branquiobdélidos, invertebrados bentónicos y peces) en los ecosistemas fluviales navarros (norte de España). En primer lugar, se hace una revisión bibliográfica sobre el estado de conocimiento y legislación vigente de las especies de cangrejo de río presentes en la Península Ibérica que sirve de capítulo introductorio. La disparidad entre los criterios de gestión de las distintas administraciones y la consideración del cangrejo señal como una especie exótica invasora y, a la vez, una especie pescable, son importantes escollos en la gestión global de esta especie. Posteriormente se estudia la distribución y abundancia del cangrejo señal en Navarra y se relaciona con los parámetros físico-químicos del agua, características del hábitat y las comunidades de peces. El hábitat óptimo del cangrejo señal se sitúa entre los tramos salmonícolas de cabecera, con aguas frías y baja proporción de materia orgánica, y los tramos ciprinícolas (tramos bajos), con aguas más cálidas, donde cohabita con otra especie invasora de cangrejo, el cangrejo rojo de las marismas *Procambarus clarkii* (Girard, 1852). La presencia del cangrejo señal está limitada en los tramos de cabecera porque las temperaturas son extremadamente bajas y hay poca materia orgánica. La existencia de unos factores ambientales limitantes en tramos altos favorece la conservación de estos ecosistemas acuáticos y su fauna nativa. Además, se encuentra una nueva relación ectosimbiótica entre el cangrejo rojo de las marismas y el branquiobdélido *Xironogiton victorienesis* (Gelder & Hall, 1990) y se estudian las relaciones biológicas que tiene este organismo ectosimbionte con su hospedador y con las variables ambientales. Aunque *X. victoriensis* se pensaba que era un ectosimbionte específico del cangrejo señal (su único hospedador hasta el momento), se observa que también puede infestar otras especies de cangrejo. Las consecuencias de estas nuevas relaciones biológicas en otros lugares y con diferentes especies se desconocen. En el siguiente trabajo, se vio que los ejemplares de cangrejo de mayor tamaño y con mejor condición corporal de cangrejo presentan mayores abundancias del ectosimbionte. El mutualismo es el mejor tipo de relación biológica que explica la relación existente entre *X. victoriensis* y su hospedador. Finalmente, se evalúa la competencia trófica y por espacio entre el cangrejo señal y las especies nativas de peces mediante el uso de grabaciones nocturnas, el estudio del contenido estomacal y el análisis de isótopos estables de carbono y nitrógeno. Se demuestra que existe una competencia por el alimento y por el hábitat/espacio entre el cangrejo señal y las especies nativas de peces, en particular con las especies bentónicas. En resumen, los resultados de esta tesis doctoral demuestran que el cangrejo señal produce impactos negativos sobre la fauna autóctona mediante la depredación, competencia trófica, competencia por espacio y, transporte de ectosimbiontes asociados que pueden causar enfermedades y/o infestaciones a las especies nativas. Por todo ello, se deben tomar medidas urgentes para controlar las poblaciones de cangrejo señal debido a que en su área de distribución habitan especies Ibéricas endémicas de peces “En Peligro de Extinción” como la lamprehuela *Cobitis calderoni* Bacescu, 1962.

Abstract

In this doctoral dissertation the main ecological relationships between the signal crayfish *Pacifastacus leniusculus* (Dana, 1852) and abiotic –physicochemical parameters of water and habitat features– and biotic factors –algae, branchiobdellidans, benthic invertebrates and fish– are dealt in the rivers of Navarra (northern Spain). First, a bibliographic review about the state of knowledge and current legislation of crayfish species of the Iberian Peninsula was made as an introductory chapter. The disparity between the management criteria of the different regional administrations and the consideration of the signal crayfish as an invasive species and at the same time as a fishable species, are important pitfalls in the overall management of this species. Subsequently, we studied the distribution and abundance of the signal crayfish in Navarra and related it with water physicochemical parameters, habitat characteristics and fish assemblages. The optimal habitat of signal crayfish is among salmonid stretches (headwaters), with cold waters and low proportion of organic debris, and cyprinid stretches (low waters) with warmer waters where inhabits with another invasive crayfish, the red swamp crayfish *Procambarus clarkii* (Girard, 1852). The presence of signal crayfish is limited to uppermost stretches by lower water temperatures and lower proportion of organic debris. The existence of a natural environmental limiting factor in upstream reaches facilitates the conservation of aquatic ecosystems and native fauna. A new ectosymbiotic association was found between the red swamp crayfish and the branchiobdellidan *Xironogiton victorienesis* (Gelder & Hall, 1990). We studied the biological relationships of the branchiobdellidan with its host and environmental variables. Although *X. victoriensis* was thought to be a specific ectosymbiont of signal crayfish (its only host to date), our finding showed that it can also infest other crayfish species. Impacts of these exotic ectosymbionts on habitat and biota at a new location are unknown, as are their consequences on native biodiversity. In the following work, we showed that the abundance of the branchiobdellidan was higher in the largest individuals and in those showing the best body condition. Our results support mutualism as the best biological relationship explaining the symbiosis between *X. victoriensis* and signal crayfish. Finally, we evaluated the trophic and spatial competition between signal crayfish and the native fish species using nocturnal recordings, stomach content and stable isotopes of carbon and nitrogen. We demonstrated that trophic and spatial competition between signal crayfish and native fishes is particularly higher with benthic species. In summary, the results of this doctoral dissertation prove that the signal crayfish causes negative impacts on native fauna by predation, trophic and spatial competition and, the transportation of associate ectosymbionts can cause diseases (*e.g.* aphanomycosis) and/or infestations of native species. For that reasons, considering that in the distribution area of this invasive crayfish inhabits endemic fish species of the Iberian Peninsula, such as the benthic *Cobitis calderoni* Bacescu, 1962, catalogued on the IUCN Red List as endangered, managers should take urgent measures to control its populations.

INTRODUCCIÓN

INTRODUCTION

Especies exóticas invasoras (EEI)

El intercambio de especies de flora y fauna mediado por el hombre de unos lugares a otros se remonta a la Prehistoria, desde los primeros movimientos humanos. Un ejemplo de estos movimientos de fauna prehistóricos son la introducción del topillo campesino *Microtus arvalis* (Pallas, 1778) en las Islas Orcadas (Orkney) desde el suroeste de Europa (Haynes *et al.* 2003, 2004) o la introducción de la musaraña enana *Sorex minutus* (Linnaeus, 1766) en Irlanda (Mascheretti *et al.* 2003). El transporte intencionado de vertebrados por el Mediterráneo está especialmente documentado desde hace miles de años (Argüelles *et al.* 2006). Por ejemplo, el perro fue domesticado hace unos 14 000 años y se introdujo en Chipre y en Córcega hace unos 9000 y 8500 años respectivamente (Pascal *et al.* 2003). Asociadas a las múltiples especies transportadas en el pasado se introdujeron patógenos, que ocasionaron diversas enfermedades en humanos y animales, y la extinción de muchas especies animales (Masseti 2002, Carvalho *et al.* 2003, Steadman & Martin 2003).

El descubrimiento de América en 1492 favoreció la expansión mundial de la civilización europea. Gracias a este acontecimiento histórico, comenzó a acelerarse la transferencia de diversas especies de animales y plantas entre América y Europa. En los siglos XIX y XX las mejoras tecnológicas posibilitaron un aumento en la rapidez de los medios de transporte y en la frecuencia de los movimientos transcontinentales y, con ello, las probabilidades de introducir nuevas especies exóticas (Argüelles *et al.* 2006). En las últimas décadas, el transporte internacional se ha incrementado de forma drástica tanto por vía aérea, marítima como terrestre, debido al proceso de globalización. Este hecho ha favorecido la dispersión de cientos de especies de unos lugares a otros del planeta. Actualmente, una de las mayores amenazas para la biodiversidad es la introducción, intencional o accidental, de especies exóticas que desarrollan un comportamiento invasivo, desplazando a especies nativas y causando graves daños a los ecosistemas. La creciente expansión de especies exóticas invasoras a escala planetaria es sin lugar a dudas uno de los problemas más graves a los que se enfrenta la biodiversidad a nivel mundial (Argüelles *et al.* 2006, Gherardi 2010). De hecho, las especies exóticas invasoras se consideran la segunda causa más significativa de extinción de especies después de la destrucción del hábitat (Clavero & García-Berthou 2005). Además, este problema no sólo afecta a la biodiversidad, sino que también desencadena problemas económicos, sanitarios y sociales (Reinhardt *et al.* 2003).

Según la Unión Internacional para la Conservación de la Naturaleza (UICN) una especie exótica invasora (EEI) es una “especie exótica que se establece en un ecosistema o hábitat natural o seminatural; es un agente de cambio y amenaza la diversidad biológica nativa”. Las especies exóticas invasoras son animales, plantas u otros organismos introducidos por el hombre en lugares fuera de su área de distribución natural, donde se establecen y se dispersan, provocando un impacto negativo en el ecosistema y especies locales (IUCN 2011). Las especies exóticas invasoras de animales se caracterizan por tener una amplia tolerancia a las condiciones ambientales, omnivorismo, rápido crecimiento y dispersión, reproducción rápida y prolífica, y otros rasgos relacionados con el oportunismo. Además, la alta eficacia depredadora y la falta de depredadores favorecen aún más su invasión (Ilhéu *et al.* 2007). Por otra parte, en esta tesis se considera como especie “exótica, alóctona, introducida, no nativa o foránea” (términos sinónimos) a aquella no originaria del lugar en la que se encuentra de forma natural (introducida por el hombre) y para la que no se ha demostrado hasta ese momento su carácter invasor.

Las vías de entrada de EEI se pueden clasificar en tres: (1) intencionales (por ej. sustitutos ecológicos de especies nativas casi desaparecidas; control biológico; caza y pesca recreativa), (2) no intencionales (por ej. especies incrustantes en cascos de embarcaciones; transporte en vehículos, equipos o calzado) y (3) negligentes (por ej. escapes de granjas con fauna alóctona). Sin embargo, hay que tener en cuenta que muchas de las especies exóticas introducidas nunca llegan a naturalizarse. Se ha estimado que, en promedio, un 10% de las especies introducidas se convierten en invasoras (Williamson & Fitter 1996).

Los ecosistemas acuáticos son especialmente vulnerables a la introducción de especies exóticas (Welcomme 1988). En algunos grupos, como los peces y cangrejos, el número de especies exóticas supera el 50% de las especies nativas de este grupo presente en la Península Ibérica. En concreto, las aguas continentales de la Península Ibérica presentan al menos 46 especies exóticas de invertebrados y 28 especies de vertebrados, de las cuales 23 son peces (García-Berthou 2007). De las 46 especies de invertebrados, cuatro especies son cangrejos de río: el cangrejo rojo de las marismas *Procambarus clarkii* (Girard, 1852), el cangrejo señal *Pacifastacus leniusculus* (Dana, 1852), el cangrejo de los canales *Orconectes limosus* (Rafinesque, 1817) y el cangrejo australiano o Yabbie *Cherax destructor* Clark, 1936.

Cangrejos de río: distribución y clasificación

Actualmente, están descritas un total de 593 especies de cangrejo de río en el mundo, que habitan de forma natural en todos los continentes, excepto en el continente africano (a excepción del género *Astacoides* en Madagascar) y la Antártida. Existen dos grandes superfamilias de cangrejos de río: Astacoidea en el Hemisferio Norte y Parastacoidea en el Hemisferio Sur. Dentro de la superfamilia Astacoidea se diferencian la Familia Astacidae (Europa y Oeste de Norteamérica) y la familia Cambaridae (Este de Norteamérica y Este de Asia). Y dentro de la superfamilia Parastacoidea únicamente se encuentra la familia Parastacidae (Australia, Madagascar y Sudamérica). El mayor número de especies están presentes en Norteamérica y pertenecen a la familia Cambaridae con un total de 409 especies descritas (Souty-Grosset *et al.* 2006).

Las especies europeas nativas de cangrejo de río son el cangrejo de río autóctono europeo o cangrejo noble *Astacus astacus* (Linneo, 1758), el cangrejo turco o de patas delgadas *Astacus leptodactylus* Eschscholtz, 1823, el cangrejo de los torrentes *Austropotamobius torrentium* (Schrank, 1803) y, los cangrejos de patas blancas *Austropotamobius pallipes* (Lereboullet, 1858) y *Austropotamobius italicus* (Faxon, 1914). Todas ellas sufrieron en el siglo pasado un fuerte declive en sus poblaciones debido, en gran medida, a la introducción de especies Norteamericanas de cangrejo (*P. clarkii*, *P. leniusculus* y *O. limosus*) portadoras del patógeno responsable de la afanomicosis, el *Aphanomyces astaci* Schicora, 1906 (Edgerton *et al.* 2002). De hecho, este organismo está incluido dentro de las 100 especies más invasoras del mundo (Lowe *et al.* 2000). Aunque otros factores, como la contaminación de las aguas y la alteración del hábitat, también han influido negativamente sobre las poblaciones nativas europeas (Souty-Grosset *et al.* 2006).

Ecología del cangrejo de río

Los cangrejos de río desempeñan un importante papel en la ecología de los ecosistemas fluviales y son considerados como especies clave: especies cuyo impacto en su comunidad o ecosistema es más importante del que cabría esperar si sólo se tiene en cuenta su abundancia relativa o biomasa total (Power *et al.* 1996). En muchos ecosistemas, estas especies ocupan una posición central en la cadena trófica, siendo al mismo tiempo presas y depredadores (Geiger *et al.* 2005). Forman parte del enlace entre la energía acuática y la energía terrestre, es decir, son uno de los eslabones que unen los ecosistemas acuáticos con los ecosistemas terrestres (Larson & Olden 2011). Además de la función ecológica, el cangrejo de río desempeña un importante papel económico y social, sobre todo en algunos países nórdicos como Finlandia. Por ejemplo, en algunas regiones de España, como en las marismas del Guadalquivir, la producción del cangrejo de río es muy elevada y su venta proporciona un aporte extra muy importante a las economías locales (Cano & Ocete 1994). También hay que tener en cuenta que su pesca recreativa ha estado siempre muy arraigada en la cultura popular de la Península Ibérica (Alonso *et al.* 2000).

Estos crustáceos de agua dulce son nocturnos y su nivel de actividad está determinado principalmente por la temperatura del agua (Guan & Wiles 1998, Withnall 2000). Son omnívoros, oportunistas, y con un amplio espectro trófico. Fundamentalmente se alimentan de la materia orgánica presente en los lechos de los ríos, realizando una importante labor detritívora, ya que eliminan los restos muertos de materia orgánica vegetal y animal, aunque también se alimentan de invertebrados bentónicos (Larson & Olden 2011, Guan & Wiles 1998). Ocasionalmente, los cangrejos de río pueden tener un comportamiento caníbal cuando las poblaciones son muy densas o cuando la alimentación disponible es insuficiente (Geiger *et al.*2005, Guan & Wiles 1998, Withnall 2000). Los individuos recién mudados sin caparazón protector son los más vulnerables a los ataques caníbales (Withnall 2000).

Respecto al cangrejo señal *P. lenisuculus*, objeto de estudio de esta tesis, es endémico del noroeste de EE.UU. y suroeste de Canadá. Vive en un amplio rango de hábitats, desde pequeños arroyos hasta grandes ríos y lagos. Presenta mayor capacidad de adaptación que el cangrejo rojo de las marismas a los tramos fluviales altos. Ha sido introducido en Europa y en Japón (Souty-Grosset *et al.* 2006). Está presente en 29 regiones lo que hace que sea la especie invasora de cangrejo de rio más expandida en Europa (Kouba *et al.* 2014). Se introdujo por primera vez en Suecia en 1959 y posteriormente fue expandiéndose por otros países europeos. Las principales razones biológicas de su potencial invasor son su rápido crecimiento y madurez sexual (6–9 cm de longitud total), su comportamiento agresivo y su gran tolerancia a condiciones ambientales adversas como temperaturas extremas, salinidad y contaminación (Souty-Grosset *et al.* 2006).

En España, el cangrejo señal fue introducido entre los años 1974 y 1975 en dos astacifactorías, situadas respectivamente en el río Cifuentes (Guadalajara) y en el río Ucero (Soria), con ejemplares procedentes de la astacifactoría sueca de Simmontorp (Alonso *et al.* 2000). En los años posteriores se hicieron nuevas introducciones, pero en esta ocasión procedían directamente de Norteamérica. Por otro lado, los programas de repoblación de algunas administraciones, como la de Navarra, País Vasco y Castilla y León, facilitaron su expansión (Alonso *et al.* 2000). Actualmente, a pesar de que la distribución del cangrejo señal en la Península Ibérica no es muy extensa, las poblaciones de dicho cangrejo son muy densas en el tercio norte peninsular. En el año 2004 ya estaba presente en Orense, Castilla y León, Cantabria, País Vasco, Navarra, Teruel, Lérida, Castellón, Valencia, Cuenca, Guadalajara, Albacete, Madrid y Granada (Alonso *et al.* 2000).

Como ya se ha mencionado, algunas Comunidades Autónomas llevaron a cabo un programa de introducción del cangrejo señal a partir de los años 80, con el objetivo de crear una “barrera ecológica” entre el cangrejo rojo de las marismas y el cangrejo de patas blancas *A. italicus*. Por otro lado, se pensaba que el cangrejo señal era el “homólogo ecológico” perfecto del cangrejo de patas blancas y, además era resistente a la afanomicosis (Alonso *et al.* 2000). Sin embargo, no hay datos científicos que demuestren que exista tal “barrera ecológica” porque no se ha demostrado que exista ninguna dominancia entre el cangrejo rojo y el cangrejo señal. De hecho, se ha observado que ambas especies pueden vivir en el mismo tramo de un río (Oscoz *et al.* 2008). Además, al igual que el cangrejo rojo de las marismas, el cangrejo señal es portador de *A. astaci*, el agente responsable de la afanomicosis que redujo drásticamente las poblaciones de cangrejo nativas europeas del género *Austropotamobius* y *Astacus*. Este cangrejo produce efectos negativos sobre los ecosistemas fluviales y en particular sobre algas macrófitas, macroinvertebrados, culebras y larvas de anfibios (Johnsen & Taugbøl 2010). Sin embargo, las interacciones existentes entre el cangrejo señal y las comunidades de peces no están bien definidas. Mientras que unos autores sugieren que el cangrejo señal afecta negativamente a las comunidades de peces (por ej. Guan & Wiles 1997), otros señalan que no hay ningún tipo de interacción (por ej. Degerman *et al*. 2006).

Lámina 1. Imágenes de los principales trabajos de campo realizados. Pescas eléctricas, muestreos nocturnos de cangrejo señal *Pacifastacus leniusculus*, grabaciones nocturnas con cámaras de vídeo modificadas para visión nocturna e iluminación infrarroja y caracterización del hábitat.

Impactos y gestión

Las introducciones de especies de cangrejos de río alóctonas en los ríos peninsulares ha sido una fuente de recursos importante para las especies de los niveles tróficos superiores como los peces, aves y mamíferos (Geiger *et al.* 2005). Por otro lado, se ha observado que en estos lugares disminuyen las algas macrófitas, los herbívoros y los carnívoros primarios (Rodríguez *et al.* 2005). Por ejemplo, tras la introducción del cangrejo rojo de las marismas en el lago Chozas (León) se observó una disminución en las poblaciones de aves herbívoras (patos, fochas, etc.), debido a una reducción drástica en su cantidad de alimento y a una pérdida de hábitat importante para anidar (Rodríguez *et al.* 2005). En el caso del cangrejo señal se conoce su carácter invasor por otros estudios (por ej. Johnsen & Taugbøl 2010), pero se desconocen los efectos que tiene sobre las especies acuáticas nativas peninsulares como la trucha común *Salmo trutta* Linnaeus, 1758, la locha *Barbatula quignardi* (Bacescu-Mester, 1967), la lamprehuela *Cobitis calderoni* Bacescu, 1962, el desmán de los pirineos *Galemys pyrenaicus* (Geoffroy, 1811) y los invertebrados bentónicos.

Actualmente, las especies exóticas dominan en muchas partes del mundo (Didham *et al.* 2005). El control de las especies invasoras exige medidas caras y complicadas, y en muchas ocasiones no existen medidas eficaces para controlarlas y mucho menos para erradicarlas (Gherardi 2010). Lamentablemente, aún no sabemos lo bastante sobre los mecanismos que intervienen en las invasiones, por lo que es preciso dedicar mucha más atención y recursos a los estudios sobre el tema.

Como ya se ha comentado, existe multitud de bibliografía que indica que las especies exóticas invasoras de cangrejo de río producen un impacto negativo sobre los ecosistemas fluviales en los que se establecen (Rodríguez *et al.* 2005). Por ello, uno de los objetivos primordiales de las administraciones debería ser la de erradicar las especies introducidas y evitar la entrada de nuevas especies invasoras de cangrejo de río. Sin embargo, en muchas Comunidades Autónomas, ni tan siquiera se ha realizado un seguimiento de los ríos para conocer la distribución de las especies exóticas y nativas.

Otro de los aspectos que se debería contemplar en la gestión es la prevención de nuevas introducciones de especies exóticas de cangrejo. La prevención es muy importante ya que hay que tener presente que una vez que una nueva especie exótica se establece en el medio natural, los mecanismos de erradicación y control son muy complicados debido a que el sistema es muy complejo y es difícil encontrar un método específico que no afecte al resto de la biodiversidad. Finalmente, la educación ambiental también debería ser un pilar muy importante de la gestión si tenemos en cuenta que el hombre es el principal vehículo de la propagación de las especies exóticas de cangrejo de río.

Lámina 2. Algunas de las principales actividades de laboratorio realizadas en esta tesis doctoral. Observación del contenido estomacal de cangrejos, determinación de invertebrados bentónicos y preparación del material para el análisis de los isótopos estables de carbono y nitrógeno.

Presentación de la tesis doctoral

En esta tesis doctoral se abordan las principales relaciones ecológicas existentes entre el cangrejo señal y el medio ambiente que le rodea. En primer lugar, se hace una revisión bibliográfica sobre el estado de conocimiento de las especies de cangrejo de río presentes en la Península Ibérica que sirve de capítulo introductorio (Capítulo 1). Posteriormente se estudia la distribución y abundancia del cangrejo señal en el área de estudio y se relaciona con variables ambientales y de hábitat (Capítulo 2). En el siguiente capítulo, se describe la detección de una nueva relación ectosimbiótica entre el cangrejo rojo de las marismas y el branquiobdélido *X. victoriensis* (Capítulo 3), y posteriormente se estudian las relaciones biológicas que tiene este organismo ectosimbionte con el hospedador (Capítulo 4). Finalmente, se estudia la dieta del cangrejo señal (depredación y competencia trófica) y la competencia por hábitat con especies de peces nativas (Capítulo 5). En definitiva, se abordan las relaciones existentes entre la especie invasora de cangrejo y los factores abióticos y bióticos en los ecosistemas fluviales navarros (Figura 1).

Figura 1. Esquema general de las relaciones ecológicas estudiadas entre el cangrejo señal *Pacifastacus leniusculus* y los factores abióticos y bióticos.

- En el Capítulo 1 se revisa el estado de conocimiento en relación a los cangrejos de río presentes en la Península Ibérica: especies autóctonas y alóctonas, y descripción de sus principales características: origen, taxonomía, área de distribución e impactos asociados. También se analiza la función ecológica y económica de las diferentes especies de cangrejo de río. Finalmente, se revisa la legislación vigente en relación a la gestión de todas las especies presentes en la Península Ibérica y se evalúa las implicaciones que esto conlleva en la conservación de los ecosistemas acuáticos y su fauna nativa.

- En el Capítulo 2 se estudia la distribución y abundancia del cangrejo señal en los principales ríos de la vertiente mediterránea de Navarra y su abundancia relativa se relaciona con variables abióticas y bióticas. La identificación de los requerimientos de hábitat de las especies invasoras es esencial para evaluar su propagación y analizar la vulnerabilidad de los ecosistemas. Se estudia si esta especie es capaz de colonizar los tramos de cabecera donde habitan poblaciones de especies catalogadas por la Lista Roja de la UICN como el Desmán de los Pirineos (Vulnerable) o la lamprehuela (En Peligro) (IUCN 2011).

- En los capítulos 3 y 4 se estudian las relaciones ecológicas entre los cangrejos de río y un ectosimbionte obligado de los mismos. En muchas ocasiones se centra toda la atención sobre las propias especies invasoras, pero se presta muy poca atención sobre las especies que están asociadas a ellas. Se analiza la evolución de la distribución de una especie exótica de gusano ectosimbionte obligado del cangrejo señal, *Xironogiton victoriensis* (Gelder & Hall, 1990), en la Cuenca del Ebro desde el año 2005 al 2013. En estos muestreos, se detecta y describe una nueva asociación biológica a nivel mundial entre el exótico *X. victoriensis* y el cangrejo rojo de las marismas *P. clarkii*, y se alerta de la posibilidad de que infeste a otras especies de cangrejo (Capítulo 3). De este importante descubrimiento surge la necesidad de estudiar en detalle la relación biológica entre el gusano, el hospedador y las condiciones ambientales (Capítulo 4).

- En el capítulo 5 se estudia el comportamiento del cangrejo señal y sus interacciones con las especies nativas de peces [*S. trutta*, *B. quignardi*, *Phoxinus bigerri* Kottelat, 2007, *Gobio lozanoi* Doadrio & Madeira, 2004 y *Parachondrostoma miegii* (Steindachner, 1866)] mediante grabaciones nocturnas con iluminación con focos de infrarrojos y cámaras nocturnas (para no alterar el comportamiento, ya que esta fuente de iluminación es imperceptible por los cangrejos y los peces). También se estudia la dieta del cangrejo señal mediante la observación directa a la lupa del contenido estomacal (previa disección y separación de los estómagos) y el uso de isótopos estables de carbono y nitrógeno.

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CAPÍTULO 1ST CHAPTER

REVISIÓN DEL ESTADO DE CONOCIMIENTO DE LAS ESPECIES DE CANGREJO DE RÍO EN LA PENÍNSULA IBÉRICA

Review of the state of knowledge of crayfish species in the Iberian Peninsula

El Anexo 1 del artículo se encuentra en el Anexo 1 de la tesis (pag. 173)

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Resumen

En este artículo se ha hecho una profunda revisión de toda la información relevante para la gestión de las cuatro especies de cangrejo de río presentes en la Península Ibérica. El cangrejo rojo de las marismas Procambarus clarkii*, el cangrejo señal* Pacifastacus leniusculus *y el cangrejo Yabbie* Cherax destructor *son consideradas especies exóticas invasoras en la legislación española. Se mencionan los impactos negativos más significativos que estas especies están produciendo sobre los ecosistemas acuáticos y se proponen algunos métodos de gestión. Además, se exponen los diferentes estudios científicos relacionados con el origen y taxonomía del cangrejo de patas blancas* Austropotamobius italicus italicus. *Las poblaciones ibéricas de* A. i. italicus *están muy debilitadas a causa de la enfermedad de la afanomicosis que comenzó a expandirse a partir del año 1978. Sin embargo, esta tendencia comenzó a cambiar hace unos pocos años y las poblaciones actualmente están creciendo. Finalmente, se explica y discute la legislación vigente relativa a la conservación y pesca de las especies de cangrejos de río.*

Palabras clave: Austropotamobius italicus, Procambarus clarkii, Pacifastacus leniusculus, Cherax destructor, *especies invasoras, afanomicosis, legislación pesquera.*

Abstract

This paper reviews information relevant to managing the four crayfish species present in the Iberian Peninsula. The red swamp crayfish *Procambarus clarkii*, signal crayfish *Pacifastacus leniusculus*, and Yabbie *Cherax destructor*, are classified as invasive alien species by Spanish legislation. The most significant negative impacts of these species in freshwater ecosystems are noted, and some management methods are proposed. In addition, scientific studies addressing the origin and taxonomy of the Iberian white-clawed crayfish *Austropotamobius italicus italicus* are discussed. The Iberian populations of *A. i. italicus* have been severely diminished since the onset of the expansion of the aphanomycosis disease in 1978. However, this trend began to undergo reversal a few years ago, and the populations of this species are now growing. Finally, current legislation concerning the fishing and conservation of crayfish species is explained and discussed.

Keywords: *Austropotamobius italicus*, *Procambarus clarkii*, *Pacifastacus leniusculus*, *Cherax destructor*, invasive species, aphanomycosis, fishing regulations.

Introduction

The increasing spread of invasive alien species on a global scale is one of the most serious problems facing global biodiversity. In fact, invasive alien species are considered the second most significant cause of extinctions after habitat destruction (IUCN 2011). This problem not only affects biodiversity but also leads to economic, health and social problems.

Aquatic ecosystems are particularly vulnerable to the introduction of invasive alien species. Fifty percent of the invasive species present in the Iberian Peninsula are fishes and crayfishes. In the inland waters of the Iberian Peninsula, in particular, there are at least 45 invasive invertebrate species and 28 invasive vertebrate species (García-Berthou, 2007). Of the 45 invasive species of invertebrates, three are crayfish. These three non-native crayfish species are the red swamp crayfish, *Procambarus clarkii*, signal crayfish, *Pacifastacus leniusculus*, and Yabbie, *Cherax destructor*.

Crayfishes are considered keystone species because they play an important ecological role in freshwater ecosystems. The influence of these crustaceans on ecosystems is more important than might be expected considering their relative abundance or total biomass. These species occupy a central position in the food chains of many ecosystems, simultaneously acting as both prey and predators (Geiger *et al.* 2005). Crayfishes constitute one component of the connecting link between aquatic and terrestrial ecosystems, facilitating the energy flow between them (Larson & Olden, 2011).

In addition, crayfishes play an important economic and social role. For example, in some areas, such as the Guadalquivir marshes, the red swamp crayfish represents an important food resource, and its trade provides an extra contribution to the local economy (Cano & Ocete 1994). Additionally, recreational crayfish fishing is deeply rooted in local culture (Alonso *et al.* 2000).

The specific goals of this paper are as follows: 1) to review the current knowledge of crayfishes in the Iberian Peninsula by describing the main characteristics of native and alien crayfishes in terms of their origin, taxonomy, distribution range and associated impacts; 2) to analyse and evaluate the ecological function performed by crayfish in aquatic ecosystems; 3) to note some commercial, economic and production-related information about crayfish; and 4) to review the existing legislation addressing the management of all crayfish species present in the Iberian Peninsula and assess its implications for aquatic ecosystem conservation.

In summary, the principal aim of this paper is to analyse the current knowledge regarding the conservation status of crayfish conservation status in the Iberian Peninsula to develop a better set of scientific criteria with which to implement adequate management policies.

Materials and methods

The Iberian Peninsula is a geographically isolated area located in southwestern Europe. It is surrounded by the Mediterranean Sea and Atlantic Ocean and is connected to the rest of Europe by the Pyrenees in the northeast. The Iberian Peninsula covers an approximate surface area of 583 254 km^2, and it displays an extensive river system with 12 main hydrographic basins.

More than 150 scientific articles and 15 books were reviewed to evaluate the current state of knowledge about crayfishes and their ecological role in the Iberian Peninsula. This literature search was completed using the ISI Web of Knowledge academic database and Google Scholar search engine. Library resources and printed publications available at the Library of Sciences of the University of Navarra were also employed.

To analyse Iberian and global crayfish stocks and production, the official database and fishery statistics of the Food and Agriculture Organization of the United Nations (FAO 2012) were consulted. This database includes the volume of aquatic species caught by country or area, by species, by major FAO fishing areas and by year for all types of fishing, *i.e.* commercial, industrial, recreational and subsistence. It also includes aquaculture production and the breeding of other types of fish.

Finally, to analyse the existing legislation related to crayfish in the Iberian Peninsula, the following sources were consulted: 1) the “Boletín Oficial del Estado” (BOE) (Official Bulletin of the State), to check whether any of the crayfish species are included in the Spanish Catalogue of Endangered Species or in the Spanish Catalogue of Invasive Alien Species; 2) “El Diário da República Portuguesa” (Diary of the

Portuguese Republic); and 3) the Bulletins of the regional Spanish administrations were checked to obtain information on fishing regulations and the conservation status of crayfish species in the Iberian Peninsula. The Official Bulletins of Andalucía, Aragón, Asturias, Cantabria, Castilla-La Mancha, Castilla y León, Cataluña, Extremadura, Galicia, La Rioja, Madrid, Murcia, Navarra and the Comunidad Valenciana were consulted for this purpose. In the País Vasco, where each province exhibit different legislations, the Official Bulletins of Álava, Vizcaya and Guipúzcoa were analysed separately.

Results

Crayfish species in the Iberian Peninsula

Of the three families of crayfish that exist in the world (Parastacidae, Cambaridae and Astacidae), only the Astacidae family is native to Europe (Souty-Grosset *et al.* 2006). The four crayfish species that live in the Iberian Peninsula are described below.

Austropotamobius italicus italicus (Faxon, 1914)

The white-clawed crayfish, *Austropotamobius italicus italicus*, is endemic to freshwater ecosystems in southwestern Europe (Alonso, 2011). Its populations have been substantially reduced during recent decades, mainly because of the crayfish plague and other factors such as pollution or habitat alteration (Alonso *et al.* 2000). However, this trend has changed because the number of populations has been growing in the last few years (Alonso, 2012). Prior to this decline, *A. i. italicus* was widely distributed throughout peninsular freshwater habitats, except in the more western areas, the highest mountain ranges and the sub-desert areas of the southeast and Ebro River valley (Matallanas *et al.* 2011). This species inhabits rivers, streams and reservoirs with clean and well-oxygenated waters and represents the main food source for many bird, fish and mammal species, thus playing a key role in aquatic ecosystems (Zaccara *et al.* 2004).

The phylogeny, taxonomy, biogeography and origin of *A. i. italicus* have been discussed at length in recent years (Grandjean *et al.* 2002). The purpose of this section is to explain the various scientific studies related to this complex topic. Based on traditional taxonomic analyses, scientists temporarily defined a group of species and subspecies of this crayfish as a specific complex, designated *Austropotamobius pallipes s.l.* (Chiesa *et al.* 2011). Mitochondrial *16S rRNA* analysis and morphological studies show that *A. pallipes* s.l. can be divided into two different species: 1) *A. pallipes*, which is present in France, Switzerland, Germany and the British Isles, and 2) *A. italicus*, which is found in Spain, France, Italy, south of the Alps and the Balkans (Grandjean *et al.* 2000, 2002, Zaccara *et al.* 2004, Fratini *et al.* 2005, Schulz & Grandjean 2005, Trontelj *et al.* 2005). The distribution range of the Iberian white-clawed crayfish differs if we consider this specific complex to be separated into one or two species.

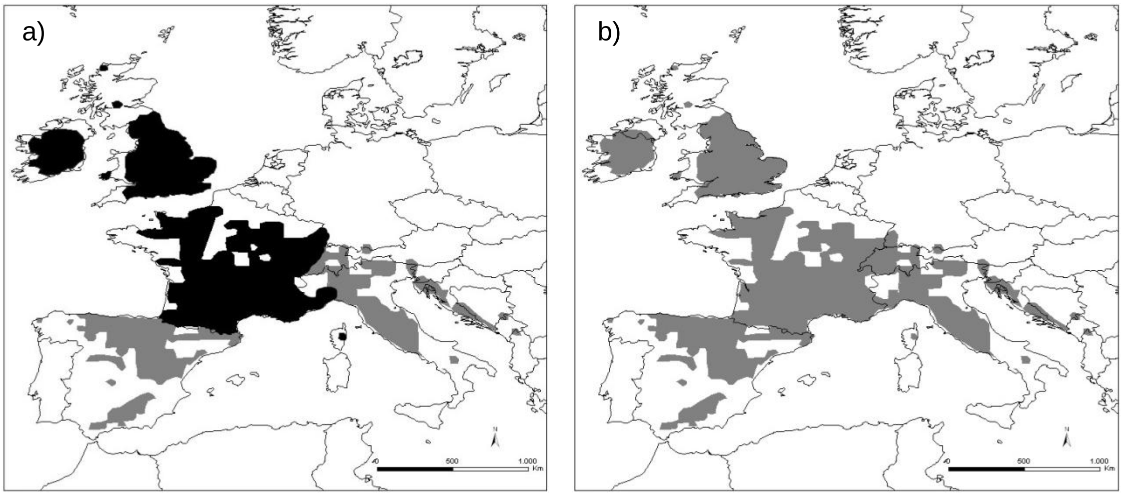

Figure 1. a) Distribution range of the genus *Austropotamobius* in Europe according to Grandjean *et al.* (2002). Black colour indicates the *Austropotamobius pallipes* distribution range, and grey colour represents the *Austropotamobius italicus* distribution range. b) The *A. pallipes* distribution range in Europe according to Souty-Grosset *et al.* (2006).

Distribución del género Austropotamobius *en Europa de acuerdo con Grandjean* et al. *(2002). El color negro indica el rango de distribución de* Austropotamobius pallipes *y el color gris representa el rango de distribución de* Austropotamobius italicus. *(b) Rango de distribución de* A. pallipes *en Europa de acuerdo con Souty-Grosset* et al. *(2006).*

Figure 1a shows the global distribution of *A. italicus* and *A. pallipes* (Grandjean *et al.* 2002, Trontelj *et al.* 2005, Alonso, 2011). In addition, *A. italicus* exhibits a high intraspecific genetic oscillation, separating it into three (Grandjean *et al.* 2002) or four (Fratini *et al.* 2005) subspecies, with *A. italicus italicus* being the subspecies found in the Iberian Peninsula (Grandjean *et al.* 2002, Fratini *et al.* 2005). This subspecies is distributed in Italy, southern Switzerland and Spain (Fratini *et al.* 2005). In contrast, figure 1b shows the global distribution of *A. pallipes* when it is considered as a single species in Europe (Souty-Grosset *et al.* 2006, Chiesa *et al.* 2011). *A. pallipes* and *A. italicus* present significant differences in their *16S rRNA* mitochondrial genes.

Grandjean *et al.* (2002) observed a difference of 3.6-5.4% between the two species, and a difference of 3.5% was detected by Ghia *et al.* (2006). Diéguez-Uribeondo *et al.* (2008) & Trontelj *et al.* (2005) observed mitochondrial *DNA* variations of 11.3% and 7.0%, respectively. It is estimated that 2.75% variation in the *16S rRNA* mitochondrial gene sequence indicates isolation of one million years between species. Therefore, these two species could have been isolated over two million years ago (Grandjean *et al.* 2002, Schulz & Grandjean 2005). Due to its wider acceptance, the taxonomic hypothesis represented in figure 1a will be employed in the present paper.

The origin of the Iberian populations of the white-clawed crayfish (*A. i. italicus*) has been widely discussed in the scientific community. Some authors believe that the Iberian populations of *A. i. italicus* were the result of human translocations coming from northwestern Italy in the 17th century. The first molecular studies based on mitochondrial data found very low genetic diversity within the Iberian populations of *A. i. italicus,* in addition to shared haplotypes and non-significant differentiation between the Iberian and the Italian sub-species (Grandjean *et al.* 2000, 2002, Fratini *et al.* 2005, Trontelj *et al.* 2005). Moreover, the absence of historical sources and fossil records supports this hypothesis, given the importance of this crustacean in other European cultures. The “Relaciones Topográficas de Felipe II” is an exhaustive record of Spanish fisheries and their exploitation that was registered for all the areas under the king's mandate, but no specific mention of the presence of this crayfish were found in this resource (Miguel Clavero, pers. comm., Campos 2003). The first mention of crayfish in the Iberian Peninsula was made in the Calatayud (Asso, 1784). In the geographical-statistical-historical Dictionary of Spain written by Madoz (1845-1850), crayfish were cited as a fishery resource in the Ebro basin and in some localities situated in the Duero and Tajo basins (Maldonado *et al.* 2008). Moreover, none of the main languages spoken in the Iberian Peninsula has a specific vernacular name for crayfishes. They are referred to as “cangrejos de río” (literally "river crabs") in Spanish, with similar translations being found in Basque, Catalan and Galician, or “lagostim” in Portuguese (a generic name for all the species of Astacidea group), most likely related to their similarity with some types of marine shrimps.

Other authors consider *A. i. italicus* to be the only native crayfish species in the Iberian Peninsula (Beroiz *et al.* 2008, Diéguez-Uribeondo *et al.* 2008, Pedraza-Lara *et* *al.* 2010, Matallanas *et al.* 2011, 2012, Alonso 2011). Beroiz *et al.* (2008) used RAPD-PCR to show that there was higher genetic variability in Spanish populations of the white-clawed crayfish than reported by previous authors, who employed different markers. Mitochondrial analyses also suggest a possible natural origin for this species as a consequence of a complex biogeographic and demographic history and further recent natural or human-mediated processes that might have shaped its current genetic structure (Pedraza-Lara *et al.* 2010). Genetic analyses of peninsular populations employing nuclear and mitochondrial markers (*COI* and *16S rDNA*) have shown similar or even higher genetic diversity compared to that reported in previous studies on other Spanish and European populations (Matallanas *et al.* 2011, 2012). Eight different mitochondrial *DNA* haplotypes have been found in this species, and it would be impossible to explain this degree of genetic diversity as having occurred within a few hundreds of years (Matallanas *et al.* 2011).

The source of the crayfish plague (aphanomycosis) is the water mould *Aphanomyces astaci* (Schicora, 1906). This mould is included among the 100 worst invasive species in the world (Lowe *et al.* 2000). This pathogen has decreased the populations of native European crayfish species during the past several decades, especially since the introduction of non-native crayfishes infected with the pathogen. However, other factors such as saprolegniasis, pollution, habitat alteration, climatic drought and overfishing also affect white-clawed crayfish populations (Diéguez-Uribeondo *et al.* 1997, Alonso *et al.* 2000, Edgerton *et al.* 2002).

The *A. i. italicus* populations found in Spain started to decrease dramatically in 1978 (Diéguez-Uribeondo *et al.* 1997). According to Alonso *et al.* (2000), the decline of these crayfish populations was more pronounced in the central and southern areas of the Iberian Peninsula. Between 1990 and 2000, there were an estimated 700 remaining populations of white-clawed crayfish (Alonso *et al.* 2000). However, this negative trend showed a change at the beginning of this century as a consequence of administrative restoration plans, and a total of 1086 populations were estimated to inhabit this region in 2010 (Alonso, 2011). However, these populations remain fragmented and restricted to small river sections, which are usually isolated from the main stream where pathogen-carrying alien crayfish are present, and their average individual size is decreasing.

It is believed that the introduction of aphanomycosis was spatially and temporally linked to the first introduction of North American crayfishes to Europe (Diéguez-Uribeondo *et al.* 1997). The first isolation of *A. astaci* obtained from white-clawed crayfish corpses in Spain (Burgos and Álava) came from signal crayfish. Thus, North American crayfish introductions could have been the route through which this disease entered the Iberian Peninsula (Diéguez-Uribeondo *et al.* 1997).

The American crayfishes, *Pacifastacus leniusculus* and *Procambarus clarkii*, serve as vectors of the aphanomycosis pathogen during their whole lifespan (Diéguez-Uribeondo *et al.* 1997, Edgerton *et al.* 2002, ISSG 2011, Longshaw 2011). However, American crayfishes are resistant to this disease as a result of coevolution between the pathogen and host. As a consequence of this coevolution, the pathogen is restricted exclusively to the cuticle of these crayfishes, except when they experience stress (Diéguez-Uribeondo *et al.* 1997, Edgerton *et al.* 2002, Longshaw 2011).

This pathogen exhibits three different stages: the mycelium, zoospore and cyst stages (Oidtmann *et al.* 2002). Many zoospores are formed when an infected crayfish is dying or has recently died. These zoospores constitute the dispersion structures of the pathogen. They are able to survive for up to two weeks in the mud, and they are motile in the water for three days at 10 ºC (Edgerton *et al.* 2002). Nevertheless, it is difficult for this pathogen to survive for a long period of time in a natural environment in the absence of a suitable host. Its spores and mycelium die when exposed to extreme temperatures and desiccation (Oidtmann *et al.* 2002). Sodium hypochlorite, $MgCl_2$, iodine and malachite green can be used to sterilise and/or reduce sporulation (Edgerton *et al.* 2002), although Oidtmann *et al.* (2002) suggested that the best decontamination method is to boil materials at 100 ºC for one minute.

The infection generated by *A. astaci* can be spread easily from one river basin to another through the movements of crayfishes and fishes or when attached to contaminated equipment, such as nets, boots, clothes and other items (*e.g.* Oidtmann *et al.* 2002). Although it is difficult to determine whether a crayfish is infected, symptoms such as brown melanisation of the exoskeleton or whitening of the tail muscle are sometimes observed (Edgerton *et al.* 2002). Once the infection has begun, crayfish usually die after one or two weeks (Edgerton *et al.* 2002).

The protection status of the white-clawed crayfish varies among different entities. This species has been catalogued as endangered in the World Red List (IUCN 2011) since 2010 (it was previously catalogued as vulnerable). In Europe, it is included in the Annex II-species of community interest, meaning that its conservation requires the designation of Special Areas of Conservation (SAC), Annex V of the Habitats Directive and Annex III of the Berne Convention. In Spain, *A. i. italicus* is catalogued as vulnerable in the National Catalogue of Endangered Species, and its protection status differs among different regional administrations.

Procambarus clarkii (Girard, 1852)

The red swamp crayfish, *Procambarus clarkii*, is native to the wetlands of the southern United States of America (U.S.A.) and northeastern Mexico (Larson & Olden, 2011). It is the most invasive crayfish species in the world and is found in Africa, Asia, Europe and North and South America (Lowe *et al.* 2000, Larson & Olden 2011). The features responsible for the high invasive potential of this species are its high fecundity, rapid growth, short life cycle, high aggressiveness and ability to adapt to poor environmental conditions (*e.g.* low oxygen, salinity, acidity and pollution) and to tolerate sudden changes in water levels (*e.g.* Geiger *et al.* 2005). With regard to this last characteristic, it is important to note that *P. clarkii* can withstand long periods of drought by excavating galleries more than 2 m deep to increase its proximity to the water table, where the humidity is higher (Barbaresi & Gherardi 2000, Souty-Grosset *et al.* 2006). In summary, this invasive species can survive in a wide range of environmental conditions and habitats, such as crop areas, lakes, canals, rivers, marshes and swamps (ISSG 2011).

The red swamp crayfish is native to Louisiana marshes (U.S.A.), and its first introduction to Europe occurred in southern Spain. It was introduced for aquaculture production between 1972 and 1974 in the marshes of the Guadalquivir River and quickly established itself in the wild (Barbaresi & Gherardi 2000, Geiger *et al.* 2005, Souty-Grosset *et al.* 2006, Holdich *et al.* 2009). Several years later, *P. clarkii* colonised other areas of the Iberian Peninsula, such as the Albufera of Valencia (1978), the Ebro delta, Zamora (1979) and Lugo (Alonso *et al.* 2000). Human translocations and the sale of live specimens at fish markets supported its expansion (Geiger *et al.* 2005, Souty-

Grosset *et al.* 2006). *P. clarkii* is currently the most abundant crayfish species in the Iberian Peninsula (Alonso *et al.* 2000, Souty-Grosset *et al.* 2006), showing especially high abundance in the middle and lower stretches of rivers (Alonso *et al.* 2000, Oscoz *et al.* 2008). Figure 2a shows that most of its European populations are found in the Iberian Peninsula.

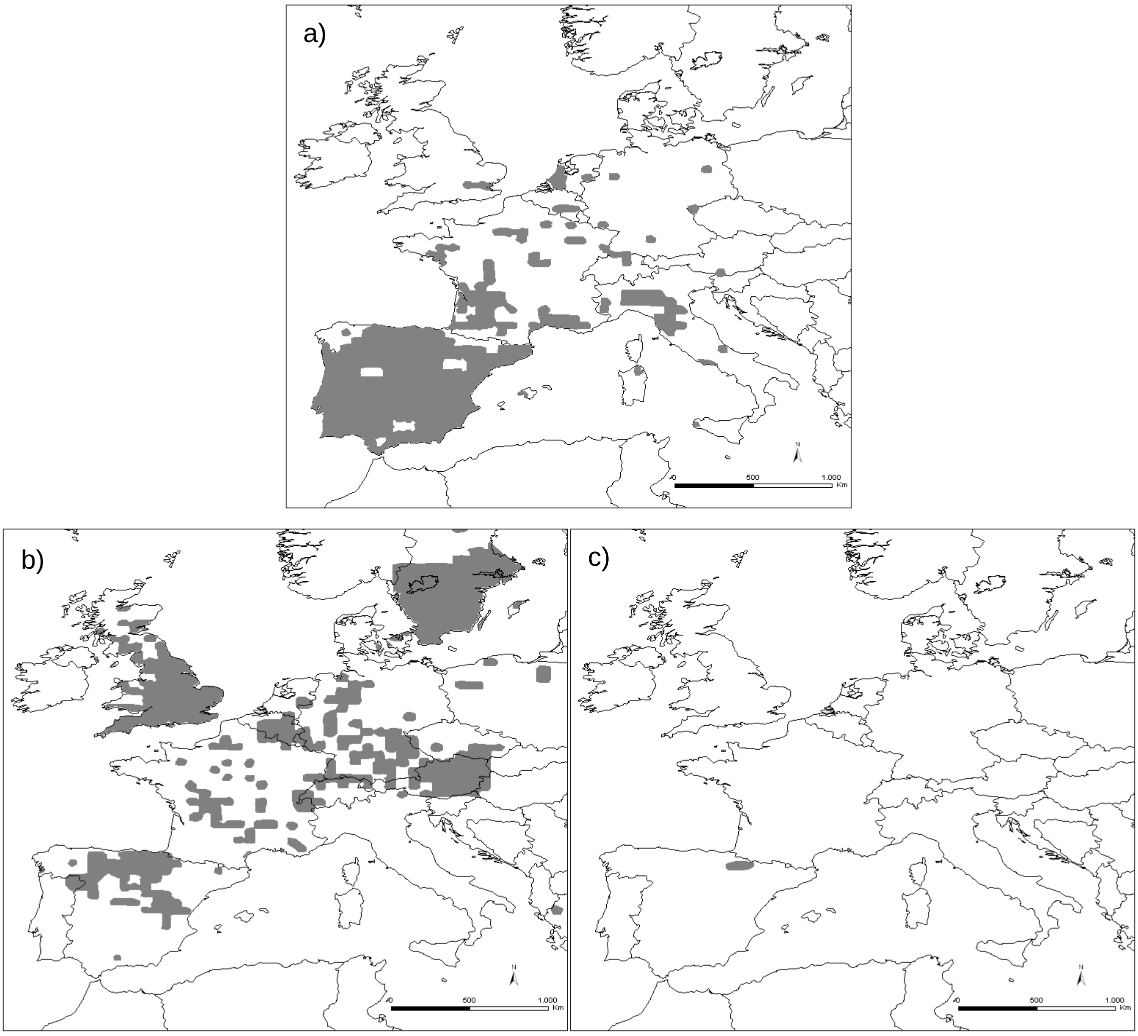

Figure 2. a) Red swamp crayfish, *Procambarus clarkii*, b) signal crayfish, *Pacifastacus leniusculus*, and c) Yabbie, *Cherax destructor* distribution range in Europe. The signal crayfish distribution range in Norway, Slovakia, Croatia, Estonia and Denmark is not represented on the map; © Scientific publications MNHN, Paris, 2006 (modified from Holdich *et al.* 2009 for *P. clarkii* and *P. leniusculus* and from Souty-Grosset *et al.* 2006 for *C. destructor*).

Distribución del cangrejo rojo de las marismas Procambarus clarkii*, cangrejo señal* Pacifastacus leniusculus *y Yabbie* Cherax destructor *en Europa. El área de distribución del cangrejo señal en Noruega, Eslovaquia, Croacia, Estonia y Dinamarca no está representada en el mapa © Publicaciones científicas MNHN, Paris, 2006 (modificado de Holdich* et al. *2009 para* P. clarkii *y* P. leniusculus *y de Souty-Grosset* et al. *2006 para* C. destructor*).*

The ecological characteristics of the red swamp crayfish have triggered a series of negative socio-economic, health and environmental impacts since its introduction to the Iberian Peninsula (Geiger *et al.* 2005, Larson & Olden 2011). Furthermore, its introduction to the lower Guadalquivir has caused a conflict of interests: *P. clarkii* produces high economic losses and is responsible for massive devastation of rice crops, but is also an important source of wealth as a fishing resource (Cano & Ocete 1994).

As noted above, rice crops are severely impacted by the red swamp crayfish. These impacts are produced indirectly as a consequence of the abundant galleries that this species digs within the cultivation areas of this crop, thus damaging the water drainage capability of these fields (Barbaresi & Gherardi 2000). In addition, the red swamp crayfish feeds on rice, reducing the germination and establishment of rice seedlings (Cano & Ocete 1994, Souty-Grosset *et al.* 2006). It also damages various types of infrastructure, such as water canals, bridges, roads and highways (Cano & Ocete 1994).

In 1997, a tularemia outbreak occurred in the province of Cuenca in association with *P. clarkii* handling (Díaz de Tuesta *et al.* 2001). *Francisella tularensis tularensis* (McCoy & Chapin, 1912) is the bacterium that causes this serious infectious disease. This bacterium has been found in the stomach, hepatopancreas and water of *P. clarkii*, but not in its exoskeleton. These findings indicate that the red swamp crayfish is not a direct transmission vector of tularemia, and the most likely cause of this outbreak was the contamination of river water with animals killed by tularemia (lagomorphs), which then also infected *P. clarkii*.

Furthermore, this non-native species generates negative environmental impacts on macrophytic algae, amphibians, molluscs and macroinvertebrate diversity in aquatic ecosystems (Rodríguez *et al.* 2005, Souty-Grosset *et al.* 2006, Correia *et al.* 2005, 2007). Some authors suggest that the red swamp crayfish increases water turbidity, hence reducing light penetration and macrophytic algae productivity (Geiger *et al.* 2005, Larson & Olden 2011, Marchi *et al.* 2011). It also modifies biogeochemical cycles and unbalances the food chain, decreasing the complexity of ecosystems (Marchi *et al.* 2011).

Pacifastacus leniusculus (Dana, 1852)

The signal crayfish, *Pacifastacus leniusculus*, is endemic to the northwestern U.S.A. and southwestern Canada and inhabits a wide range of habitats, from small streams to large rivers and lakes. This species is found in 24 European countries and is the most widespread alien species in Europe (Souty-Grosset *et al.* 2006, Holdich *et al.* 2009, Johnsen & Taugbøl 2010) (Figure 2b). The signal crayfish is better adapted than the red swamp crayfish to upstream stretches. This crustacean exhibits higher rates of growth and sexual maturation than the white-clawed crayfish, but lower rates than the red swamp crayfish. Its ecological characteristics include tolerance of salty water, a wide temperature range, resistance to pH levels above six and a high capacity for enduring long periods out of water (Souty-Grosset *et al.* 2006).

The signal crayfish was introduced to Spain between 1974 and 1975 at two crayfish hatcheries, which were located in the Cifuentes River (Guadalajara) and Ucero River (Soria), respectively, with the introduced individuals coming from Simmontorp, a Swedish crayfish hatchery (Alonso *et al.* 2000). In later years, new signal crayfish introductions were carried out, but with the individuals involved coming directly from North America. The restocking programs of some administrations, such as Navarra, País Vasco and Castilla y León, facilitated its expansion (Alonso *et al.* 2000). By 2004, it was present in Orense, Castilla y León, Cantabria, País Vasco, Navarra, Teruel, Lleida, Castellón, Comunidad Valenciana, Cuenca, Guadalajara, Albacete, Madrid and Granada. The first reference to signal crayfish populations in southern Spain in the literature comes from 2000, in the Riofrio River (Loja, Granada) (Dana *et al.* 2010), though its presence in other southern locations has been reported as well. While the distribution range of signal crayfish in the Iberian Peninsula is not currently as wide as the distribution of the red swamp crayfish (Figure 2b), its populations are undergoing expansion in the central peninsula as a result of illegal translocations. The fishing legislations related to this species have been changing. The initial mode of management was fishing area limitations, whereas free fishing is currently allowed (Annex 1, www. limnetica.net/internet).

As mentioned previously, some regional administrations conducted a signal crayfish introduction program in the 1980s with the aim of creating a "green barrier" between the red swamp crayfish and the white-clawed crayfish (Cuéllar & Cuéllar

2000). The signal crayfish was thought to represent the perfect "ecological counterpart" of the white-clawed crayfish and to be resistant to aphanomycosis. Nevertheless, there is no scientific evidence supporting the actual creation of this "green barrier", as both species, *P. clarkii* and *P. leniusculus*, have been observed to coexist within the same stretch of river without any appreciable difficulties (Oscoz *et al.* 2008, Larson & Olden 2011). In fact, Alonso & Martínez (2006) studied shelter competition between red swamp crayfish and signal crayfish under laboratory conditions, and in most experiments, the red swamp crayfish was both the first species to be observed in a shelter (70.8%) and the long-term winner (62.5%).

Additionally, *P. leniusculus* also carries the pathogen responsible for crayfish plague in its cuticle. The introduction of these non-native crayfish species has further complicated the situation because they were introduced together with branchiobdellidan species. The branchiobdellidans, or crayfish worms, are obligate ectosymbionts related to both oligochaetes and hirudinids (Souty-Grosset *et al.* 2006). There are seven endemic European species of Branchiobdellidae belonging to the genus *Branchiobdella*. In some river stretches, signal crayfish are infected by a non-indigenous species of Branchiobdellidae, *Xironogiton victoriensis* (Gelder & Hall, 1990) (Oscoz *et al.* 2010). This species causes a negative impact among fishermen who reject “infected” individuals for consumption.

The signal crayfish also produces negative effects in macrophytes, macroinvertebrates, snakes, benthic fishes and amphibian larvae (Johnsen & Taugbøl 2010).

Cherax destructor (Clark, 1936)

The Yabbie, *Cherax destructor* is native to eastern Australia and is found in New South Wales, Victoria and South Australia (Souty-Grosset *et al.* 2006). This crustacean inhabits marshes, streams, rivers and reservoirs with sandy and muddy bottoms where the water exhibits a moderate turbidity level, thus offering better protection from predators such as fish and birds (Withnall 2000). The Yabbie can withstand temperatures between 1 ºC and 35 ºC, with its optimum range for growth falling between 20 ºC and 30 ºC. When the water temperature drops below 16 °C, its metabolism decreases to a level corresponding to partial hibernation (Withnall 2000).

The first introduction of *C. destructor* into Spain and Europe took place in Girona (Catalonia) in 1983, involving specimens from California (Souty-Grosset *et al.* 2006). Subsequently (1984-1985), another introduction was conducted in the province of Zaragoza, where a population was eventually stabilised in Gordués, near the Sos del Rey Católico (Holdich *et al.* 2009). Because *C. destructor* is sensitive to aphanomycosis, some of its populations have been eradicated using the crayfish plague pathogen. However, some populations persist to the present day (Holdich *et al.* 2009). It is believed that there are at least four stable populations in Spain: one in Aragon and three in Navarra (Souty-Grosset *et al.* 2006) (Figure 2c). Moreover, this species has been detected in other European countries in recent years. For example, a population was detected in Italy in 2008, and one individual was reported in Lake Geneva, Switzerland (Scalici *et al.* 2009).

The Yabbie is commercially produced in some regions of Australia (New South Wales, Victoria and South Australia), and it is considered a delicacy and sold live in North America, Switzerland, Germany and England (Souty-Grosset *et al.* 2006).

This species displays highly aggressive, competitive and predatory behaviour. Similar to the American crayfishes (*P. clarkii* and *P. leniusculus*), *C. destructor* is able to dig a network of tunnels with depths of 0.5 m to 2 m to survive dry summer periods (Withnall, 2000). These galleries damage crops, lawns, dams and irrigation canals (Gherardi, 2007).

The Yabbie is considered a serious threat to native *Cherax tenuimanus* populations in western Australia because of its high reproductive potential and high tolerance to various environmental conditions. For these reasons, this invasive species is considered a serious threat to European aquatic ecosystems (Souty-Grosset *et al.* 2006).

Impacts and management of crayfishes

The introduction of non-native crayfishes to freshwater ecosystems has provided an important food resource for species at higher trophic levels, such as fish, birds and mammals (Geiger *et al.* 2005). Energy from detritus is transferred directly to higher trophic levels, thereby reducing the number of trophic levels. Consequently, a decline in the importance of macrophyte algae, herbivores and primary carnivores takes place, altering the overall structure and function of aquatic food chains (Rodríguez *et al.* 2005, Geiger *et al.* 2005).

The red swamp crayfish exhibits more herbivorous habits than the white-clawed crayfish, thus producing significant changes in aquatic ecosystems (Rodríguez *et al.* 2005). For example, following the introduction of the red swamp crayfish into Lake Chozas (León), a decline in the populations of herbivorous birds (*e.g.* ducks, coots) was observed due to a reduction of food and nesting habitats (Rodríguez *et al.* 2005).

Furthermore, there are studies showing that non-native crayfishes have threatened and even eradicated amphibian species in many rivers and lakes in the Iberian Peninsula (Ilhéu *et al.* 2007). For example, Rodríguez *et al.* (2005) found that four frog species disappeared after the introduction of *P. clarkii*.

Nevertheless, the presence of non-native crayfishes has a positive impact in some cases on certain fish-eating species, such as the otter *Lutra lutra* L., the bittern *Botarus stellaris* L., the little egret, *Egretta garzetta* L., and the heron *Ardea purpurea* L. (Barbaresi & Gherardi, 2000, Correia *et al.* 2005, Geiger *et al.* 2005, Rodríguez *et al.* 2005).

Eradication methods could become an alternative means of eliminating non-native crayfishes, although such methods are not always effective (Peay & Hiley 2001). For example, applying heavy fishing pressure could appear to be a good control mechanism for these populations *a priori*, although this increased pressure may have negative effects because fishermen often act as dispersal agents (Alonso *et al.* 2000). A total of 25 492 signal crayfishes were eliminated from the Riofrío (Loja, Granada) between 2005 and 2008, as shown by the “Plan Andaluz para el Control de las Especies Exóticas Invasoras”. In eliminating these crayfishes, workers used a combination of methods, such as minnow traps, electrofishing, hand catching and artificial shelters. Thus, the size of the signal crayfish population and the risk of its dispersion were dramatically minimised, as the population was brought near or below the minimum size for reproduction. However, other studies have suggested that manual removal has not effective been at any level of effort (*e.g.* Peay & Hiley 2001). To control non-native crayfish populations, management decisions should be focused on controlling the main factor involved in their expansion, which is human translocations. Nevertheless, other effective methods can be applied. Corkum (2004) and Stebbing *et al.* (2003) have suggested that capturing signal crayfish using pheromones could become a widely applied eradication method in the future because pheromones also act as repellents for local crayfish populations (Johnsen & Taugbøl 2010). The possibility of isolating and developing a biological control agent specifically to influence invasive species should be considered (Freeman *et al.* 2007). Additionally, the construction of dams has proven to be effective in restraining the expansion of *P. clarkii* to headwaters, thereby preventing new aphanomycosis infections in white-clawed crayfish populations (Dana *et al.* 2011). Peay & Hiley (2001) considered manual removal, habitat destruction, barriers, predators, diseases, pheromones and biocides for the eradication of signal crayfish populations in the UK. They concluded that the eradication or control of a signal crayfish population is only likely to be achieved through chemical control (biocides). Peay *et al.* (2006) studied a new methodology based on the use of natural pyrethrum for eradicating signal crayfish in ponds.

For the eradication of *C. destructor,* Scalici *et al.* (2009) proposed that direct pathogen application or the introduction of infected crayfish should be implemented. However, these methods may represent a high risk to the local populations of *A. i. italicus*, which is also susceptible to the disease (Oscoz *et al.* 2010). Thus, following this procedure to eradicate *C. destructor* would only be carried out in areas where no evidence of the white-clawed crayfish species is found. Although low water temperatures constitute natural barriers to the spread of this species, there is no way to prevent human translocations, which are the main means of the spreading of all crayfish species (Scalici *et al.* 2009).

Global and Spanish crayfish production

According to FAO statistics, the red swamp crayfish has been the species with the highest global production since the 1970s (ISSG 2011, FAO 2012). The global production of *A. i. italicus* and *A. pallipes* during the 1950s, 1960s and 1970s was also remarkable, considering that their range was limited to a small region in Europe (Figure 1). For example, an average of 1380 *t* of *A. i. italicus* and *A. pallipes* was produced annually during the 1950s, compared to 16 *t* of *P. clarkii*. However, this pattern was dramatically reversed a few years later, when the production of *A. i. italicus* and *A.*

pallipes decreased dramatically, mainly in response to aphanomycosis disease. In contrast, the production of *P. clarkii* increased exponentially, reaching annual global production of 173 938 *t* (Table 1). In contrast to the red swamp crayfish, there has historically been little or no global production of *P. leniusculus* and *C. destructor*, although there has been an increasing trend in the last three decades.

European crayfish production is lower than Asian or American production. Nevertheless, the crayfish market has been very important to the economy of some Spanish regions (Cano & Ocete 1994, Souty-Grosset *et al.* 2006) (Table 1). Until 2002, almost all production data for *P. clarkii* came from America (U.S.A.) and, to a lesser extent, from Europe. However, the FAO started recording production statistics in Asia (China) in 2003, and the global production data were multiplied six times (Table 1).

Table 1. Evolution of the average annual production of crayfish species present in the Iberian Peninsula by countries (tonnes, *t*) (FAO 2012).

Evolución de la producción promedio anual por países (toneladas, t) de las especies de cangrejo de río presentes en la Península Ibérica (FAO 2012).

COUNTRY	SPECIES	50-59	60-69	70-79	80-89	90-99	00-09	2010
Kenya	*P. clarkii*				49	33	17	20
U.S.A.	*P. clarkii*	16	242	3775	28 663	24 438	33 995	52 942
China	*P. clarkii*						139 726	563 281
Indonesia	*C. destructor*						2	8
Spain & Italy	*P. clarkii*			59	2533	2263	200	1509
Finland	*P. leniusculus*				1.7	5.8	137	226
	A. italicus	1380	440	679	37			
Australia	*C. destructor*				5	185	143	51

There is no other place in America where the red swamp crayfish has as great an impact on the economy as it has in the southern states. This species is produced and consumed as food in several areas. The state of Louisiana dominates both aquaculture and wild capture fisheries of this crayfish, which contribute over 150 million dollars annually to the state economy. In Louisiana, approximately 48 000 ha are used to cultivate this crustacean, representing 90-95% of U.S. production (McAlain & Romaire 2007). Nevertheless, China is the largest crayfish producer in the world, with a 2010 total production that was ten times higher than that of the U.S.A. (Table 1).

Figure 3 shows a chronology of the annual production of the white-clawed crayfish and red swamp crayfish in Spain in tonnes (*t*). Since data have been available (starting in 1980), *A. i. italicus* has shown an annual production of between 500 *t* and 3000 *t*. However, the production of the signal crayfish was only one tonne per year between 1998 and 2002. The first production data for the red swamp crayfish in Spain were obtained in 1977, indicating an annual production of 22 *t*. Subsequently, the annual production of this species increased to 400 *t* in 1980 and 5662 *t* in 1991 (Figure 3). In recent years, red swamp crayfish production has been estimated to be 1500 *t* per year, although this is most likely an underestimation.

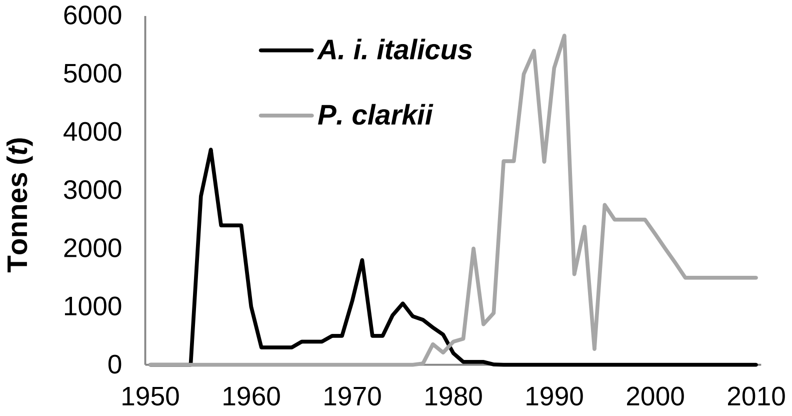

Figure 3. Evolution of the annual production (tonnes, *t*) of *A. i. italicus* and *P. clarkii* in the Iberian Peninsula between the years 1950 and 2010 (FAO 2012).

Evolución de la producción anual (toneladas, t) de A. i. italicus y P. clarkii en la Península Ibérica entre los años 1950 y 2010 (FAO 2012).

The Ministry of Agriculture, Fisheries and Food (2007) reported an index of commercialisation for the red swamp crayfish of 2 or 3 *t*/year in El Palmar (Valencia) and more than 6 *t*/year in Catalonia between 2005 and 2006. Additionally, the region of Castilla y León produces a total of $3 \cdot 10^5$ crayfish per year, with a value of 57 660 Euros.

Table 2. Summary of crayfish species present in the Iberian Peninsula and catalogued in the world (IUCN), Spain (Real Ordinance 139/2011 and 1628/2011) and Portugal (Ordinance-Law nº 49/2005 and Ordinance-Law 565/99). LC: least concern; VU: vulnerable; EN: endangered; S: south; NE: northeast; NW: northwest; E: east.

Tabla resumen de las especies de cangrejo de río presentes en la Península Ibérica y su catalogación mundial (IUCN), española (Real Decreto 139/2011 y 1628/2011) y portuguesa (Decreto-Ley nº 49/2005 y Decreto-Ley 565/99). LC: preocupación menor; VU: vulnerable; EN: en peligro; S: sur; NE: noreste; NW: noroeste; E: este.

	*A. i. italicus**	*P. clarkii*	*P. leniusculus*	*C. destructor*
Origin	SW Europe	S U.S.A., NE Mexico	NW U.S.A. and SW Canada	E Australia
Family	Astacidae	Cambaridae	Astacidae	Parastacidae
Year of introduction	-	1973-1974	1974-1975	1984-1985
Aphanomycosis	Sensitive	Resistant (carrier)	Resistant (carrier)	Sensitive
Global legislation	EN	LC	LC	VU
Spanish legislation	VU	*Invasive*	*Invasive*	*Invasive*
Portuguese legislation	Community interest	*Invasive*	*Exotic*	-

* Referring to *A. pallipes*.

Legislation and commercialisation

The legislation addressing white-clawed crayfish conservation is complex because it includes regulations related to conservation, national- and regional-scale commercialisation and fishing regulations at the provincial level. While many of the regional administrations have developed their own laws for fishing and conservation, the marketing of this species is still nationally regulated. Limitation in live crayfish commercialisation is important for the control of non-native crayfish populations. Although the sale of red swamp crayfish is allowed in Spain, the commercialisation of signal crayfish is not permitted by national legislation. This prohibition could explain the different patterns of dispersion observed for the two species.

Management and conservation

Table 2 shows the global, Spanish and Portuguese cataloguing of crayfish species that inhabit the Iberian Peninsula. Furthermore, it includes the most important features of each species. Annex 1 (supplementary material: www.limnetica.net/internet) summarises the principal laws for these species included in the local and national legislation of Spain and Portugal.

Fishing regulations

All the information concerning authorisations regarding fish sizes, numbers of catches and fishing periods for the red swamp crayfish and signal crayfish is provided within the same Annex.

Discussion

The origin of Iberian *A. i. italicus* populations has been a topic of debate in recent years. First, it is essential to clarify whether *A. i. italicus* is an introduced species or the only native species of crayfish in the Iberian Peninsula, as crayfish management policies should be completely different depending on the answer to this question. Some authors believe that the Iberian populations of this species are the result of human translocations from Italy because mitochondrial data have shown very low genetic diversity between Iberian and Italian populations (Grandjean *et al.* 2002, Fratini *et al.* 2005, Trontelj *et al.* 2005). In addition, certain authors believe that there are no fossils or historical references available to help track the biogeographic evolution of this species (Madoz 1845-1850). Nevertheless, there are many reviews that criticise these studies due to the small number of samples used (eight populations and only one individual for each one). According to Chiesa *et al.* (2011), the mitochondrial gene sequencing method used by most authors to study the phylogeny of the *Austropotamobius* genus is not reliable, and they argue that this technique only provides a partial view because it solely reflects the maternal phylogeny. Furthermore, these studies did not consider the major reduction of population sizes and subsequent human translocations of Iberian *A. i. italicus*, which could also reduce their genetic diversity, causing a bottleneck (Diéguez-Uribeondo *et al.* 2008, Pedraza-Lara *et al.* 2010). In addition, new references to the white-clawed crayfish in the Iberian Peninsula have been found in medieval and Arabic texts (Diéguez-Uribeondo *et al.* 2008). The question of vernacular names complicates the situation but does not prove that the white-clawed crayfish is introduced. Other recent studies have shown that this species could be native to the Iberian Peninsula because the genetics of its peninsular populations differ sufficiently from its Italian populations to infer that their differentiation occurred more than a few hundreds of years ago (Beroiz *et al.* 2008, Pedraza-Lara *et al.* 2010, Matallanas *et al.* 2011, 2012). Thus, these authors suggest that there are no strong arguments calling into question the indigenous status of the white-clawed crayfish.

There is a long-standing consensus suggesting that the main cause for the decline of *A. i. italicus* populations has been the *Aphanomyces astaci* water mould. The life cycle and dispersal mechanisms of *Aphanomyces astaci* are well known, and it is considered to be a pathogen that requires a host to survive. Thus, it is important to implement management measures to account for the fact that non-native crayfishes, specifically *Procambarus clarkii* and *Pacifastacus leniusculus*, are carriers of the crayfish plague. However, *C. destructor* and *A. i. italicus* are sensitive to crayfish plague.

The protection status of the white-clawed crayfish differs among the different regional administrations of Spain. *A. i. italicus* is catalogued as "endangered" in nine regional administrations and "vulnerable" in six regional administrations. In addition, five regional administrations have a recovery plan for this species. To reduce these efforts and considering Spain together with Portugal as an ecological unit, it seems logical to develop a single recovery plan for the species and make the regional administrations responsible for implementing and applying adequate measures.

Many data suggest that the introduction of the red swamp and signal crayfishes between 1972 and 1975 was directly related to the beginning of the crayfish plague because white-clawed crayfish populations began to decline sharply after 1978 (Diéguez-Uribeondo *et al.* 1997). This is a clear example of how the introduction of non-native species negatively affects the environment. Some regional administrations have carried out signal crayfish restocking under the following two hypothetical goals: 1) to create a "green barrier" between the red swamp crayfish and the native crayfish and 2) to introduce an "ecological substitute" for the white-clawed crayfish. Nevertheless, the signal crayfish does not prevent the passage of the red swamp crayfish, and it carries the pathogenic crayfish plague agent. The current aim is to find a way to eradicate these non-native populations by procuring a potential habitat where *A. i. italicus* could be reintroduced and recovered. Some possible eradication methods are mentioned in the management section.

The negative impacts caused by signal crayfish in freshwater ecosystems are well known (Dunn *et al.* 2009, Johnsen & Taugbøl 2010). However, this species has been subjected to incorrect management policies for several years, focused on its utilisation and the conservation of its populations as an exploitable resource. It seems logical that all non-native Iberian crayfish species, *i.e.*, *Procambarus clarkii*, *Cherax destructor* and *Pacifastacus leniusculus*, should be addressed using the same management policies, directing all efforts towards their eradication.

The current knowledge of Iberian *C. destructor* crayfish populations seems to be sparse. Considering the aggressive, competitive and predatory behaviour of this species, its presence and spreading could have negative effects in the future. Thus, the implementation of measures to prevent its spread is urgently required, despite the fact that its populations are currently very restricted.

The data on Spanish crayfish production were obtained from an official source (FAO), whose accuracy is not high. These data were collected from the Agriculture Minister through annual direct polling of responsible managers in each province and it is known that some provinces have provided data without conducting any control of recreational fishing in the past. For this reason, subsequent interpretation of these data must be conducted with caution.

Legislation: conservation and fisheries

Analysis of the existing legislation related to the conservation and management of Iberian crayfish species yields ambiguous results. The Spanish Catalogue of Invasive Alien Species (Boletín Oficial del Estado 2011), as described in its transitional provision, includes all of the introduced crayfish species in the Iberian Peninsula prior to the implementation of Law 42/2007 addressing hunting, fishing and forestry. Under this provision, "in order to prevent these invasive species from spreading beyond their current range, hunting, fishing and forestry strategies are proposed as management, control and eradication measures".

This provision also states that if hunting, fishing and forestry activities involving one of the species cited therein are promoting its expansion and establishment outside its current range, the General Directorate of the Environment and Forestry Policies must take appropriate steps to manage this species.

There is a large body of literature suggesting that non-native crayfish species have a negative impact on freshwater ecosystems (Geiger *et al.* 2005, Rodríguez *et al.* 2005, Souty-Grosset *et al.* 2006, Ilhéu *et al.* 2007). Therefore, a primary management objective should be to eradicate introduced species and prevent the establishment of new invasive ones. It must be kept in mind that once a new species is established in the wild, eradication becomes very challenging because of the complexity of ecosystems and the difficulty of finding a method that only affects the target species. Thus, it appears that is quite difficult to eradicate *P. clarkii* and *P. leniusculus* populations, though it is easier to prevent new introductions of non-native species. Some authors suggest that the best way to avoid human translocations is through fishing prohibition to avoid generating expectations related to fishing. At present, there are few populations of *C. destructor*, and it is very important to prevent the expansion of this species.

Fishing regulations for the signal crayfish have been changing during recent years in a manner that differs between the Spanish regional administrations. For several years, the fishing regulations published by regional administrations established limitations such as quotas and sizes to regulate and preserve the populations of signal crayfish. However, the recent policies of most regional administrations allow fishing of signal crayfish, without any type of restriction. On the other hand, in the regional administrations of Andalucía, Castilla-La Mancha, Comunidad Valenciana, Extremadura, Guipúzcoa and Murcia, signal crayfish fishing is forbidden (Annex 1).

In conclusion, to properly manage crayfish populations in the Iberian Peninsula, we propose the following actions: 1) eradication of non-native crayfish populations, 2) strictly monitoring watercourses to prevent invasion by these or other species of alien crayfishes and 3) informing and warning the human population, especially fishermen, about the serious effects that the introduction of non-native crayfishes causes in the environment.

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CAPÍTULO 2ND *CHAPTER*

FACTORES AMBIENTALES QUE AFECTAN A LA DISTRIBUCIÓN Y ABUNDANCIA DEL INTRODUCIDO CANGREJO SEÑAL (*PACIFASTACUS LENIUSCULUS*) EN EL NORTE DE LA PENÍNSULA IBÉRICA

Environmental factors influencing the regional distribution and abundance of the introduced signal crayfish Pacifastacus leniusculus in the North of Iberian Peninsula

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En segunda revision en la revista *Marine & Freshwater Research*

Resumen

La identificación de los requerimientos ambientales de las especies invasoras es esencial para evaluar su potencial de expansión y la vulnerabilidad de los ecosistemas receptores. Estudiamos la distribución y abundancia del invasor cangrejo señal Pacifastacus leniusculus *en 43 lugares del norte de España, y analizamos sus relaciones con varios parámetros abióticos y bióticos en los ecosistemas acuáticos. Los resultados indicaron que la abundancia del cangrejo señal estaba positivamente relacionada con la cobertura vegetal y negativamente relacionada con grandes rocas. También su abundancia fue positivamente correlacionada con la temperatura del agua, materia orgánica, cationes (e.g. sodio), aniones (e.g. sulfatos) y la abundancia de algunas especies nativas de peces* (Parachondrostoma miegii *and* Luciobarbus graellsii). *Concluimos que el hábitat del cangrejo señal está entre los tramos salmonícolas (cabecera) con aguas frías y baja proporción de materia orgánica y restos vegetales, y los tramos ciprinícolas (tramos bajos) con aguas más cálidas donde cohabita con otra especie invasora de cangrejo, el cangrejo rojo de las marismas* Procambarus clarkii. *Nuestro análisis señala que la presencia del cangrejo señal está limitada en los tramos de cabecera porque las temperaturas son extremadamente bajas y hay poca materia orgánica. La existencia de un factor ambiental limitante en tramos altos favorece la conservación de estos ecosistemas acuáticos y la fauna nativa.*

Palabras clave: *conservación ecosistemas dulceacuícolas, requerimientos de hábitat, especies exóticas invasoras,* Pacifastacus leniusculus, *España.*

Abstract

The identification of habitat requirements of invasive species is essential to evaluate their spread and the assessments of the vulnerability of recipient ecosystems. We study the distribution and abundance of the invasive signal crayfish *Pacifastacus leniusculus* in 43 sites in northern Spain and analyse the relationships with several abiotic and biotic parameters of the aquatic ecosystems. Our results indicated that the abundance of signal crayfish was positively associated with vegetation cover and negatively associated with boulders. Also its abundance was positively correlated with water temperature, organic matter, cations (*e.g.* sodium), anions (*e.g.* sulphates) and abundance of some native fish species (*Parachondrostoma miegii* and *Luciobarbus graellsii*). We concluded that the habitat of signal crayfish is among salmonid stretches (headwaters), with cold waters and low proportion of organic debris, and cyprinid stretches (low waters) with warmer waters where inhabits with another invasive crayfish, the red swamp crayfish *Procambarus clarkii*. Our analysis points out that the presence of signal crayfish is limited in uppermost stretches by lower water temperatures and lower proportion of organic debris. The existence of a natural environmental limiting factor in upstream reaches facilitates the conservation of aquatic ecosystems and native fauna.

Key words: freshwater ecosystems conservation, habitat requirements, invasive alien species, *Pacifastacus leniusculus*, Spain.

Introduction

The main threats to native freshwater fauna are water retention structures, water removal, pollutants, habitat degradation and the introduction of non-native species (Gherardi 2007). Invasive species are considered the second most significant cause of extinctions after habitat destruction and this problem is particularly relevant in inland waters (Gherardi 2007, Hanfling *et al.* 2011). Crayfish species are the most common alien crustaceans in freshwater ecosystems and are considered keystone species because they produce significant ecological and socioeconomic impacts (Geiger *et al.* 2005). Many studies show the changes and problems to the receiving ecosystems caused by invasive crayfish species (Geiger *et al.* 2005, Twardochleb *et al.* 2013). Better understanding of habitat quality requirements of these species is essential to prevent the spread of invasive crayfish species as well as to evaluate the vulnerability of aquatic ecosystems and native fauna (Haddaway *et al.* 2015, Maceda-Veiga *et al.* 2013, Římalová *et al.* 2014).

The signal crayfish *Pacifastacus leniusculus* (Dana, 1852) is one of the most successful crayfish invaders in Europe being present in 29 European countries (Kouba *et al.* 2014). It was firstly introduced to Sweden in 1959 and nowadays it keeps extending their range in Europe (Holdich *et al.* 2009, Kouba *et al.* 2014). The signal crayfish is native from the cool temperate regions of Western North America and inhabits a wide range of habitats, from small streams to large rivers and lakes (Souty-Grosset *et al.* 2006). Invasive crayfish species have many ecological impacts such as competitive interactions for habitat and food, predation, transmission of diseases and parasites, habitat alteration and introgressive hybridization, changing the functionality of the ecosystems in which they are introduced (*e.g.* Geiger *et al.* 2005, Gherardi 2007, Lodge *et al.* 2012). In particular, the signal crayfish triggers negative effects in macrophytes, macroinvertebrates, snakes, benthic fishes and amphibian larvae (Johnsen & Taugbøl 2010). This crustacean is considered a non-burrowing species in North America, but in Europe it constructs burrows under rocks or in river and lake banks (Souty-Grosset *et al.* 2006). This behaviour can collapse the river banks when the burrow density is high (Guan 1994).

The main biological characteristics of signal crayfish are its fast growth and sexual maturity, aggressive behaviour (dominate the use of food and shelter), large size and high tolerance to adverse environmental conditions such as pollution (Guan & Wiles 1996). However, signal crayfish have generally been identified specifically as slow or K-life history species in contrast to fast or r-life history crayfish like red swamp crayfish *Procambarus clarkii* (Girard,1852), but it grow faster and mature earlier than the European Astacidae (Momot 1984, Pintor *et al.* 2011). Size at maturity is usually 6-9 cm (total length) at an age of 2-3 years, although in some individuals maturity can occur as early as one year (Souty-Grosset *et al.* 2006). It is particularly tolerant of brackish water and temperature, although it does not occur in waters with a pH lower than 6.0 (Souty-Grosset *et al.* 2006).

The first introductions of signal crayfish in Europe were made to replace the decreasing stocks of the Indigenous Crayfish Species (ICS) in many lakes and streams (Holdich *et al.* 2009, Olsson *et al.* 2009, Kouba *et al.* 2014). However, the signal crayfish, as all American crayfish species, carries the pathogen *Aphanomyces astaci* Schikora, 1906 responsible for crayfish plague or aphanomycosis which has caused an important decline in native European crayfish populations (Holdich *et al.* 2009). Further, Kataria (2007) demonstrated that in the long term signal crayfish will out-compete the native noble crayfish *Astacus astacus* (Linneo, 1758). Also, other factors such as the degradation of aquatic habitats and water quality have increasingly become one of the most important factors influencing the decline of ICS (Holdich & Reeve 2006, Římalová *et al.* 2014).

Nowadays, native species inhabiting headwaters could be endangered by North American crayfish. For example, these crayfishes are carriers of *Batrachochytrium dendrobatidis* (Longcore, 1999), the causative agent of chytridiomycosis in amphibians (McMahon *et al.* 2013). Habitat modifications could affect seriously the populations of native species catalogued on the IUCN Red List, as the Pyrenean desman *Galemys pyrenaicus* (Geoffroy, 1811) or the north Iberian spined loach *Cobitis calderoni* Bacescu, 1962 (IUCN 2016). Furthermore, most of the ICS remain restricted to headwaters and small rivers (Wendler *et al.* 2015). It becomes indispensable to study if American crayfishes have the possibility to reach the headwaters streams.

When the physicochemical parameters are suitable, the abundance and size distribution of crayfish populations depends on predatory fish, cannibalism, food supply, refuge availability and stream morphology (France 1985, Lodge & Hill 1994, Guan & Wiles 1996, Usio & Townsend 2000, Streissl & Hödl 2002, Nystrøm *et al.* 2006). Quantitative data on the habitat requirements and physicochemical parameters of crayfish have been generally lacking for crayfish species (Usio *et al.* 2006, Jowett *et al.* 2008). The negative effects of signal crayfish in the conservation of ecosystems and native aquatic fauna enhances the importance of study the environmental features and factors that determine the presence and abundance of this species. Although over fifty-five years have gone since the first introduction of this invasive crayfish to Europe, the environmental factors affecting its distribution and abundance have been scarcely studied in Spain (but see Almeida *et al.* 2013). The understanding of its environmental and habitat requirements can help to avoid future introductions and take control measures by ecosystem managers.

The aims of this work were 1) to study the distribution and abundance of the signal crayfish populations in the medium and headwaters stretches of a northern region of Spain and, 2) to analyse the potential relationship between the presence and abundance of signal crayfish and abiotic (physicochemical parameters, habitat characteristics) and biotic (fish assemblages) factors. Firstly, we hypothesise that the presence of signal crayfish is limited by unknown factors in uppermost stretches. Secondly, its abundance is expected to be influenced by abiotic and biotic factors, although the extent of this influence is unknown.

Materials and methods

Study area

This study cover all medium and high stretches of the main Pyrenean rivers of the region of Navarra (Ebro River Basin, Spain). The surveyed sampling sites are representative of the distribution range of signal crayish the southwest of Europe covering a wide altitudinal gradient, riparian characteristics and water quality. The Ebro Basin has the largest, more abundant and oldest signal crayfish populations of the Iberian Peninsula because some regional administrations carried out stocking-programs in the 1980s (see Oscoz *et al.* 2010). A total of 43 sites were sampled in the summers of 2013 and 2014 (Figure 1). The location of each sampling site was selected to include pools, riffles and a wide range of habitat characteristics.

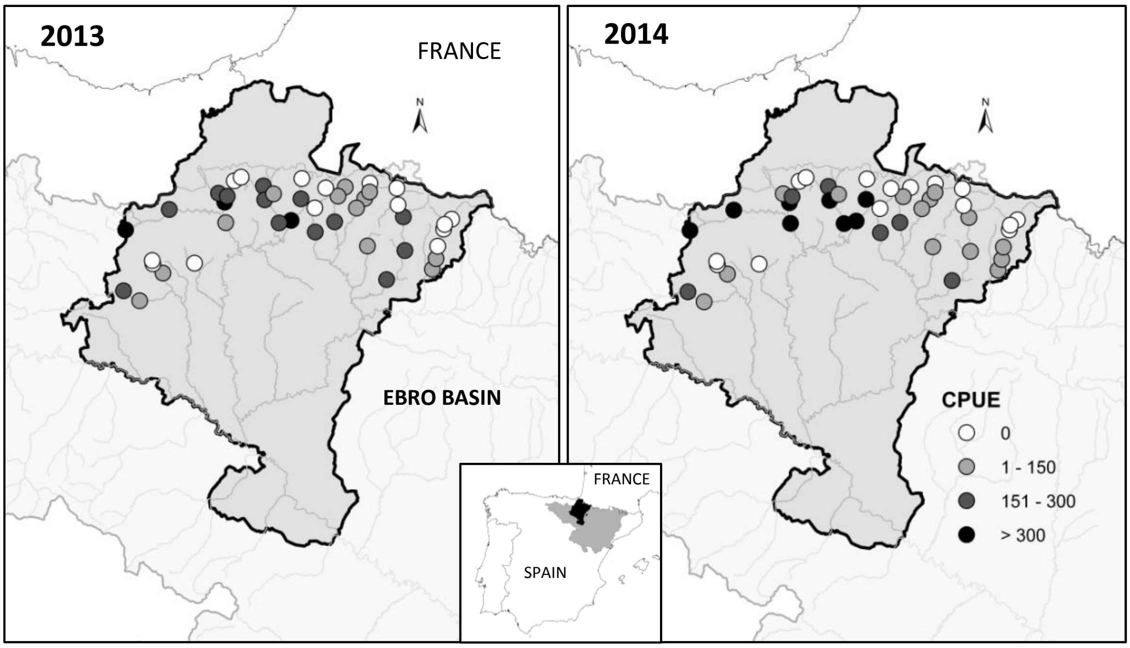

Figure 1. Location of study sites and abundance of signal crayfish *Pacifastacus leniusculus* in the North of Spain in 2013 and 2014 (region of Navarra, Ebro Basin). Greyscale dots indicate the abundance of signal crayfish expressed as Catch Per Unit Effort (CPUE, number of specimens collected in an hour). In the small picture the grey area is the Ebro River Basin and the black area is the administrative region of Navarra.

Biotic data

Specimens of signal crayfish were captured between July and August of 2013 and the same period of 2014. These samplings were conducted at the same sites than the fish surveys (see below, Figure 1), but at least 10 days spent between both samplings to avoid possible interactions in the behaviour of crayfish. At this time of year the hydrology is similar with low flow and the signal crayfish is in high activity. There are many sampling methods to quantify crayfish density in freshwater systems (Rabeni *et al.* 1997, Reynolds *et al.* 2010). However, each sampling method fits better to the type of habitat to be sampled. Reynolds *et al.* (2010) state that handling of crayfish, with practice, will be easy and efficient in some habitats. Given that the average water depths were low (15–80 cm) and that the water clarity was good, nocturnal capture with the aid of headlights was considered the most effective methodology to quantify signal crayfish abundance (Rabeni *et al.* 1997, Reynolds *et al.* 2010). Due to the invasiveness of signal crayfish, all the specimens were removed from the river and subsequently transported to the laboratory.

Fish sampling was taken by electrofishing surveys between June and July of 2013 (Hans Grassl model IG200/2D, 300-600V, 0.2–2A) following a single-run depletion methodology and estimating fish abundance. Collected fish were anesthetized with MS-222 (tricaine methanesulphonate) and subsequently counted, measured total length (TL) and released after the survey.

The fish and signal crayfish abundances at each sampling point were expressed as Catch Per Unit Effort (CPUE). The CPUE was calculated as the number of fish or crayfish captured in a sampling site per unit of time (one hour) and person. In all streams, the sampling stretches were continuous, but we ensured that all the pool and riffle habitat types were sampled.

Abiotic data

A digital multiparameter HI 98130 and an oximeter HI 9146 were used to measure four physicochemical water variables: water temperature (°C), pH, conductivity (µS/cm) and oxygen (mg/l).

The river habitat characteristics were measured after fish sampling. The values of width (m), depth (cm), water velocity (m/s), substrate type [fines (<2 mm), gravels (2–64 mm), pebbles (64–256 mm), boulders (>256 mm), bedrock] and shade (yes/no) were taken along ten perpendicular transects to the flow at 5 m intervals (three measurements per transect). We consider a shade point when there are vegetation cover in the vertical. With all these values the mean river width (m), mean river depth (cm), depth standard deviation (SD), mean water velocity (m/s), water velocity SD, percentage of substrate type and shadow (%) were calculated, providing a total of 11 variables for decribing the habitat. Water velocity was determined with an adapted velocimeter.

In addition, in 18 of the 43 sampling points, the Government of Navarra has monthly been collecting environmental variables since 1992 (Gobierno de Navarra, 2014). These variables are temperature (°C), oxygen (mg/l), pH, conductivity (µS/cm), turbidity (NTU), suspended solids (mg/l), inorganic carbon (mg/l), organic carbon (mg/l), organic matter (mg/l), biochemical oxygen demand (mg/l), ammonium (mg/l), nitrites (mg/l), nitrates (mg/l), phosphates (mg/l), total coliforms (CFU/100 ml), *Escherichia coli* (CFU/100 ml), calcium (mg/l), sodium (mg/l), potassium (mg/l), chloride (mg/l), magnesium (mg/l), sulphates (mg/l), bicarbonates (mg/l), carbonates (mg/l) and fluorides (mg/l).

Data analysis

The effect of physicochemical parameters on the presence of crayfish was studied with non-parametric tests. Kruskal-Wallis *H* test and *a posteriori* Mann-Whitney *U* tests were used to analyse if some physicochemical variables or habitat characteristics were statistically different between sites with and without signal crayfish. An exploratory analysis based on non-parametric correlations (Spearman's rho) between the environmental variables provided by the Government of Navarra (mean of the annual averages) and the mean CPUE of signal crayfish per site was performed to study the relationships between the abundance of signal crayfish and environmental data.

Of the 15 environmental variables considered, the percentage of fines (poorly represented) was eliminated to avoid problems of multicollinearity among substrate predictor variables. Subsequently, principal-components analysis (PCA) was then applied as an unconstrained ordination technique to describe the main sources of variation and relationships among the 14 environmental variables in the 27 sites with crayfish. The suitability of the PCA was confirmed by the interdependence of the environmental variables using the Kaiser–Meyer–Olkin (KMO) measure of sampling adequacy. The suitability of PCA is guaranteed when the KMO is in the range 0.5 to 1. The ''varimax'' rotation method was used to increase the interpretation of axes. Principal components with eigenvalues greater than 1 were retained as (synthetic) independent environmental variables for further analyses. A generalized linear model (GLM) with Poisson distribution was used to explore the relationships between the abundance of signal crayfish (square root of CPUE) and the independent variables (PCA scores). The normality of the residuals of the regression model was proved with a Shapiro test.

The relationship between all the biotic and abiotic variables was explored in the 43 sites by means of a Canonical Correspondence Analysis (CCA). Poorly represented fish species (less than 5 localities with the presence of the species) were removed from this analysis (*e.g. Barbus haasi*). The significance of the analyses was assessed by a permutation test (Lengendre & Legrende 1998). To improve linearity, environmental variables were log-transformed (conductivity, temperature, oxygen, pH, mean river width, mean river depth and mean water velocity). Proportional data of substrate types (%) and shadow (%) were arcsine transformed. Abundance of fish and crayfish data were root transformed.

All statistical analyses were performed with SPSS 15.0 for Windows, R 3.1.3 (http://www.r-project.org) and PAST 2.17 (Hammer *et al.* 2001). The level of significance was established at $P = 0.05$. The geographical information system software ArcGIS (version 10) was used for the preparation of the maps.

Results

Signal crayfish was found in 67% of the sampling sites. A total of 2914 specimens of signal crayfish were collected between 2013 and 2014. The Arakil and Arga rivers had the highest abundances of crayfish. Seven native fish species were found at the sampling sites: the Pyrenean stone loach *Barbatula quignardi* (Bacescu-Mester, 1967) (percentage of occurrences: 26%, total CPUE: 5.5), the Ebro barbel *Luciobarbus graellsii* (Steindachner, 1866) (16%, CPUE: 2.6), the Iberian redfin barbel *Barbus haasi* (Mertens, 1924) (12%, CPUE: 1.4), the Pyrenean gudgeon *Gobio lozanoi* (Doadrio & Madeira, 2004) (28%, CPUE: 3.7), the Ebro nase *Parachondrostoma miegii* (Steindachner, 1866) (21%, CPUE: 6), the Pyrenean minnow *Phoxinus bigerri* (Kottelat, 2007) (88%, CPUE: 74) and the brown trout *Salmo trutta* (Linnaeus, 1758) (86%, CPUE: 21.5).

The altitude distribution of signal crayfish ranged between 415 meters above sea level (m.a.s.l.) and 890 m.a.s.l. The sampling points with signal crayfish had higher values of water temperature, mean river width and depth and lower values of water oxygen (Table 1). The Pyrenean gudgeon, the Ebro nase and the Ebro barbel were only sampled in sites with crayfish (Table 1). The pH, conductivity, mean water velocity, fines, gravel, pebbles, boulders, bedrock, shadow and abundance of *Barbus haasi* were not different between sites with and without crayfish.

Table 1. Abiotic and biotic factors differences in sites with and without signal crayfish *Pacifastacus leniusculus*.

	Absence	Presence	Statistics	
	Mean ± SD	Mean ± SD	*U*	*P* value
Environmental data				
Temperature (°C)	13.09 ± 1.45	16.77 ± 2.01	106	2.91×10^{-11}
Oxygen (mg/l)	9.54 ± 0.88	8.87 ± 0.99	336	0.003
River width (m)	9.65 ± 3.32	13.39 ± 7.59	458	5.36×10^{-4}
River depth (m)	0.32 ± 0.08	0.39 ± 0.12	470	7.99×10^{-4}
	Mean ± SD	Mean ± SD	*U*	*P* value
Fish abundances CPUE				
Salmo trutta	30.50 ± 22.00	17.08 ± 18.00	423	1.54×10^{-4}
Phoxinus bigerri	67.36 ± 102.80	78.95 ± 71.01	498	0.002
Gobio lozanoi	-	5.65 ± 11.63	480	3.69×10^{-5}
Barbatula quignardi	3.64 ± 14.11	5.86 ± 10.84	563	0.001
Parachondrostoma miegii	-	9.30 ± 27.16	570	5.80×10^{-4}
Lucioarbus graellsi	-	4.10 ± 12.10	630	0.003

In the 18 sites with additional environmental variables from the regional administration new environmental relationships were established. The abundance of signal crayfish was positively correlated with water temperature, organic matter, ammonium, phosphates, sodium, potassium and sulphates (Table 2).

Table 2. Non-parametric correlations between the environmental variables provided by the Government of Navarra and the abundance of signal crayfish *Pacifastacus leniusculus* (CPUE) that were statistically significant (r_S: Spearman rank correlation coefficient).

	r_s	*P*	n
Water temperature	0.558	0.016	18
Organic matter	0.750	< 0.001	18
Ammonium	0.492	0.038	18
Phosphates	0.498	0.035	18
Sodium	0.670	0.002	18
Potassium	0.564	0.015	18
Sulphates	0.523	0.026	18

The PCA of the 27 sites with crayfish and 14 environmental variables produced three relevant axes (Table 3). The substrate types were the most determinant variables in the characterization of the analysis. PC1 (46.61%) was highly related with the predominance of gravels and pebbles over bedrock. PC2 (24.86%) accounted for river morphology (narrow stretches with abundant shadow). Finally, the presence of boulder substrates was the main factor in the PC3 (14.30%). The GLM analysis indicated that the abundance of crayfish was positively related to high PC2 values and low PC3 values (Table 4). The residuals of the regression model were statistically normal (*P* = 0.069).

Table 3. Factor loadings of environmental variables for axes obtained in the PCA. The absolute highest values for each axe are highlighted in bold.

	PC1	PC2	PC3
Conductivity	0.279	-0.010	0.017
Temperature	-0.338	-0.364	-0.108
pH	-0.492	-0.188	-0.018
Oxygen	-0.179	0.132	0.292
Mean river width	-0.139	**-0.642**	-0.080
Mean river depth	0.229	-0.140	-0.045
Depth SD	-0.034	0.244	-0.036
Mean water velocity	0.163	0.013	0.347
Water velocity SD	-0.001	0.291	0.365
Gravels	**0.593**	0.432	-0.234
Pebbles	**0.600**	0.322	0.049
Boulders	0.073	-0.183	**0.963**
Bedrock	**-0.922**	0.030	-0.377
Shadow	0.182	**0.960**	0.183

Table 4. Regression model accounting for the relationship between the abundance of signal crayfish *Pacifastacus leniusculus* and the environmental variables (PCA scores).

Fixed effects	Value	Standard error	z value	*P* value
Intercept	2.514	0.071	35.458	P < 0.001
PC1	-0.010	0.061	-0.147	0.883
PC2	0.315	0.089	3.523	P < 0.001
PC3	-0.165	0.083	-1.993	0.046

The CCA of the 43 sites with environmental variables, fish assemblages and crayfish abundances produced two axes that together explained 81.5% of the environmental dataset variation (Figure 2). The first axis (CCA1) explained 55.46% of the variance, separating cyprinid (left) and salmonid (right) sites. Salmonid stretches (upper reaches with brown trout) were related with high values of environmental variables like oxygen, water velocity, shadow and prevalence of small to medium size type of substrate. In contrast, cyprinid stretches (lower reaches with Pyrenean gudgeon, Ebro barbel and Ebro nase) were related with high values of water temperature, mean river width, conductivity, mean river depth and bedrock. Axis 2 (CCA2) explained 26.04% of the variance, influenced primarily by water temperature and shadow. Sites with signal crayfish were perfectly situated among salmonid and cyprinid reaches and were highly influenced by shadow. All sites without crayfish were located in headwater streams (salmonid stretches) (Figure 2).

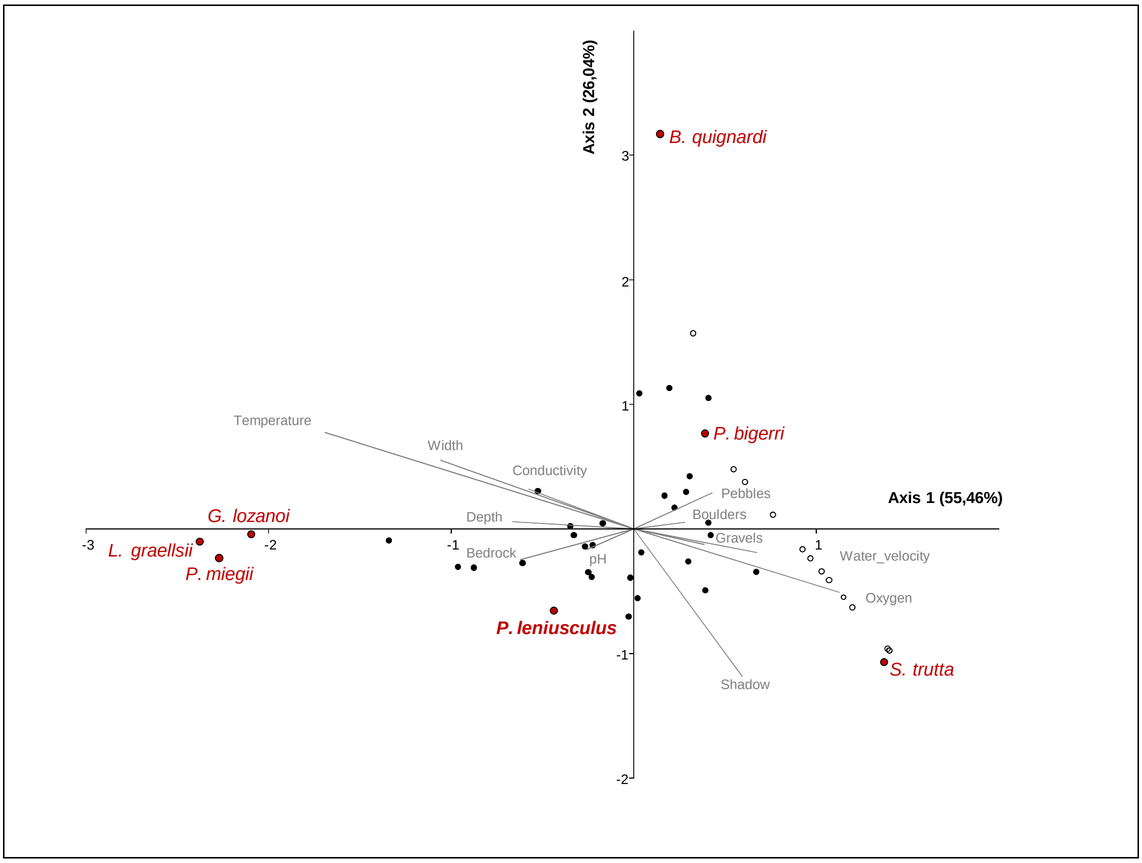

Figure 2. Canonical correspondence analysis (CCA biplot) that represents the relationships between the abundance of signal crayfish *Pacifastacus leniusculus*, fish species, physicochemical parameters (temperature, oxygen concentration, pH, conductivity) and habitat parameters (substrate type, mean river width, mean river depth, mean water velocity and shaded percentage of the river channel). White dots are sites without crayfish and black dots are sites with crayfish.

Discussion

In laboratory experiments, signal crayfish was found to have a greater overall tolerance to temperature compared to the white-clawed crayfish *Austropotamobius pallipes* (Lereboullet, 1858) and the narrow-clawed crayfish *Astacus leptodactylus* Eschscholtz, 1823, and a higher growth rate than these two species at all temperatures tested (Souty-Grosset *et al.* 2006). Nakata *et al.* (2002) found that the upper lethal temperature of signal crayfish was 31.1ºC. In addition, Firkins (1993) studied that this species has a greater thermal tolerance (12.7°C–32.5°C) than other crayfish species such as the white-clawed crayfish (13.5°C–29.2°C) and the narrow-clawed crayfish (14.3°C–29.3°C). However, in our study area the signal crayfish is unable to colonize the headwater streams with lower temperatures where in some of them inhabits the white-clawed crayfish. The mean water temperature in the summer in these upper stretches is around 13.1°C (Table 1). Similarly, in other study in Japan it was found that water temperature was the major predictor that determined the abundance of signal crayfish (Usio *et al.* 2006).

Physicochemical parameters and river habitat features directly and indirectly affect survivorship and distribution patterns of freshwater fauna (Haddaway *et al.* 2015, Usio *et al.* 2006). Physical and chemical water characteristics may regulate crayfish physiological processes such as osmoregulation, respiration and many other metabolism reactions (Lodge & Hill 1994). Temperature is a major limiting factor for aquatic poikilothermic animals and can influence survival, range distribution, growth and metabolism, food and feeding habits, reproduction and life histories, movements, migrations and behaviour (Firkins 1993, Angilletta *et al.* 2004). The maximum water temperature tolerance of signal crayfish is 33°C and its maximum activity is at 20°C (Rutledge & Pritchard 1981, Lewis 2002). Our results show an association between the abundance of signal crayfish and the environmental parameters of the river stretches. In headwaters with coldest water temperatures and low proportion of organic debris there was no crayfish, while in middle stretches with higher water temperatures, width channels and water conductivities the abundances of crayfish were high. Again, the abundance of signal crayfish decrease gradually in lowland sites with warmer water temperatures and turbidity where inhabits another invasive crayfish, the red swamp crayfish (Oscoz *et al.* 2010).

Patzner *et al.* (2005) limit the occurrence of signal crayfish in Austria between 350 and 810 m.a.s.l., but varying mainly from 500 to 600 m.a.s.l. Similarly, we observed that the altitude distribution of this invertebrate in Navarra ranged between 415 and 890 m.a.s.l. However, this range is limited by the sampling points analysed that were located in a specific area. In fact, Oscoz *et al.* (2008) observed that the presence of signal crayfish in the Ebro Basin varies from 119 to 941 m.a.s.l. On the other hand, the altitudinal limit of the invasive red swamp crayfish in the Catalonian region was established in 750 m.a.s.l. (Maceda-Veiga *et al.* 2013), although its range in the Ebro Basin varies from 35 to 900 m.a.s.l. (Oscoz *et al.* 2008).

Many studies suggest that the type of substrate and vegetation cover may influence on the abundance of crayfish. In accordance with Anastácio *et al.* (2015), signal crayfish showed a preference for riffles and pool areas with riparian vegetation cover in a Portuguese river. This association between crayfish and vegetation cover has been previously established in other crayfish species (Naura *et al.* 1998, Jowett *et al.* 2008, Usio & Townsend 2000). For example, Jowett *et al.* (2008) found that undercut banks, leaf litter, tree roots, and woody debris were strongly related to the presence of *Paranephrops planifrons* White, 1842. In fact, there are some studies that show the dependence of signal crayfish on terrestrial leaf detritus (Bondar *et al.* 2005, Larxon *et al.* 2011). In addition, Usio *et al.* (2006) found in Japan that the abundance of signal crayfish was highest when undercut bank volume was >0.61 m^3. Similarly, our study also shows that the areas with more shadow, which are directly related with more riparian vegetation and leaf litter, presented higher abundances of signal crayfish. Leaf litter might act as a direct source of food and as an indirect support of microbes, fungi and invertebrates, which are another source of food for crayfish (Kaushik & Bird 1987, Naura *et al.* 1998).

Population density is often determined by shelter availability and shelter suitability (Nyström *et al.* 2006). Shelter suitability is mainly determined by water velocity and stone morphology. Streissl & Hödl (2002) observed that *Austropotamobius torrentium* (Schrank, 1803) avoided shelters in areas of water velocities exceeding 25 cm/s and also found that large males occupied larger stones. Our results point out that the presence of boulders negatively effect on the abundance of crayfish in sunny, width and warmer stretches. Other studies with different crayfish species also observed this relationship. For example, Jowett *et al.* (2008) observed that the probability of finding *P. planifrons* was reduced sharply as the substrate became larger. Guan & Wiles (1996) showed that the densities and biomass of signal crayfish were highest in the habitat with highest density of cobbles. Nyström *et al.* (2006) caught fewer signal crayfish when the substratum was sandy or dominated by large boulders. Anastácio *et al.* (2015) suggested that boulders and large stones are an important component of the substrate which strongly reduces the visual orientation capabilities of the signal crayfish moving on the river bottom. Other studies suggest that the interstitial spaces between boulders may be large enough to allow access to fish or other predators such as otters, rendering crayfish less suitable protection (Usio & Townsend 2000, Jowett *et al.* 2008). Another hypothetical explanation that signal crayfish have a strong negative association to larger substrates could be that these sites were just harder to sample effectively for crayfish.

However, this hypothesis is unlikely because in these sites the average water depths were low and the visibility was good. Other studies have also related density of crayfish and substrate size. For example, Wooster *et al.* (2012) studied the ecology of signal crayfish in the mountains of its Pacific Northwest native range. They examined the relationship between density and size of individuals and environmental factors. Their results indicated that substrate size is one of the most important drivers of the distribution and density of signal crayfish at multiple spatial scales.

Signal crayfish like all crustaceans need calcium for the development of the crayfish’s exoskeleton during the moulting process. Laboratory experiments found that a calcium concentration between 1–2.5 mg/l was the survival thresholds for crayfish (Cairns & Yan 2009). However, the dissolved calcium was not a limiting factor in our study area because the dissolved calcium is much higher than this minimum requirement. In fact, from 1978 to 2010 the average of dissolved calcium in the site with the lower calcium values was 19.1 mg/l and the minimum was 12.8 mg/l (Arga River, locality of Urtasun).

The habitat of signal crayfish is among salmonid stretches (upper reaches with brown trout) and cyprinid stretches (lower reaches with Ebro nase, Ebro barbel and Pyrenean gudgeon). Our results show that the presence of signal crayfish is correlated with the presence of Pyrenean gudgeon, the Ebro nase and the Ebro barbel. And its abundance was negatively associated with the presence of brown trout. The effect of crayfish on fish communities has been widely reported under laboratory conditions (*e.g.* Bubb *et al.* 2009), but less emphasis has been placed on this competition in natural settings (*e.g.* Guan & Wiles 1997). Whereas some studies found substantial effects (*e.g.* Guan & Wiles, 1997), others reported that crayfish have no or small effects on fish (*e.g.* Stenroth & Nyström, 2003). The study of interactions between native and invasive species in aquatic systems is complex due to the difficulty in distinguishing if the changes in abundance of native species are determined primarily by the invaders or by uncontrolled external factors (*e.g.* environmental variables). Further investigations should be done to study the competition between signal crayfish and fishes, especially under natural conditions.

Our study shows that signal crayfish is unable to invade the coldest headwater streams. We suggest that low water temperatures and low proportion of organic debris could be the environmental limiting factors. In fact, in other introduced regions, also signal crayfish cannot survey in very cold localities, such as those in northern Scandinavia and Austria (Firkins 1993, Lowery & Holdich 1998, Weinländer & Füreder 2012). However, other factors could be influencing. For example, Light (2003) observed that signal crayfish were absent from streams with gradients >3% in a mountainous region of California, and found that water velocity and flow regime strongly affected the distribution and invasion success of this species. Pintor and Sih (2011) similarly report that high stream discharge significantly limited the abundance of signal crayfish. In our study only two of 18 rivers studied (Arga and Irati Rivers) have reservoirs, and we cannot confirm that the crayfish distribution is related by hydrologic factors, gradient or by the presence of reservoirs.

Furthermore, a limitation exists in lower stretches with warmer waters by environmental variables and biotic competition where inhabits with another invasive crayfish species, the red swamp crayfish (Capinha & Anastácio 2011, Oscoz *et al.* 2010). It has been observed that both species can be present and coexist in the same river stretch (Oscoz *et al.* 2008), although it has been studied in a laboratory experiment that the red swamp crayfish were both the first (70.8%) and the long-term winner (62.5%) (Alonso & Martínez 2006). In addition, a study in the Pacific Northwest suggested that the red swamp crayfish has potential to locally displace signal crayfish (Pearl *et al.* 2013). However, Hanshew & Garcia (2012) observed that the two crayfish species are equal competitors for shelter in a mesocosm experiment.

The medium and low stretches of European rivers have been colonized by invasive species such as signal crayfish or red swamp crayfish where native crayfish species inhabiting in the past (Holdich *et al.* 2009). However, the existence of a natural environmental limiting factor in upstream reaches would facilitate the conservation and recovery of native freshwater species. In fact, these headwater streams are the only water courses where currently inhabits endangered native European species as some crayfishes (*e.g.* noble crayfish), fishes (*e.g.* north Iberian spined loach) and amphibians (*e.g.* Pyrenean brook salamander *Calotriton asper* (Dugès, 1852)) (Wendler *et al.* 2015). In many countries these streams and small lakes are the only places free from the crayfish plague (*e.g.* Holdich & Reeve 2006). A study suggests that European catchments will become dominated by red swamp crayfish with climate warming

(Gherardi *et al.* 2013). And other studies also predict that the climate warming will facilitate that populations of signal crayfish colonize these headwater streams with endangered fauna (Capinha *et al.* 2012).

The wide distribution and dense populations of signal crayfish in Europe makes its eradication impossible. A detailed knowledge of the distribution and ecological requirements of invasive and native crayfish species will help ecologists and river managers to provide important information for guiding management and conservation decisions. A good management plan for invasive crayfish species, the identification of potential refuge areas for the endangered native crayfish species and the improvement in water quality by administrations are the best management measures in sustaining and improving native freshwater ecosystems.

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CAPÍTULO 3RD *CHAPTER*

UN BRANQUIOBDÉLIDO EXÓTICO ECTOSIMBIÓTICO (ANNELIDA: CLITELLATA) HOSPEDANDO UN CANGREJO EXÓTICO: UNA CO-INVASIÓN BIOLÓGICA CON CONSECUENCIAS IMPREDECIBLES

An alien ectosymbiotic branchiobdellidan (Annelida: Clitellata) adopting exotic crayfish: a biological co-invasion with unpredictable consequences

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Resumen

Las especies exóticas invasoras son una amenaza para la biodiversidad, especialmente cuando están implicados sus patógenos y simbiontes. Los branquiobdélidos son unos anélidos clitelados con una asociación ectosimbionte obligada sobre los cangrejos astacoideos. Existen varios ejemplos de especies de branquiobdélidos adpotando un hospedador exótico donde cangrejos nativos y exóticos cohabitan en la misma masa de agua. El primer registro de un branquiobdélido del oeste de Norteamérica, Xironogiton victoriensis*, hospedando un cangrejo del este de Norteamérica,* Procambarus clarkii*, en dos cuencas hidrográficas en España proporciona una prueba más de la tolerancia de los ectosimbiontes de infestar un hospedador exótico. Dada las amplias reintroducciones de ésta y otras especies comerciales de cangrejos de río, los limnólogos y los gestores ambientales deben de estar alerta de nuevas introducciones de* X. victoriensis *y otros branquiobdélidos. Los impactos de estas especies exóticas de ectosimbiontes sobre el hábitat y la biota en una nueva ubicación son desconocidas, al igual que se desconocen sus consecuencias sobre la biodiversidad nativa.*

Palabras clave: *conservación biológica, dispersión, dulceacuícola, especies invasoras, invertebrados,* Xironogiton victoriensis*.*

Abstract

Invasive alien species present a global threat to biodiversity, particularly where pathogens and symbionts are involved. Branchiobdellidans are clitellate annelids with an obligate ectosymbiotic association primarily on astacoidean crayfish. There are several examples of branchiobdellidan species adopting a geographically exotic host where endemic and exotic crayfishes co-habit the same water body. The first records of a western North American branchiobdellidan, *Xironogiton victoriensis*, adopting the eastern North American crayfish, *Procambarus clarkii*, in two river basins in Spain provide further evidence of the ectosymbionts tolerance to adopt an exotic host. Given world-wide translocations of these and other commercial crayfish species, limnologists and agency managers need to be alert for further introductions of *X. victoriensis* and other branchiobdellidans. Impacts of these exotic ectosymbionts on habitat and biota at a new location are unknown, as are their consequences on native biodiversity.

Key words: biological conservation, dispersal, freshwater, invasive species, invertebrates, *Xironogiton victoriensis*.

Introduction

Biological invasions are serious global phenomena that threaten biodiversity of freshwater fauna and flora in particular (Dudgeon *et al.* 2006). Invasive alien species are leading to increasing global biogeographical homogeneity, with widespread ecological and evolutionary implications (Olden *et al.* 2004). Pathogens of these alien host species are adding a further concern to the increasing number of biological invasives. In particular, symbionts associated with translocated hosts that in turn infect native hosts (Prenter *et al.* 2004).

North American crayfishes, signal crayfish *Pacifastacus leniusculus* (Dana, 1852) and red swamp crayfish *Procambarus clarkii* (Girard, 1852), are considered a major threat to stream ecosystems in Europe, where these species have been successful introduced. Native European crayfishes have been driven local extinction as a result of crayfish plague, *Aphanomyces astaci* (Schikora, 1906), from the North American crayfishes (Vedia & Miranda 2013). These crayfishes are hosts to other exotic pathogens and symbionts, an example of the latter being branchiobdellidans.

Branchiobdellidans or crayfish worms are clitellate annelids with an obligate ectosymbiotic association primarily on astacoidean crayfish (Gelder & Williams 2015). The worms form a monophyletic order, Branchiobdellida (Annelida: Clitellata) with a discontinuous Holarctic distribution (Gelder 1999). Of the 22 genera, six are endemic to the Palaearctic and 16 to the Nearctic realms. These two realms each contain two distinct regions: the Euro-Mediterranean and East Asia, and Eastern and Western North America with the latter separated by the Continental Divide (Bănărescu 1990). Genera in North America are endemic to either eastern or western regions, and three genera have species in both regions although these species are similarly restricted to only one region or the other. The ectosymbiont's crayfish hosts are also endemic to these respective regions.

Although most branchiobdellidans live on freshwater crayfish, some species have adopted other crustaceans such as isopods, crabs and shrimps (Holt 1973a, 1973b, Gelder *et al.* 2002), and some of these, *e.g. Cambarincola mesochoreus* (Hoffman, 1963) occur on both crayfish and estuarine crabs (Gelder & Messick 2006). Where a branchiobdellidan appears to be host species specific, this is more likely the result of limited host availability in the area, *e.g. Uglukodrilus hemophagus* (Holt, 1977) and *Sathodrilus* species (Holt, 1981).

Branchiobdellidans adaptability and acceptability of new hosts can be explored where endemic and exotic crayfishes co-habit the same water body. The North American spinycheek crayfish *Orconectes limosus* (Rafinesque, 1817) was first translocated to Poland in 1890 and has successfully spread in Europe to Italy, England and the Pyrenees Mountains (Souty-Grosset *et al.* 2006). Vogt (1999) observed sympatric populations of stone crayfish *Austropotamobius torrentium* (Schrank, 1803) and *O. limosus* in a tributary to Steinbeck Creek (Hess, Germany), where both hosts carried European *Branchiobdella parasita* (Henle, 1805) and *B. pentodonta* (Whitman, 1892). A similar adoption of *O. limosus* by these two *Branchiobdella* species plus *B. balcanica* (Moszynski, 1938) and *B. hexadonta* (Gruber, 1883) was found in the Czech Republic (Ďuriš *et al.* 2006). Although no endemic branchiobdellidans have been introduced on *O. limosus*, two other exotic North American crayfishes, signal crayfish and red swamp crayfish, did bring some of their endemic branchiobdellidans into Europe (Gelder *et al.* 1999). A list of branchiobdellidan species reported on these three commercially important crayfish in North America is given in Gelder (2004). Therefore, any branchiobdellidan found where these crayfishes have been introduced is likely to be found on this list.

To date, only *C. mesochoreus* has been reported on exotic *P. clarkii* in northern Italy (Gelder *et al.* 1994). Subsequently, *P. clarkii* specimens were reported in the same area where white-clawed crayfish *Austropotamobius pallipes* (Lereboullet, 1858) was found to carry *B. parasita* and *B. italica* (Canegallo, 1928). This sympatric association of crayfishes resulted in some *P. clarkii* being adopted by *B. parasita* and *B. italica*, but no *A. pallipes* were found with *C. mesochoreus* (Gelder *et al.* 1999). A new dimension to branchiobdellidan adoptions in the Euro-Mediteranean region was the first report of branchiobdellidan cocoons on Chinese mitten crabs, *Eriocheir sinensis* (Edwards, 1853), in Lake Dąbie, northwest Poland (Sobecka *et al.* 2011). This is the only non-crayfish host of branchiobdellidans in the region and indicates *Branchiobdella* species is more adaptable to adopting additional crustacean hosts than previously though.

Although *P. clarkii* were introduced into Japan in 1927 (Kawai *et al.* 2003) and soon spread across the country, no exotic branchiobdellidans have been found on them.

In contrast, *P. leniusculus* were also introduced into Japan during the late 1920's (Kamita 1970) and they were subsequently found to have carried three branchiobdellidan species (Ohtaka *et al.* 2005). However, none of these exotic branchiobdellidans have been recorded on endemic *Cambaroides japonicus* (De Haan, 1841) or exotic *P. clarkii*. Signal crayfish in Europe with *Xironogiton victoriensis* (Gelder & Hall, 1990), are widely distributed from southern Scandinavia to Italy and westward to northern Spain (Gelder & Williams 2015).

Procambarus clarkii and *P. leniusculus* are quite common in the Iberian Peninsula (Vedia & Miranda 2013) and co-habit in several water bodies (Oscoz *et al.* 2010). However, only studies in northern Spain have found *X. victoriensis* on *P. leniusculus* (Gelder 1999, Oscoz *et al.* 2010). Strangely, while the Iberian Peninsula has only the endemic *Austropotamobius italicus* (Faxon, 1914), no branchiobdellidans are known to be associated with them (Gelder 1999). A meeting of *A. italicus* with either signal or red swamp crayfishes would almost certainly result in the death of *A. italicus*. Therefore, exotic *X. victoriensis* or any other North American branchiobdellidan would not be a problem to endemic Iberian crayfish.

From rice fields in the Júcar Basin at Sueca, Valencia (39º17'22''N; 0º19'32''W) two specimens of *X. victoriensis* were found on *P. clarkii* (7 December 2012). Moreover, during the annual ecological sampling of selected water body sites in the Ebro Basin, Spain (16 July 2013), *P. clarkii* and *P. leniusculus* were collected in the Piedra River at Cimballa, Zaragoza (41º6'8''N; 1º46'31''W) and both carried *X. victoriensis*. Also, during the samplings of 2014 in the Ebro Basin, twelve specimens of *X. victoriensis* were found on *P. clarkii* (7 August 2014) in the Zadorra river at Villodas, Álava (42º50'6''N; 2º46'55''W). These are the first records of a western North American branchiobdellidan on an eastern North American crayfish. Signal crayfish is considered a "cold water" species and this tends to keep their distribution separate from "warm water" red swamp crayfish. While true in general term, environmental conditions at some locations enable both crayfishes to cohabit (Oscoz *et al.* 2010). Such a situation has also been reported in Hokkaido, Japan (Nakata *et al.* 2006).

Because *X. victoriensis* on *P. clarkii* in the Júcar Basin (Sueca) is 450 km from the reported Ebro Basin location (Villodas), such adoptions are likely more wide spread than currently appreciated, and more should be expected to arise. Although *P. clarkii* were introduced into California (Riegel 1959) and recently found in eastern Washington State, USA (Larson & Olden 2013), it is surprising that an exchange of endemic branchiobdellidans between the two host species has not been reported in these Pacific States, USA. However, following the extensive world-wide translocations of these crayfishes it is interesting that the first record of a western branchiobdellidan onto an eastern crayfish should have been observed in Spain.

Most branchiobdellidans, particularly *X. victoriensis*, have a nutritional relationship with their host, which using ecological definitions of Boucher *et al.* (1982) can be commensal, parasitic or mutualistic (reviewed in Skelton *et al.* 2013) as prevailing conditions, *e.g.* abundance, microhabitat, and water quality, dictate. How and to what extent branchiobdellidans affect crayfish behaviour with respect to the local fauna, flora, and physical environment remain to be investigated, although effort are being made (Brown & Lawson 2010). To date, *X. victoriensis* appeared to be host specific to signal crayfish, therefore, limnologists and agency managers have a further example of an exotic branchiobdellidan on an unexpected crayfish species. The effects of branchiobdellidans on wild crayfish species are unknown, and this makes it difficult to suggest any meaningful conservation measures. However, both signal and red swamp crayfish have a significant impact on the introduced habitat’s ecology and for the moment authorities must focus on them (Usio & Townsend 2004).

Effort by the European Union to identify and track expansions of exotic host and symbiont species will help to develop strategies to maintain endemic diversity as far as is possible. Such monitoring also enables early detection of pathogens. These circumstances and our first report of an exotic branchiobdellidan and crayfish association emphasize the need for freshwater body monitoring and accurate identification of collected invertebrates across the world. Monitors sorting samples can no longer rely entirely on existing taxonomic keys for a region, but must also be capable tracking and identifying new exotic species.

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CAPÍTULO 4TH *CHAPTER*

INVADIENDO A LOS INVASORES: CONDICIONES AMBIENTALES Y RELACIONES ENTRE UN BRANQUIOBDÉLIDO EXÓTICO Y SU HOSPEDADOR EXÓTICO

Invading the invaders: relationships of an exotic branchiobdellidan with its exotic host and environmental conditions

El Apéndice 1 del artículo se encuentra en el Anexo 2 de la tesis (pag. 181)
y el Apéndice 2 en el Anexo 3 (pag. 187)

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Resumen

En este trabajo se estudian las condiciones ambientales y los rasgos del hospedador que afectan a la abundancia de un branquiobdélido exótico. Una de las especies invasoras de cangrejo de río más ampliamente distribuidas en Europa es el cangrejo señal Pacifastacus leniusculus *(Dana, 1825). Junto con esta especie invasora de cangrejo, se introducen otras especies exóticas que viven en su superficie, como bacterias, algas, hongos e invertebrados. Una de estas especies simbióticas es un gusano branquiobdélido,* Xironogiton victoriensis *(Gelder & Hall, 1990). Debido a que en un estudio previo esta especie se ha localizado en tres poblaciones diferentes y distantes de cangrejo rojo de las marismas,* Procambarus clarkii *(Girard, 1852), se hace imprescindible estudiar los factores que afectan su abundancia. Entre los años 2005 y 2013 se muestrearon 1817 lugares en la Cuenca del Ebro (España), en los cuales se estudió la distribucion de* X. victoriensis *y* P. leniusculus. *Además, muestreamos 2914 cangrejos señal en 43 localidades españolas para investigar la influencia de las condiciones ambientales y los rasgos del hospedador (sexo, tamaño, condición corporal, abundancia y muda) en la abundancia de* X. victoriensis. *La abundancia del branquiobdélido fue mayor en los individuos más grandes y aquellos que mostraban mejor condición corporal. Debido a que la condición corporal del cangrejo se incrementaba significativamente con la infestación de branquiobdélidos, se sugiere que el mutualismo podría ser la relación biológica existente entre* X. victoriensis *y* P. leniusculus. *Mientras que el 91.04% de los cangrejos adultos estaban infestados con* X. victoriensis*, sólo el 59.3% de los juveniles estaban infestados. Los cangrejos no-mudados tuvieron mayores abundancias de* X. victoriensis *que los ejemplares recién mudados; sin embargo, la abundancia del ectosimbionte no tuvo diferencias ni entre sexos ni entre abundancias del hospedador. Finalmente, la abundancia del branquiobdélido se correlacionó positivamente con una elevada demanda biológica de oxígeno, fosfatos, coliformes y potasio. Estas relaciones entre las variables ambientales y la abundancia de branquiobdélidos son muy importantes para la evaluación de riesgos y la creación de modelos de la predicción de la propagación de las especies exóticas de branquiobdélidos, ya que no han sido muy estudiadas previamente.*

Palabras clave: *relaciones biológicas, especies exóticas, mutualismo, cangrejo señal,* Xironogiton victoriensis.

Abstract

Environmental conditions and host features that influence the abundance of an exotic branchiobdellidan were studied. One of the most widespread invasive crayfish species in Europe is the signal crayfish *Pacifastacus leniusculus* (Dana, 1825). Along with this invasive crayfish, other alien species that live on the crayfish's body surface, such as bacteria, algae, fungi and invertebrates, were introduced. One of these symbiont species is a branchiobdellidan worm, *Xironogiton victoriensis* (Gelder & Hall, 1990). Because a previous study identified this exotic branchiobdellidan in 3 different and spatially separate populations of the red swamp crayfish, *Procambarus clarkii* (Girard, 1852), an investigation into the factors affecting its abundance was necessary. We sampled 1817 sites between 2005 and 2013 in the Ebro Basin (Spain), and the distributions of *X. victoriensis* and *P. leniusculus* were represented and interpreted. Furthermore, we sampled 2914 crayfish from 43 Spanish localities to investigate the influence of environmental conditions and host features (sex, body size, body condition, density and ecdysis) on the abundance of *X. victoriensis*. The abundance of the branchiobdellidan was higher in the largest individuals and in those showing the best body condition. The crayfish body condition (or welfare) increased significantly with the branchiobdellidan infestation, suggesting that mutualism is the biological relationship between *X. victoriensis* and *P. leniusculus*. Although 91.04% of the adult signal crayfish were infested with *X. victoriensis*, only 59.3% of the juveniles were infested. Nonmolted crayfish had higher branchiobdellidan abundance than that of recently molted host specimens; however, the abundance of the symbiont did not differ between sexes and host densities. Finally, biochemical oxygen demand, phosphates, coliforms, and potassium were positively related with the branchiobdellidan abundance. The relationships between some environmental variables and the abundance of branchiobdellidans have not been previously well studied, and these findings will be important for risk assessments and models predicting the spread of exotic branchiobdellidans.

Key words: biological relationships, exotic species, mutualism, signal crayfish, *Xironogiton victoriensis*.

Introduction

Invasive alien species are considered the second most significant cause of extinctions after habitat destruction (Clavero & García-Berthou 2005). This problem is particularly relevant in inland waters, and crustaceans are among the most successful invaders (Gherardi 2007, Hanfling 2011). Invasive species have many ecological impacts, which include competitive interactions (for habitat and food), predation, transmission of diseases, habitat alteration, and changes in ecosystem function (*e.g.* Geiger *et al.* 2005). Invasive crayfish species not only affect biodiversity but also lead to economic, health, and social problems (Cano & Ocete 1994).

The signal crayfish, *Pacifastacus leniusculus* (Dana, 1825), is one of the most successful crayfish invaders in Europe and was first introduced to Sweden in 1959 (Holdich *et al.* 2009). This crayfish is endemic to the northwestern United States and southwestern Canada and has negative effects on macrophytes, benthonic invertebrates, amphibians, reptiles, and benthic fishes (*e.g.* Karjalainen *et al.* 2015). Although the ecological impacts of invasive crayfish species have received increasing attention, less is known about the symbiotic organisms associated with these invaders. Invasive crayfishes are covered with a hard, nonliving exoskeleton that is a suitable substrate for the growth of organic matter and alien organisms such as bacteria, diatoms, filamentous algae, protozoa, fungi, and symbiotic invertebrates (Edgerton *et al.* 2002). North American crayfish species are carriers of exotic pathogens and symbionts, including *Batrachochytrium dendrobatidis*, the causative agent of chytridiomycosis in amphibians (McMahon *et al.* 2013), and *Aphanomyces astaci* (Schikora, 1906), the causative agent of crayfish plague in native European crayfish species (Edgerton *et al.* 2004). In particular, the epibiotic organisms found by Cuellar *et al.* (2002) in the exoskeleton of *P. leniusculus* were Mastigophora, Ciliophora, Turbellaria, Rotifera, Annelida, Acarina and Ostracoda.

Symbionts are organisms living together with other larger species (host) that provide protection, including sites for reproduction and feeding (*e.g.* Young 1966). The abundance and prevalence (percentage of hosts occupied by symbionts in a population) of symbiotic species is influenced by host features and environmental conditions. Host body size and population densities are considered the most influential host features (*e.g.* DeWitt *et al.* 2013). Although the role of environmental conditions is generally considered irrelevant to endosymbionts, a few studies show that the abundance and prevalence of nonparasitic freshwater ectosymbionts (ostracods) are markedly affected by climate and water chemistry (*e.g.* Mestre *et al.* 2014). Ectosymbionts such as branchiobdellidans are directly exposed to external and variable freshwater conditions; however, the relationships between the abundance of branchiobdellidans and physicochemical water characteristics have not been previously well studied.

An example of this alien ectosymbiotic biota in the signal crayfish is the branchiobdellid *Xironogiton victoriensis* (Gelder & Hall, 1990). Branchiobdellidans, or crayfish worms, are a small group of leech-like clitellate annelids that are obligate ectosymbionts of freshwater crustaceans (Govedich *et al.* 2010). A list of branchiobdellidan species reported on signal crayfish is given in Gelder (2004). *X. victoriensis* was the first alien North American branchiobdellidan reported in Europe, and that report was from *P. leniusculus* in Sweden (Franzén 1962). In subsequent years, new reports have shown that *X. victoriensis* is widely distributed in Europe and has been identified in Spain (Gelder 1999), Finland (Kirjavainen & Westman 1999), Italy (Oberkofler *et al.* 2002, Quaglio *et al.* 2002, Gelder 2004), and France (Laurent 2007, Subchev 2008). Other alien species of branchiobdellidans have been found recently in Europe. Gelder *et al.* (2012) reported the first records in France of the North American branchiobdellidans *Cambarincola gracilis* (Robinson, 1954) and *Cambarincola okadai* (Yamaguchi, 1933) on signal crayfish. Additionally, 2 other North American branchiobdellidans, *X. victoriensis* and *Sathodrilus attenuatus* (Holt, 1981), were reported on the signal crayfish in Japan (Ohtaka *et al.* 2005).

Experimental evidence suggests that crayfish fitness and survival can be affected by branchiobdellidans, environmental conditions, and host characteristics such as age or sex (Brown *et al.* 2002, 2012, Lee *et al.* 2009, DeWitt *et al.* 2013). Understanding how branchiobdellidan abundances are influenced by biotic and abiotic factors may therefore be useful for understanding the symbiosis between crayfish and branchiobdellidans. Branchiobdellidan abundance varies temporally (Bishop 1968, Koepp & Schlueter 1977, DeWitt *et al.* 2013) and across branchiobdellidan species, host individuals (DeWitt *et al.* 2013), host populations, and host species (Brown *et al.* 2002, Skelton *et al.* 2013). Temporal variation is also influenced by several factors, including the branchiobdellidan life cycle, the host molting cycle (Koepp 1975, Koepp & Schlueter

1977), and environmental variables (*e.g.* water temperature, DeWitt *et al.* 2013). The relative importance of these variables may vary spatially, by species, or in relationship to intraspecific and interspecific interactions.

Crayfish–branchiobdellid symbiosis can shift from mutualism to parasitism depending on symbiont density (Brown *et al.* 2012). At low to moderate densities, the worms appear to clean debris and epibionts effectively from the crayfish gills; however, at higher densities, resources such as detritus and epibionts on the exoskeleton and gills may become limiting for the worms (Brown *et al.* 2012). Two branchiobdellidans, *Cambarincola ingens* (Hoffman, 1963) and *Xironodrilus* sp., increased the growth rate of their crayfish hosts, the New River crayfish, *Cambarus chasmodactylus* (James, 1966), and the Chauga River crayfish, *Cambarus chaugaensis* (Prins & Hobbs, 1969), at low branchiobdellidan density but decreased host growth at high density (Brown *et al.* 2012). Keller (1992) showed in a laboratory experiment, however, that *Cambarincola fallax* (Hoffman, 1963) did not change the growth rates of the rusty crayfish, *Orconectes rusticus* (Girard, 1852). Lee *et al.* (2009) and Brown *et al.* (2002) suggested that the relationship between crayfish and branchiobdellidans can fluctuate between commensalism and mutualism, depending primarily on the fouling pressure in the environment. In an experiment with an excess of organic matter in a simulated stream bed, Lee *et al.* (2009) observed that the growth rates in crayfish infested with branchiobdellidans were higher than in noninfested crayfish.

A few studies have inferred the importance of biotic and abiotic factors to branchiobdellidan abundance (but see DeWitt *et al.* 2013). Furthermore, a few studies have examined the underlying mechanisms that must ultimately drive alien branchiobdellidan abundance (*e.g.* dispersal, fecundity, and mortality rates), or how these mechanisms are influenced by the environment (but see Thomas *et al.* 2013). The goals of this research were (1) to analyze the distribution and new records of *X. victoriensis* in the Ebro Basin from 2007 to 2013 and (2) to study the environmental conditions and host features that influence on the abundance of the alien branchiobdellidan *X. victoriensis*.

Materials and methods

Data were collected from the Ebro Basin from 2005 to 2013 to analyze the distribution of *X. victoriensis*. The Ebro River is located in the northeastern Iberian Peninsula and flows from northcentral Spain to the northwestern Mediterranean, forming a delta of 320 km^2 (Sabater *et al.* 2009). With a length of 928 km, the Ebro River is the largest river in Spain, draining an area of 85 362 km^2 (Figure 1). Macroinvertebrate samplings have been conducted since 1991 to determine the ecological quality of the waterbodies throughout the Ebro Basin to meet the EU Water Framework Directive (CHE 2013). Additionally, the presence of crayfish and branchiobdellidan species has been recorded annually from 2005 to 2013. The same specialist studied 1817 sampling points between 2005 and 2013 (Table 1; Appendix 1). All study sites were examined for the presence of crayfish and branchiobdellidan species, although the number of crayfish studied and sampling effort in each site were lower than in the specific crayfish samplings in the province of Navarra (north of Spain; Figure 2).

Table 1. Summary of the sampling points with *Xironogiton victoriensis* and with *Pacifastacus leniusculus* in the Ebro River Basin samplings.

Year	Sampling points	With *P. leniusculus*	With *X. victoriensis*
2005	191	27 (14.1%)	None
2006	108	9 (8.3%)	None
2007	272	19 (7%)	1 (5.3%)
2008	314	19 (6.1%)	2 (10.5%)
2009	309	42 (13.6%)	10 (23.8%)
2010	180	15 (8.3%)	8 (53.3%)
2011	152	20 (13.2%)	10 (50%)
2012	107	22 (20.6%)	8 (36.4%)
2013	184	27 (14.7%)	10 (37%)

In addition, to analyze the factors that influence branchiobdellidan abundance, we sampled 43 river stretches located in the north of Spain (province of Navarra) in 2013 and 2014 (Figure 2, Appendix 2). Sampling efforts were concentrated to minimize the seasonality effect on abundance and prevalence of *X. victoriensis.* Signal crayfish were caught between 29 July and 19 August 2013 and between 17 July and 20 August 2014. Given that the average water depths were low (15–80 cm) and that the water clarity was good, nocturnal capture with the aid of artificial light was considered the most effective methodology (Reynolds *et al.* 2010). The signal crayfish abundances at each sampling point were expressed as catch per unit effort (CPUE), calculated as the number of crayfish captured in a sampling site per unit of time (1 hr) and person. In all streams, the sampling stretches were continuous, but we ensured all pool and riffle habitat types were sampled.

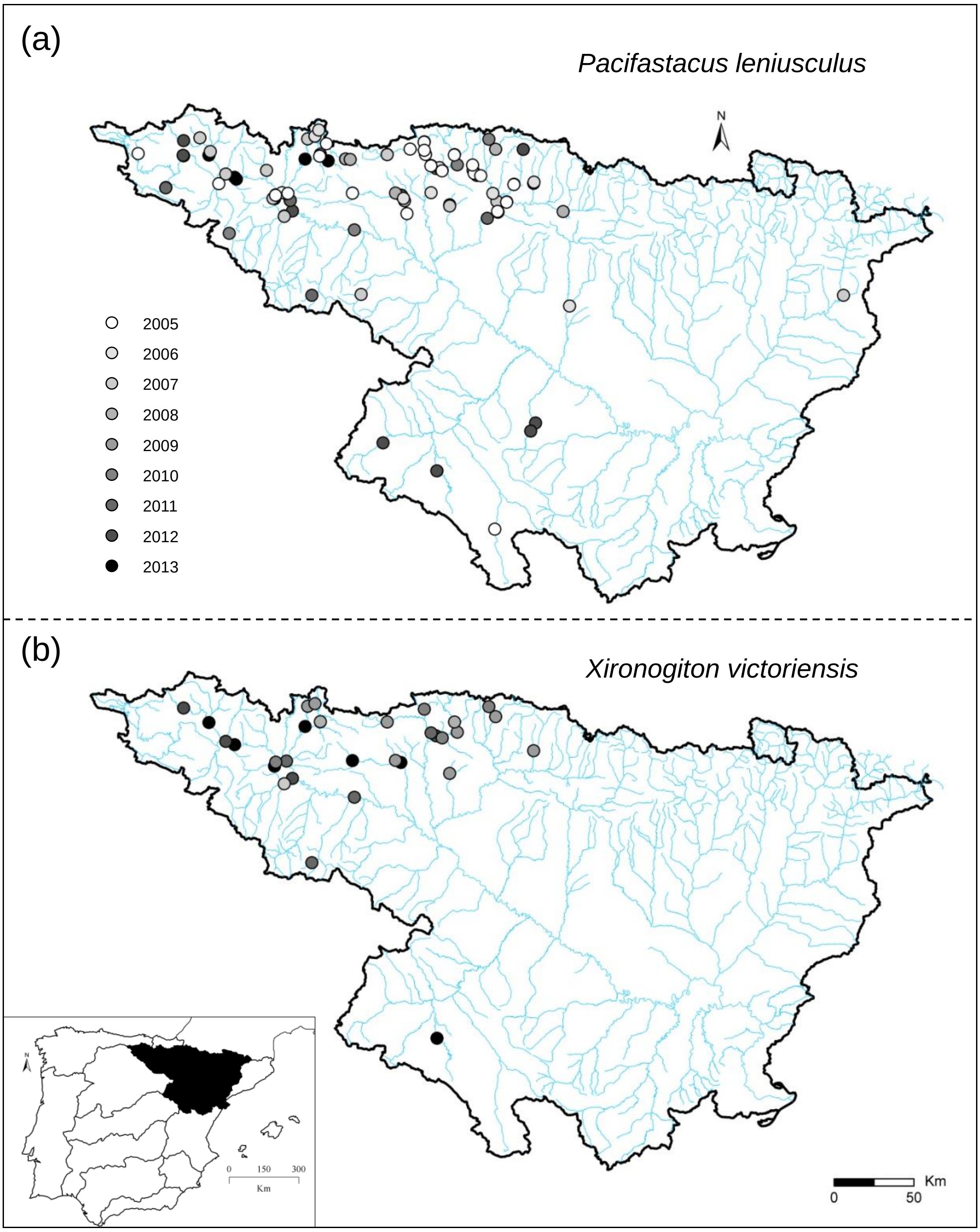


Figure 1. (a) Signal crayfish (*Pacifastacus leniusculus*) records from 2005 to 2013 and (b) *Xironogiton victoriensis* records from 2007 to 2013 in the Ebro River Basin.

Due to the invasiveness of signal crayfish, all specimens were removed from the river and subsequently transported to the laboratory. The variables measured were sex (male, female), carapace length (CL, in mm), total length (TL, in mm), wet fresh body weight (TM, in g), ecdysis (molting, nonmolting), and branchiobdellidan abundance (%). The ecdysis status was estimated by exoskeleton hardness, and the branchiobdellidan abundance was measured by estimating the proportion (expressed in percentage) of the bottom of both claws, propodus (“palm” and “fixed finger” of chela) and dactyl (“movable finger” of chela), for *X. victoriensis*. For statistical analysis, the branchiobdellidan abundance was classified according to 4 infestation classes: none (0%), low (1–4%), medium (5–9%), and high (>10%). The same researcher measured the abundance of the worms to avoid subjectivity in data collection. The branchial chambers of several signal crayfish specimens were inspected. Finally, all specimens were frozen. Branchiobdellidans collected in both samplings (Ebro Basin and Navarra) were preserved in 70% ethanol and later mounted for identification. One-fourth of the total number of branchiobdellidans collected from each site was identified according to the diagnostic characters described by Gelder & Hall (1990).

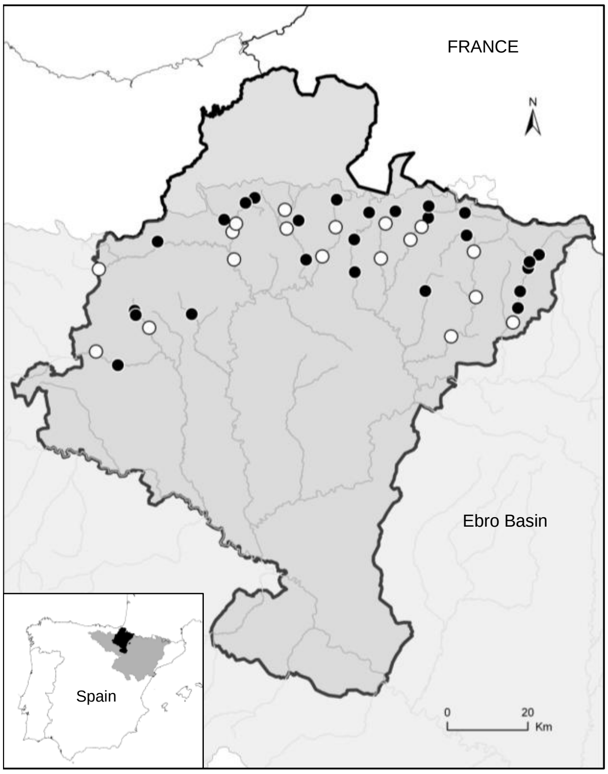


Figure 2. Location of the 43 river stretches in Navarra. These are the sampling points used to analyze the environmental and host factors that influence on the abundance of *Xironogiton victoriensis*. White circles represent the 18 sites where additional environmental variables were analyzed.

A contingency table analysis was used to explore the observed and expected frequencies of the 3 categorical variables ecdysis, sex, and stage (juvenile: TL <60 mm; adult: TL >60 mm) across the categorical variables for branchiobdellidan abundance (none, low, medium, and high; Souty-Grosset *et al.* 2006). A *t*-test was used to compare the allometric coefficients of the weight–length relationships obtained separately for males and females (Zar 1998). The statistical analyses used to compare signal crayfish body condition with the branchiobdellidan abundance of *X. victoriensis* (%) followed those used in previous studies (*e.g.* De Miguel *et al.* 2013). Individuals with only 1 claw, without claws, or with 1 or 2 regenerated claws were discarded from the analysis. The crayfish length–weight relationship is considered a good indicator of body condition and animal welfare (Peig & Green 2010).

The condition of the signal crayfish was represented by the residuals (Kr) obtained from a least squares regression between the log-transformed TM and the log-transformed TL. For this analysis, all crayfish individuals were in the nonmolted stage. A regression between log-transformed TL and log-transformed body condition was calculated to analyze the influence of body size on body condition. Nonparametric tests, a Kruskal-Wallis *H* test and a Mann-Whitney *U* test, were used to compare body condition among the 4 classes of branchiobdellidans. Because certain authors have cautioned against calculating residuals to analyze body condition (García-Berthou 2001), a univariate analysis of covariance (ANCOVA) using log-transformed TM as the independent variable, log-transformed TL as the covariate, and the branchiobdellidan class as the dependent variable was performed to determine whether body mass and length affect the branchiobdellidan abundance. This analysis was performed separately for males and females.

Temperature, conductivity, pH, and dissolved oxygen to study the relationship between the abundance of *X. victoriensis* and the environmental variables (Appendix 2) were measured *in situ* at all sampling points in Navarra. Additionally, among the 43 sites, 18 sampling points were selected to analyze the relationship between the abundance of the branchiobdellidan and some new specific environmental variables. The 18 points used were coincident with some of the 43 points sampled. Since 1992, the Government of Navarra has recorded the following environmental data monthly at these 18 sampling points: temperature (°C), oxygen (mg/L), pH, conductivity (µS/cm), turbidity (NTU), suspended solids (mg/L), inorganic carbon (mg/L), organic carbon (mg/L), organic matter (mg/L), biochemical oxygen demand (mg/L), ammonium (mg/L), nitrites (mg/L), nitrates (mg/L), phosphates (mg/L), total phosphates (mg/L), total coliforms (CFU/100 mL), *Escherichia coli* (CFU/100 mL), calcium (mg/L), sodium (mg/L), potassium (mg/L), chloride (mg/L), magnesium (mg/L), sulfates (mg/L), bicarbonates (mg/L), carbonates (mg/L) and fluorides (mg/L; GN 2014). The averages of the minimum and maximum annual values and the averages of the environmental data were used for the analysis. The annual averages of the minima and maxima were calculated for the variables temperature, conductivity, suspended solids, organicmatter, biochemical oxygen demand, and nitrates. The remaining variables did not have sufficient data for an accurate estimation of the maximum and minimum annual values. First, an exploratory analysis based on nonparametric correlations (Spearman’s rho) between all environmental variables and *X. victoriensis* abundance was performed. Subsequently, the correlated variables were selected, and a linear regression model analysis of log-transformed variables was used to show the relationship of each environmental variable to the abundance of the branchiobdellidans.

The statistical analyses were conducted with SPSS (v15.0) for Windows and PAST (v2.17). The geographical information system software ArcGIS (v10) was used for map elaboration. Data for the layer of administrative areas were downloaded from the GADM database of the Global Administrative Areas (GADM 2014).

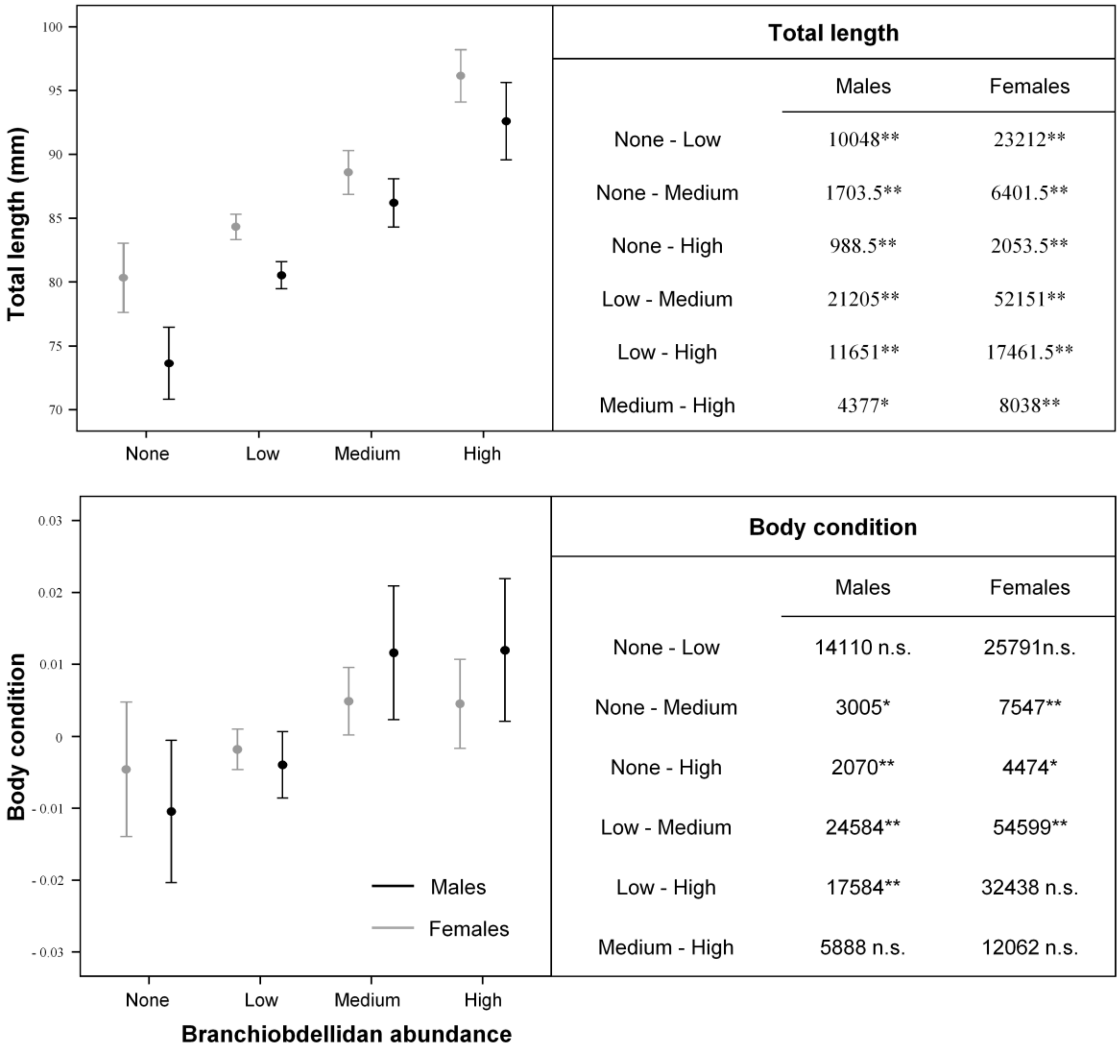
Results

All branchiobdellidan specimens examined belonged to the species *X. victoriensis*. Sites where *X. victoriensis* were found on signal crayfish in the Ebro Basin from 2005 to 2013 (Table 1, Figure 1, Appendix 1) show that the first record of *X. victoriensis* in the Iberian Peninsula occurred in the Altube River (Nervión River Basin, Province of Vizcaya) (Gelder *et al.* 1999). We analyzed 43 river samples from the specifically designed crayfish-sampling program in the province of Navarra. Of these sampling points, 29 had signal crayfish and 27 had crayfish infested with *X. victoriensis.* In all, 81.4% of the total number of signal crayfish individuals was infested.

To analyze the relationship between *X. victoriensis* and the host, 2914 signal crayfish were collected between 2013 and 2014. Individuals with only 1 claw, without claws, or with 1 or 2 regenerated claws were discarded from the analysis. The statistical analyses were performed on 1785 adult specimens (TL >60 mm; 756 males and 1029 females) and 258 juveniles (132 males and 124 females). Most specimens of *X. victoriensis* were located under the claws, although some were located over the carapace, tail, or antennae. No branchiobdellidans were found in the host's branchial chambers.

Although 91.04% of the adult signal crayfish were infested with *X. victoriensis* (689 males and 936 females), only 59.3% of the juveniles were infested (77 males and 74 females). Additionally, the abundance of *X. victoriensis* (%) was higher in adults than in juveniles ($\chi^2 = 525.93$, df = 3, $P = 1.14 \times 10^{-13}$). Because juveniles molt more frequently than adults (Young 1966), only adult specimens were used to analyze the branchiobdellidan abundance between nonmolted and recently molted crayfish individuals. Nonmolted signal crayfish had a higher branchiobdellidan abundance than recently molted host specimens ($\chi^2 = 9.28$, df = 3, $P = 0.026$); however, the abundance of the brachiobdellidan (%) did not differ significantly between adult males and adult females ($\chi^2 = 4.09$, df = 3, $P = 0.251$). Similarly, the symbiont abundances (%) were not correlated with the host abundances (CPUE; $r_s = 0.006$, $P = 0.977$, n = 29) and host population prevalence ($r_s = -0.351$, $P = 0.067$, n = 29).

Only data on adult and nonmolted signal crayfish were used to compare the crayfish body condition among the 4 classes of branchiobdellidan abundance. The slopes of the weight–length logarithmic relationships obtained for males (weight = 3.52 × length − 5.40, $r^2 = 0.95$, error b = 0.03, n = 756) and females (weight = 3.07 × length − 4.61, $r^2 = 0.97$, error b = 0.02, n = 1029) differed significantly ($t = -14.55$, $P = 2.08 \times 10^{-45}$). For that reason, the body condition was estimated separately for males and females. As expected, the body condition of the signal crayfish was not correlated with the total length ($F = 0.029$, $P = 0.865$). The body condition differed significantly among the 4 branchiobdellidan infestation abundance classes in adult males ($\chi^2 = 21.09$, df = 3, $P = 1.009 \times 10^{-4}$). The Mann-Whitney *U* tests showed statistically significant differences between the classes none and medium, none and high, low and medium, and low and high (*i.e.* none = low < medium = high, Figure 3). Similarly, the body condition differed significantly among the 4 branchiobdellidan infestation abundance classes in females ($\chi^2 = 15.24$, df = 3, $P = 0.002$). The Mann-Whitney *U* tests showed statistically significant differences in body condition between the classes none and medium, none and high, and low and medium (Figure 3).



Total length	Males	Females
None - Low	10048**	23212**
None - Medium	1703.5**	6401.5**
None - High	988.5**	2053.5**
Low - Medium	21205**	52151**
Low - High	11651**	17461.5**
Medium - High	4377*	8038**

Body condition	Males	Females
None - Low	14110 n.s.	25791n.s.
None - Medium	3005*	7547**
None - High	2070**	4474*
Low - Medium	24584**	54599**
Low - High	17584**	32438 n.s.
Medium - High	5888 n.s.	12062 n.s.

Figure 3. Left: Average of the total length (mm) and body condition of signal crayfish in the 4 abundance classes of *Xironogiton victoriensis* for males (black) and females (grey). Right: Values of the Mann-Whitney *U* test comparisons of total length and body condition among the 4 abundance classes of *X. victoriensis*. **P* < 0.05; ***P* < 0.01; n.s. = nonsignificant.

The TL differed significantly among the 4 branchiobdellidan infestation abundance classes in adult males ($\chi^2 = 100.52$, df = 3, $P = 1.20 \times 10^{-21}$). The Mann-Whitney *U* tests showed statistically significant differences in TL between the classes none and low, none and medium, none and high, low and medium, low and high, and medium and high (*i.e.* none < low < medium < high, Figure 3). Similarly, the TL differed significantly among the 4 branchiobdellidan infestation abundance classes in adult females ($\chi^2 = 102.26$, df = 3, $P = 5.08 \times 10^{-22}$). The Mann-Whitney *U* tests showed statistically significant differences in TL between the classes none and low, none and medium, none and high, low and medium, low and high, and medium and high (*i.e.* none < low < medium < high, Figure 3).

In agreement with the results presented above, an ANCOVA showed significant differences in the dependent variable TM among the 4 classes of branchiobdellidan abundance for adult males ($P = 1.43 \times 10^{-4}$) and adult females ($P = 0.024$).

Only the averages for the variables biochemical oxygen demand ($r_s = 0.710$, $P = 9.6 \times 10^{-4}$, n = 18), phosphates ($r_s = 0.571$, $P = 0.013$, n = 18), total coliforms ($r_s = 0.713$, $P = 8.9 \times 10^{-4}$, n = 18), and potassium ($r_s = 0.561$, $P = 0.015$, n = 18) were positively correlated with the branchiobdellidan abundance. Similarly, the regressions were statistically significant for the same variables (Figure 4). The remaining variables did not show significant correlation with the abundance of *X. victoriensis*.

Discussion

More than one species of branchiobdellidan is usually found on the same host, and although *P. leniusculus* in North America has been reported with several branchiobdellidan species (Gelder 2004), notably, only *X. victoriensis* has been found on *P. leniusculus* in Spain as of this study. Since the first report of this alien branchiobdellidan in Spain from the Altube River (Nervión River Basin, Province of Vizcaya; Gelder 1999), new records of this species have been increasing every year (Figure 1).

Only 40% of the signal crayfish populations had *X. victoriensis* in the samples from the Hydrographic Confederation of the Ebro between 2005 and 2013, whereas 93% of signal crayfish populations sampled in Navarra had the branchiobdellidan. These differences could be explained because in Navarra the former samplings were checked in detail for branchiobdellidans, and the number of seized crayfish was significantly higher; therefore, we consider these data with caution because the sampling effort has varied between samplings. The big difference between regions cannot be explained only by the different sampling effort, however; it is also possible that both regions had different proportion of signal crayfish populations infested with the branchiobdellidan because Navarra has more populations with high abundances. These dense populations were facilitated with the reintroductions of *P. leniusculus* made by the regional administration of Navarra in the 1980s (Vedia & Miranda 2013). Apparently, not all the signal crayfish populations introduced in the past were infested with the branchiobdellidan, and some could have been infested subsequent to introduction. Alien invaders are known to contain a reduced subsample of potential symbionts and parasites because of the chance selection of the individuals transported (Torchin *et al.* 2003). This mechanism is plausible because branchiobdellidans are considered too delicate to survive on the crayfish during commercial transport (Holt 1981, Gelder *et al.* 2012).

Branchiobdellidan abundance (%) had a positive relationship with host TL, as suggested by previous authors (Keller 1992, DeWitt *et al.* 2013). Similarly, adult signal crayfish had significantly more branchiobdellidans than juveniles. This pattern has been observed in other branchiobdellidan species of the genus *Cambarincola* (Young 1966, Keller 1992) and also in other crayfish ectosymbionts, such as the ostracod *Ankylocythere sinuosa* (Aguilar-Alberola *et al.* 2012, Mestre *et al.* 2014). Adults have more branchiobdellidans because (1) the increased body size of the larger hosts offers more surfaces for feeding and reproductive processes, and (2) host molting seems a critical time for these worms (Young 1966).

Gherardi *et al.* (2002) reported that males have more branchiobdellidans than females; however, Lee *et al.* (2009) found that the number of resident branchiobdellidans did not vary with the sex of the crayfish, as we also observed.

Although some authors have suggested that an important factor affecting symbiont prevalence is host density (Mestre *et al.* 2014), our results showed that the abundance of *P. leniusculus* (CPUEs) did not affect the prevalence of *X. victoriensis*. This lack of relationship has also been recorded in other ectosymbionts with low transmission rates compared with host reproductive and dispersal rates (Stanko *et al.* 2006).

Ecdysis and cleaning ability are considered major factors that could influence the difference in branchiobdellidan abundance between juveniles and adults (Young 1966, Keller 1992, Farrel *et al.* 2013). The frequency at which molts occur determines the abundance of branchiobdellidans because molting completely rids the body of fouling organisms (Keller 1992). Intermolt periods for older crayfish are longer (11 molts during their first year and 2 or 1 molts per year by age 3 and 4, respectively), allowing time for a greater population buildup of branchiobdellidans and fouling organisms (Young 1966, Souty-Grosset *et al.* 2006). Our results also show that the abundance of *X. victoriensis* is influenced by ecdysis because recently molted signal crayfish have a lower branchiobdellidan abundance on the claws. Keller (1992) observed that during the initial stage of ecdysis, the worms moved from the bottom to the top of the carapace, and most of the worms transferred within 5 minutes after the molt. Factors such as crayfish behavior (scraping or eating), competition between branchiobdellidan species, and environmental fouling can influence the colonization of the worms (Gelder *et al.* 1999, Thomas *et al.* 2013).

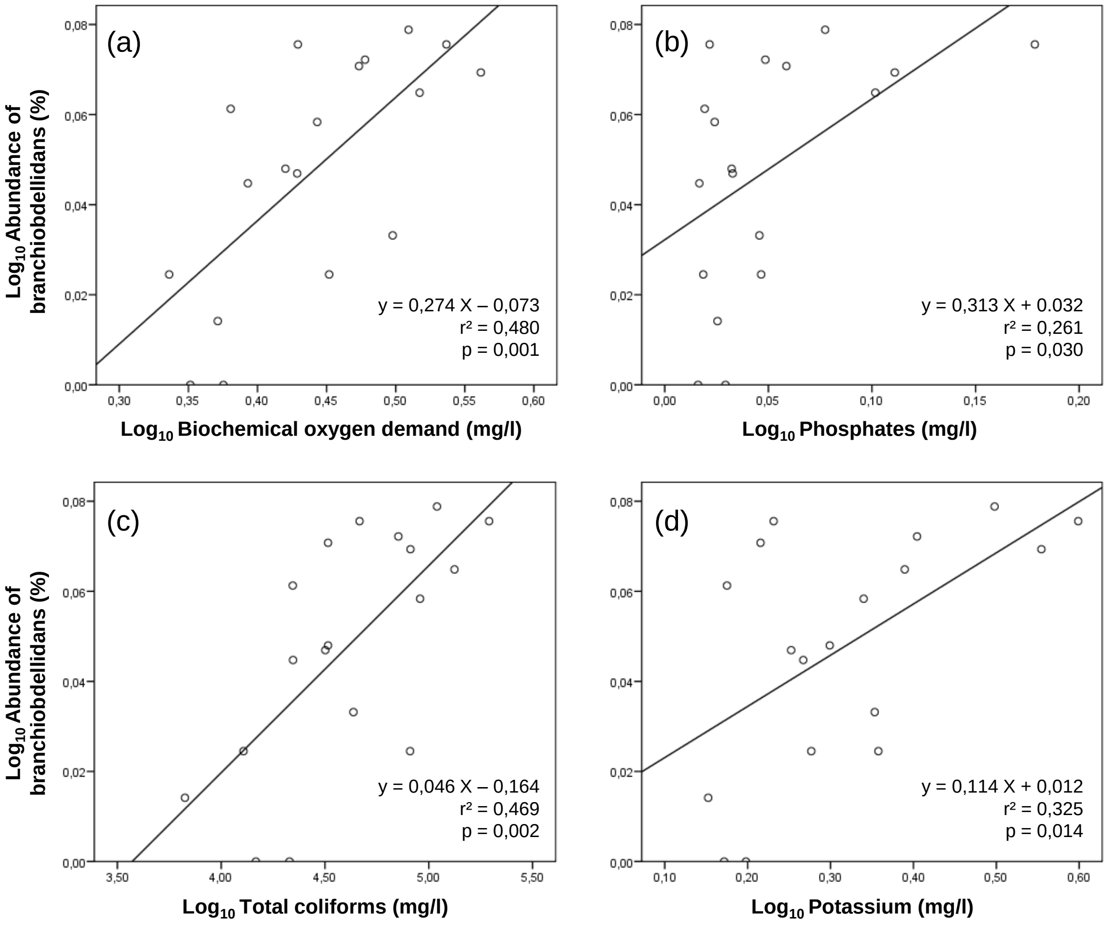


Figure 4. Relationships between the abundance of *Xironogiton victoriensis* and the environmental variables (a) biochemical oxygen demand, (b) phosphates, (c) total coliforms, and (d) potassium.

In decapod crustaceans, general body grooming is performed by the minor chelipeds and by brushes on the posterior walking legs (Skelton *et al.* 2013). Skelton *et al.* (2014) concluded that only small crayfish can effectively remove all cleaners using the dactyls of the walking legs because large crayfish are unable to detect cleaners or lack the dexterity to remove them. Gelder (1999) observed that few places on the body of 4 crayfish species were effectively out of reach of cleaning behavior; Jones and Lester (1996) found that the grooming limbs of the red claw crayfish, *Cherax quadricarinatus* (Von Martens, 1868), were able to reduce the prevalence of an ectosymbiotic flatworm; and Bauer (2002) found that the body grooming performed by the red swamp crayfish, *Procambarus clarkii* (Girard, 1852), was not sufficient to resist heavy fouling pressures from tenacious exotic fouling organisms, whether macroscopic or microscopic.

Previous studies suggest that the abundance of branchiobdellidans depends on the symbiont's life cycle, the host's biological characteristics, and also on environmental conditions (DeWitt *et al.* 2013). Biochemical oxygen demand, phosphates, total coliforms, and potassium were the environmental variables significantly related to the abundance of *X. victoriensis*. All these variables are related to the greater organic matter coming from human activities or greater riparian vegetation cover. Given that branchiobdellidans are omnivorous and feed on debris and epibionts growing on the crayfish host carapace, greater amounts of organic matter are directly associated with a greater abundance of available food for branchiobdellidans (Brown *et al.* 2012). Thomas *et al.* (2013) observed that the branchiobdellidan density of *Cambarincola ingens* (Hoffman, 1963) was higher under high-fouling conditions, but under low-fouling conditions, large worms may compete for limited epibiotic resources. Grazing activity or "cleaning" by branchiobdellidans is beneficial to the crayfish (Brown *et al.* 2002) because the epibiotic fouling that develops inside the gill chamber can cause severe respiratory problems (Bauer 1989). Consequently, the abundance of these worms is related to organic matter, which determines the degree of fouling on the exoskeleton surface of crayfishes (Bauer 2002). In the same way, other crayfish ectosymbionts, such as the ostracods, are affected by environmental conditions (Mestre *et al.* 2014). These findings suggest that the relationships between worm abundance and environmental variables (such as organic matter) will be useful for risk assessments and models predicting the spread of exotic branchiobdellidans.

Our results showed that well-feed crayfish have more branchiobdellidan abundance. This result has 2 possible explanations: (1) the species *X. victoriensis* selected and preferred the well-fed and larger signal crayfish because these individuals offer more feeding surface and leftover food in addition to having a lower molt frequency than that of juveniles; and (2) the branchiobdellidans are causing the crayfish to be in better condition. Although both explanations are possible, it is more probable that branchiobdellidans were the responsible of improving the health of the crayfish if we take into consideration the limited ability of movement of branchiobdellidans between crayfish hosts and the results of previous studies showing that worms can enhance the growth and survivorship (positive cleaning activity) of crayfish (Lee *et al.* 2009, Brown *et al.* 2012, Vedia *et al.* 2014). Our results support mutualism as the best biological relationship explaining the symbiosis between *X. victoriensis* and *P. leniusculus*; however, further analysis of the costs and benefits of the association to both organisms is required to answer this question in more detail.

Geasa (2014) has suggested that *X. victoriensis* is a surface dweller because the oral papilla and sensory ganglia in *Xironogiton* are suitable for external parasitism on the hard cuticle of the chelae and body surfaces such as the cephalothorax and legs of *P. leniusculus*. In the specimens analyzed in this study, no branchiobdellidans were found in the host’s branchial chambers.

Several specimens of *X. victoriensis* have been found in at least 3 red swamp crayfish in 3 widely separated localities of the Iberian Peninsula (Vedia *et al.* 2014); therefore, other crayfish species could be infested in the near future with nonnative branchiobdellidan species because the presence of branchiobdellidans on crayfish has not been considered when the hosts are transported. Because new associations between *X. victoriensis* and other crayfish species could have unpredictable consequences, scientists should be alert to these hypothetical future infestations because an exotic species may have different effects on different host species (Vedia *et al.* 2014). Generally, the relationship between branchiobdellidans on wild crayfish is considered between mutualism and parasitism (Brown *et al.* 2012), but the effects on crayfishes are poorly known, and this lack of information hinders suggesting any meaningful conservation measures. Because of the influential role that crayfish play in aquatic systems, branchiobdellidans may have indirect effects on aquatic communities and ecosystem processes via their direct effects on crayfish. Efforts by environmental agencies to identify and track expansions of exotic host and symbiont species will assist with the development of strategies to maintain endemic diversity as much as possible.

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Supplementary Material

Supplementary material is available for download via the Inland Waters website, https://www.fba.org.uk/journals/index.php/IW: Appendix 1 and 2.

CAPÍTULO 5TH *CHAPTER*

INTERACCIONES TERRITORIALES Y TRÓFICAS ENTRE EL CANGREJO SEÑAL (*PACIFASTACUS LENIUSCULUS*) INTRODUCIDO Y LOS PECES NATIVOS BAJO CONDICIONES NATURALES EN RÍOS IBÉRICOS

*Spatial and trophic interactions between the introduced signal crayfish (*Pacifastacus leniusculus*) and native fishes under natural conditions in Iberian rivers*

El Anexo 1 del artículo se encuentra en el Anexo 4 de la tesis (pag. 191)

Vedia I, Rodeles AA, Leunda PM, Baquero E, Galicia D,

Oscoz J, Elustondo D, Santamaría JM, Almeida D, Miranda R

Enviado a *Freshwater Biology*

Resumen

Las interacciones de competencia entre peces y cangrejos tienen lugar sobre dos recursos principalmente: alimento y espacio. En concreto, estudiamos la competencia trófica y por el hábitat entre el cangrejo señal Pacifastacus leniusculus *–uno de los cangrejos invasores más exitosos de Europa– y los peces nativos. Las interacciones de comportamiento interespecíficas entre el cangrejo y los peces han sido ampliamente estudiadas en acuarios experimentales. Sin embargo, pocos estudios han analizado estas interacciones en condiciones naturales. Esta investigación es el primer estudio que analiza las interacciones por el espacio entre un cangrejo introducido y peces nativos en condiciones naturales. El objetivo de este estudio fue analizar las interacciones entre* P. leniusculus *con peces nativos, como el lobo de río (*Barbatula quignardi*), el gobio (*Gobio lozanoi*), el piscardo o chipa (*Phoxinus bigerri*) y la trucha común (*Salmo trutta*). Además, evaluamos el solapamiento trófico entre el cangrejo introducido y las especies nativas de peces, usando tanto el contenido estomacal como los isótopos estables de carbono y nitrógeno. Para el estudio etológico, utilizamos cámaras de vídeo, que fueron modificadas para visión nocturna, y luces de infrarrojo, para no alterar el comportamiento natural de los animales. La mayoría de los encuentros entre* P. leniusculus *y peces nativos fueron sin interacción (63%). Sin embargo, en el 66% de los encuentros con interacción, el cangrejo era el dominante. El resultado de la interacción fue altamente influenciado por el estadio del cangrejo (juvenil/adulto) y por las especies de peces, pero no fue influenciado por el sexo del cangrejo. El solapamiento de la dieta se calculó con el índice de Schoener, mostrando un alto solapamiento de los contenidos estomacales entre* P. leniusculus y, B. quignardi, G. lozanoi *y* P. bigerri, *y un bajo solapamiento con* S. trutta. *Se tomaron muestras de la musculatura abdominal de peces y cangrejos para medir las proporciones de los isótopos estables de carbono y nitrógeno. En general, se observaron unas dietas y nichos isotópicos similares (solapamiento trófico) entre el cangrejo introducido y los peces nativos, sugiriéndose que puede haber una competencia trófica entre estas especies cuando hay una limitación de los recursos alimenticios. Demostramos que la competencia entre* P. leniusculus *y los peces nativos es particularmente alta con las especies bentónicas. Teniendo en cuenta que las especies de peces bentónicas de la Península Ibérica, algunas de ellas en peligro de extinción (e.g.* B. quignardi*), habitan en el área de distribución del cangrejo invasor, los gestores de la conservación deberían tomar medidas urgentes para controlar las poblaciones del cangrejo.*

Palabras clave: *especies exóticas invasoras, peces endémicos, competencia interespecífica, comportamiento, solapamiento trófico, análisis de isótopos estables.*

Abstract

Competitive interactions between fish and crayfish occur over two main resources: food and space. In particular, we study the habitat and trophic competition between the signal crayfish *Pacifastacus leniusculus* –one of the most successful crayfish invaders in Europe– and native fishes. The interspecific behavioural interactions between crayfish and fish species have been widely reported in experimental aquaria. However, few studies have analysed these interactions under natural conditions. This investigation is the first study to analyse the spatial interactions between an introduced crayfish and native fishes under natural conditions. The aims of this study were to analyse the interactions of *P. leniusculus* with native fishes, such as the Pyrenean stone loach (*Barbatula quignardi*), the Pyrenean gudgeon (*Gobio lozanoi*), the Pyrenean minnow (*Phoxinus bigerri*) and the brown trout (*Salmo trutta*). Furthermore, we evaluate the trophic overlap between the introduced crayfish and the native fish species using both stomach content and stable isotopes of carbon and nitrogen. For the ethological study, we used video cameras that were modified for night vision and infrared lights to illuminate without disturbing the natural behaviour of the animals. Most of the encounters between *P. leniusculus* and native fishes involved no interaction (63%). However, in 66% of the encounters that involved an interaction, *P. leniusculus* was dominant. The result of the interaction was strongly influenced by the crayfish life stage (juvenile/adult) and by the fish species but not by the sex of crayfish. The dietary overlap was calculated with the Schoener index, revealing a high overlap in stomach content between *P. leniusculus* and *B. quignardi*, *G. lozanoi* and *P. bigerri* and a low overlap with *S. trutta*. Samples of the abdominal muscles of the crayfish and fish were collected to measure stable isotope ratios of carbon and nitrogen. In general, similar diets and isotopic niches (trophic overlap) were observed between the introduced crayfish and the native fishes, suggesting that trophic competition may occur between these species when food resources are limiting. We demonstrate that competition between *P. leniusculus* and native fishes is particularly high with benthic species. Considering that endemic benthic fish species of the Iberian Peninsula, many of which are seriously endangered (*e.g. B. quignardi*), inhabit the distribution area of this invasive crayfish, conservation managers should take urgent measures to control the crayfish populations.

Key words: invasive alien species, endemic fishes, interspecific competition, behaviour, trophic overlap, stable isotope analyses.

Introduction

Recent extinction rates are several orders of magnitude higher than estimates for pre-human times in many different taxonomic groups and environments (Pimm *et al.* 1995, Barnosky *et al.* 2011). After habitat destruction, invasive species are considered to be the most significant cause of these extinctions (Clavero & García-Berthou 2005). This problem is particularly relevant in inland waters (Gherardi 2007). In fact, Mediterranean endemic freshwater fish are among the most endangered biota in the world and are especially threatened by habitat fragmentation and invasive species (Smith & Darwall 2006). Crayfish species are the most common alien crustaceans in freshwater ecosystems and are considered to be keystone species because of their significant ecological and socio-economic impacts: competition with native fauna for habitat and food, predation, disease transmission, habitat alteration, and changes in ecosystem function (Geiger *et al.* 2005).

The mechanisms by which fish and crayfish interact may be direct (*e.g.* predation or competition) or indirect through the alteration of habitats and interactions with the ecosystem (Reynolds 2011). Competitive interactions typically occur in the context of two main resources: food and space. When both crayfish and fish are omnivorous, there will be competition and mutual predation, depending on the relative sizes of the species. Depending on the species, the predation balance between the fish community and crayfish may be more or less unequal. Thus, while native fish populations may be affected by predation and competition from exotic crayfish, alien predatory fishes may negatively impact native crayfish (Guan & Wiles 1997).

The effect of crayfish on fish communities, particularly benthic fish species, has been widely reported under laboratory conditions. Bubb *et al.* (2009) studied interspecific behavioural interactions and competition for shelter between the benthic fish the bullhead (*Cottus gobio*) and the white-clawed crayfish (*Austropotamobius pallipes*) and signal crayfish (*P. leniusculus*) in experimental aquaria. Those authors observed that both crayfish species were dominant over *C. gobio*, but *P. leniusculus* made more aggressive approaches than *A. pallipes*. In addition, a negative relationship between the densities of *P. leniusculus* and *C. gobio* was observed, with high abundance of *C. gobio* where crayfish were absent or where *A. pallipes* was present at a low density (Bubb *et al.* 2009). Griffiths & Collen (2004) observed under controlled conditions that the proportion of Atlantic salmon (*Salmo salar*) sheltering was significantly lower in the presence of *P. leniusculus* when refuge was limiting. In contrast, those authors showed that the proportion of *P. leniusculus* sheltering was independent of the presence of *S. salar*. Bryan & Robinson (2002) found that virile crayfish (*Orconectes virilis*) and rainbow trout (*Oncorhynchus mykiss*) influence the behaviour of Little Colorado spinedace (*Lepidomeda vittata*) in artificial streams. *L. vittata* not only decreased its movements in and out of refuge but also decreased its activity rates. Using a smaller-scale *in situ* experiment, Stenroth & Nyström (2003) did not find any evidence that *P. leniusculus* affects the survival and growth of juvenile brown trout (*Salmo trutta*), even though crayfish significantly reduced the abundance of benthic invertebrates.

Laboratory experiments have limitations concerning their applicability to natural ecosystems because the limitation of space reduces the ability of the prey to escape from the predator and exaggerates predation effects. As a consequence, fights among specimens of a crayfish species were shorter, less intense and less likely to end with a tailflip in nature than in the laboratory (Bergman & Moore 2003). Nonetheless, very few attempts have been made to evaluate the real effects of introduced crayfish species on native fishes under natural conditions. In a study carried out in a British lowland river, Guan & Wiles (1997) found an inverse correlation between the abundances of the introduced *P. leniusculus* and the benthic *C. gobio* and stone loach (*Barbatula barbatula*). Those authors showed that *C. gobio* was absent from a stretch of river invaded by *P. leniusculus*, where *C. gobio* had previously co-existed with *A. pallipes*. Others authors found that where the *P. leniusculus* density is high, the density of juvenile *S. trutta* is correspondingly low (Peay *et al.* 2009). On the other hand, Degerman *et al.* (2006) showed no effects of crayfish presence, crayfish species or crayfish density on fish population densities in streams in Sweden. Thus, where some studies find substantial effects (*e.g.* Guan & Wiles 1997), others report that crayfish have no or small effects on fish (*e.g.* Stenroth & Nyström 2003, Degerman *et al.* 2006). All of these previous field studies are based primarily on traditional comparisons of the abundances of crayfish and fish populations. However, the study of interactions between native and invasive species in aquatic systems is complex due to the difficulty in distinguishing if the changes in abundance of native species are determined primarily by the invaders or by uncontrolled external factors (*e.g.* environmental variables).

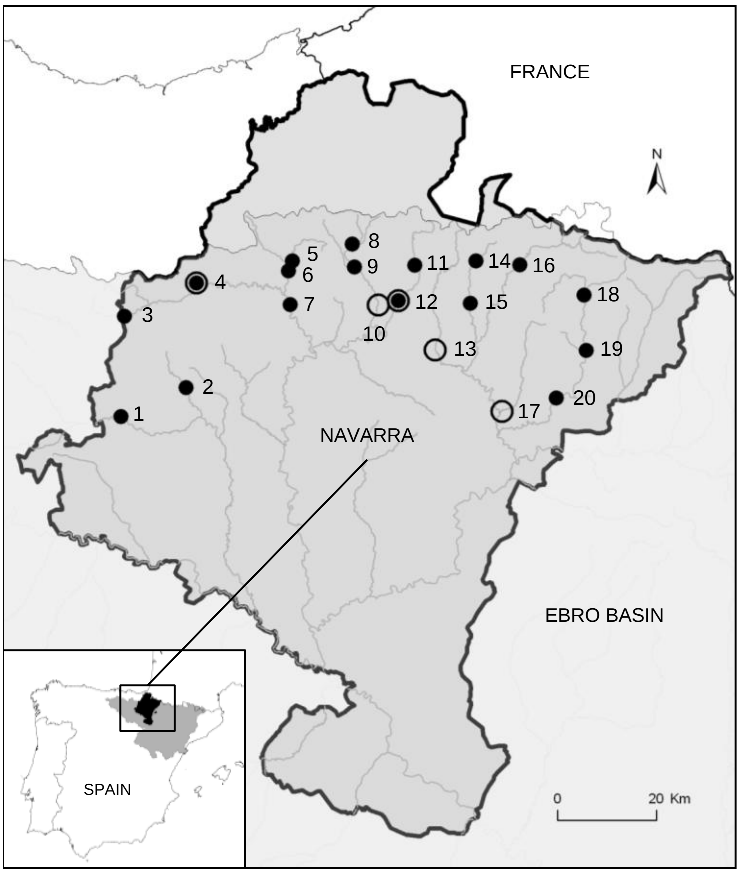
Quantifying the feeding ecology of animals has traditionally focused on direct observations (*i.e.* stomach content analysis), but these methods do not adequately reflect cryptic species and the quantity of nutrients assimilated into tissues (Bearhop *et al.* 2004). In the last years, ecologists have successfully employed stable isotope analyses of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) to define the dietary niches of species (Peterson & Fry 1987, Layman *et al.* 2007). The concept of ecological niche as conceptualized Hutchinson (1957) is a hypervolume set in n-dimensional space where each of the axes represents an environmental parameter. Increases in $\delta^{15}N$ between 2.0‰ and 3.4‰ (Post 2002) with each subsequent trophic level have been documented but vary by species, tissue, and location (*e.g.* Caut & Angulo *et al.* 2009). In addition, the isotopic biplot of $\delta^{15}N$ and $\delta^{13}C$ and the extent of isotopic niche overlap between species suggest interspecific dietary similarity, as well as the potential for coexistence or competitive exclusion of species in new interactions (Ercoli *et al.* 2014).

As noted above, the mechanisms by which crayfish and fish interact have been studied extensively under laboratory conditions, but less emphasis has been placed on this competition in natural settings. Almeida *et al.* (2014) highlighted the usefulness of direct observations in the wild for assessing the effects of invasive species on aquatic communities. To our knowledge the present investigation is the first study to analyse the spatial interactions between an introduced crayfish and native fishes under natural conditions. For that reason, the main goal of this study is to analyse the interaction between *P. leniusculus* and native fishes under natural conditions by (1) studying the spatial competition between *P. leniusculus* and indigenous fishes and (2) evaluating the trophic overlap between the crayfish and these native species. Firstly, we hypothesise that *P. leniusculus* will compete for space with native fishes, especially benthic species, as previous aquarium experiments have shown. However, this competition is expected to be less intense in the field than in aquarium experiments. Secondly, a trophic overlap is expected due to the omnivorous diet of the crustacean, although the extent of this overlap is unknown.

Materials and methods

Study area

This study covered all medium and high stretches of the main Pyrenean rivers of the region of Navarra (northern Spain). All of the sampled localities (*n* = 20) are in rivers that drain to the Ebro River, which is one of the main Iberian basins, with an area of 85 362 km^2. The following rivers were sampled: Ega, Urederra, Arakil, Lizarrusti, Basaburua, Larraun, Ulzama, Arga, Erro, Urrobi, Irati, Areta and Salazar (Figure 1, Annex 1). The flow regime type in the study area is pluvio-nival, and the period of greatest discharge lasts from November until April (Bejarano *et al.* 2010). Riparian vegetation is abundant in all of the rivers, but in the upper stretches, livestock use occurs on grasslands, and in the lower stretches, cereal fields are dominant.



Localities	**Rivers**
1. Zuñiga	Ega
2. Artavia	Urederra
3. Ziordia	Ega
4. Lizarragabengoa	Lizarrusti
5. Udabe	Basaburua
6. Latasa	Larraun
7. Oskia	Arakil
8. Iraizoz	Ulzama
9. Latasa	Ulzama
10. Olave	Ulzama
11. Urtasun	Arga
12. Idoi	Arga
13. Lizoáin	Erro
14. Espinal	Urrobi
15. Uriz	Urrobi
16. Aribe	Irati
17. Rípodas	Areta
18. Ezcaroz	Salazar
19. Guesa	Salazar
20. Aspurz	Salazar

Figure 1. Distribution of the seventeen sites where crayfish and native fishes were sampled to evaluate their trophic overlap using stomach contents and stable isotopes (black circles). The five night recording sites are marked with black circumferences. The sites used for both studies (trophic and spatial overlap) are marked both with black circles and circumferences. In Annex 1, the names of the localities and rivers of each sampling point are also presented.

The Ebro Basin has the largest, more abundant and oldest signal crayfish populations of the Iberian Peninsula (Vedia & Miranda 2013). In the studied rivers, the fish community is composed of benthic fishes [the Pyrenean stone loach (*Barbatula quignardi*) (Least Concern, LC) and the Pyrenean gudgeon (*Gobio lozanoi*) (LC)], bentho-pelagic fishes [the Ebro nase (*Parachondrostoma miegii*) (LC)], pelagic fishes [the Pyrenean minnow (*Phoxinus bigerri*) (LC) and *S. trutta* (LC)], and rarely, the endangered north Iberian spined loach *Cobitis calderoni* (endangered, EN) (Leunda & Miranda 2007, Leunda *et al.* 2012).

Habitat competition

The study of habitat competition was conducted during the months of June-July 2015 because during this period of the year, the water clarity is generally good, and the activity of fishes and crayfishes is high (Bubb & Thom 2004). We made recordings in five rivers located in the region of Navarra (northern Spain), from headwaters to middle reaches (towns in parentheses): Lizarrusti (Lizarragabengoa, 4), Ulzama (Olave, 10), Arga (Idoi, 12), Areta (Rípodas, 17) and Erro (Lizoáin, 13) (Figure 1).

As both *P. leniusculus* and *B. quignardi* are more active at night, a total of 10 recording sessions (2 recordings per site on consecutive days) were made in five locations immediately after sunset. At each site, a total area of 18 m^2 of riverbed was recorded for 7 hours (3.5 hours and 9 m^2 each day). Infrared lamps were used to illuminate and to avoid disturbing the normal behaviour of crayfish and fishes (Bergman & Moore 2003, Huber & Davis 2007). The employed recording equipment consisted of three cameras placed 1 m from the riverbed in a zenithal position (GoPro4 CHDHX-401-M modified for night vision, with a 2.5 mm lens) and six infrared lamps (IR100, 890 nm, 2AH, 12V) installed in a fully adjustable metallic structure. A battery (80 amperes/hour) provided energy to the infrared lamps (Figure 2).

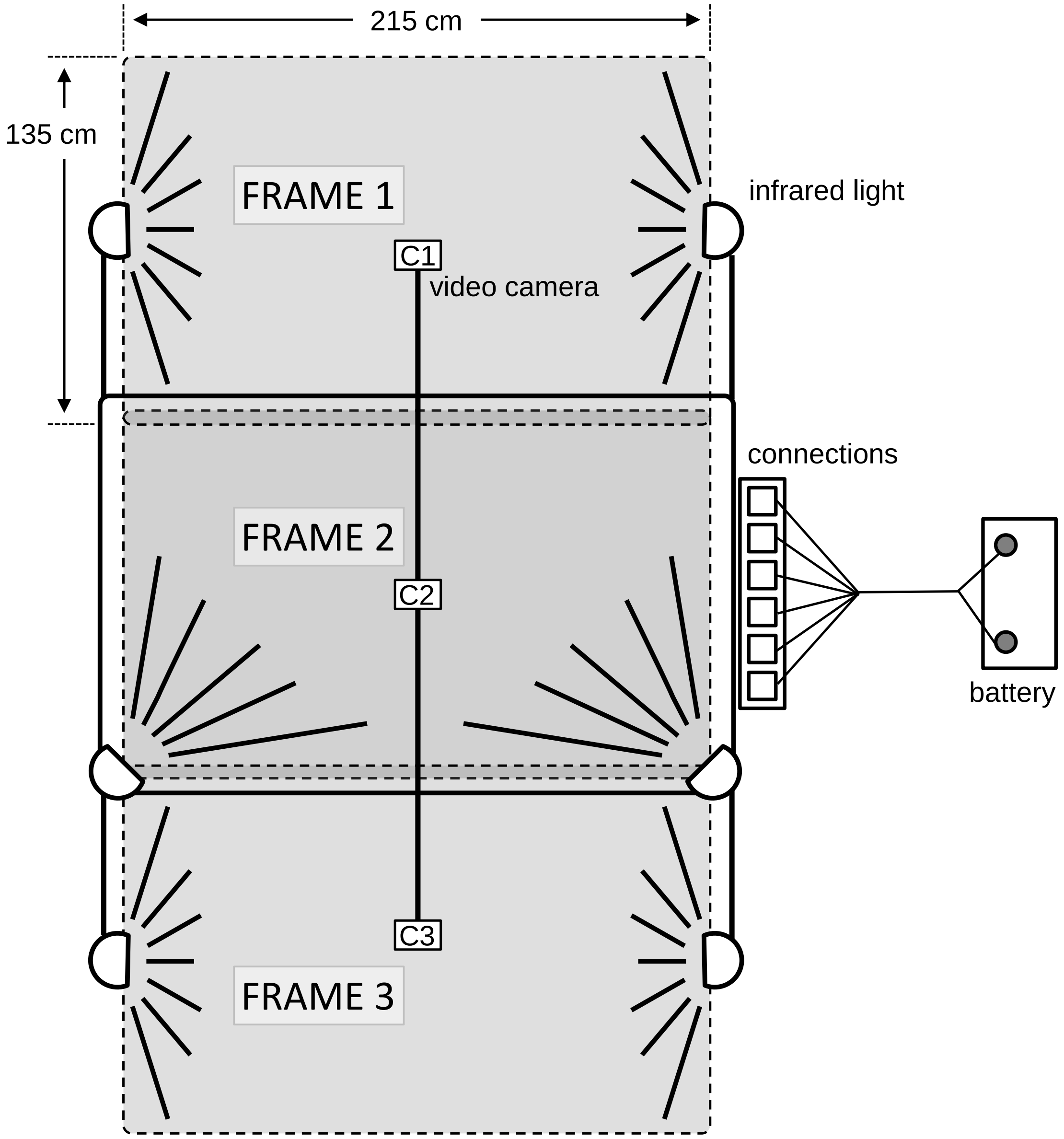


Figure 2. The night recording system consists of three video cameras adapted for night vision in the zenithal position and six infrared lights connected to a battery.

The videos obtained using the three cameras were merged with the program Adobe Premiere Pro CS6. The videos were visualized with VLC media player (version 2.2), and all interactions of each individual crayfish with native fauna were identified and analysed. The life stage (juvenile/adult) and the sex (male/female) of adult crayfish individuals were annotated. The categories of life stage and sex were classified visually by an observer experienced in previous field surveys to provide full certainty during video visualizations. The encounters were defined from the point of view of the fishes. These encounters were catalogued as (1) ‘fish evasion’ (only the fish changes its position and/or normal behaviour in the presence of the crayfish), (2) ‘fish dominance’ (only the crayfish changes its position and/or normal behaviour in the presence of the fish) and (3) ‘no interaction’ (neither the crayfish nor the fish changes its position and/or normal behaviour in the presence of the other).

Trophic competition

Fish and crayfish were collected from 17 sampling sites in July-August 2014 to evaluate the trophic competition between *P. leniusculus* and the native fishes (Figure 1, Annex 1). Given that the average depths were very low (15–80 cm) and that the water clarity was good, nocturnal hand sampling of crayfish with headlights was considered to be the most effective methodology in these types of river stretches (Reynolds *et al.*, 2010). The crayfish variables measured were ‘sex’ (male/female), ‘total length’ (TL, mm) and ‘weight’ (W, g). Fish sampling was performed via electrofishing surveys (Hans Grassl model IG200/2D, 300–600V, 0.2–2A). For the development of this study, the lowest number of native fish specimens was collected. All collected fish and crayfish were euthanized properly with low temperatures (cooler) because the use of anaesthetics was not recommended for the subsequent stable isotope analyses. Field samplings were carried out by trained personnel to avoid altering the native ecosystems and were performed in compliance with Spanish and European regulations.

All crayfish (*n* = 85; 5 per site) were stored in a laboratory freezer until the time of the study of stomach contents and the stable isotope analysis of carbon and nitrogen. The pyloric and cardiac stomachs of the crayfish were extracted individually and temporarily preserved in a vial of 70% ethanol. During the analysis, the stomachs were split, and the number of each food type was counted visually using a dissecting microscope (Table 1). The presence/absence of algae and detritus was annotated for each stomach. Benthic invertebrates were identified to the family level using available keys (Oscoz *et al.* 2011). The relative abundance of each food type was calculated as a percentage.

The abdominal muscle of the same crayfish specimens was collected to determine the carbon and nitrogen stable isotopes, which reflect distinct aspects of a consumer’s long-term trophic niche. The ratios of carbon and nitrogen isotopes provide a time- and space-integrated representation of primary carbon sources and relative trophic position, which rely on the enrichment of ^{15}N with trophic transfer (Peterson &

Fry 1987). We used muscle tissue samples because such samples exhibit less variation in $\delta^{13}C$ and $\delta^{15}N$ than other tissues, reflecting more accurately the diet over a longer period of time (Yokoyama *et al.* 2005, Phillips & Eldridge 2006). The muscle samples were not acidified, and no debris from exoskeletons containing inorganic carbonates was included (Stenroth *et al.* 2006). All samples were dried at 60°C for 72 h and milled with tungsten carbide balls (Ø 3mm) in a Retsch Mixer Mill MM400 (100–240 VAC, 50/60 Hz). The fish sample size was n = 85 for *P. bigerri*, n = 60 for *S. trutta*, n = 25 for *G. lozanoi*, n = 20 for *B. quignardi* and n = 10 for *P. miegii*. The weight and total length of the fish were measured, and a piece of white muscle tissue was dissected immediately behind the operculum. The same sample processing described for crayfish was performed for these fish samples.

The concentrations of carbon (%C) and nitrogen (%N), as well as their isotopic signatures (values of $\delta^{13}C$ and $\delta^{15}N$), were estimated for each sample. The total N and C contents (% dry weight) were determined using an elemental analyser (varioMICROCube, Elementar) with an analytical precision of 0.1%. Calibration was performed using sulfanilic acid. A plant reference material (algae- 14th Intercomparison exercise AEO, MAT Control, University of Barcelona) was used to ensure that the accuracy was within 7% of the total concentrations. Stable C and N isotope abundances were measured using an isotope ratio mass spectrometer (Isoprime 100, Isoprime, Ltd.) operating in continuous flow mode. The reference gas (high-purity N_2 gas) was calibrated to the atmospheric N_2 standard using IAEA-N-1 (International Atomic Energy Agency) and USGS 40 (U.S. Geological Survey) as reference materials. An accuracy below 3% was found for $\delta^{15}N$ and $\delta^{13}C$ using the same algae that was used as a reference material as a standard. The precision of the analysis was estimated based on the relative standard deviation (RSD) of duplicated samples and was found to be within 3% for both the total content and isotopic signatures. Isotope data are given as $\delta^{13}C$ and $\delta^{15}N$ values, which represent the ratio expressed per mil (‰) between the isotopic composition of the sample and that of the PDB standard (Pee Dee Belemnite) for carbon and that of atmospheric N_2 for nitrogen:

$$\delta^{13}C\ (‰\ \text{vs. V-PDB}) = [(R_{sample}/R_{standard}) - 1] \times 1000$$

$$\delta^{15}N\ (‰\ \text{vs. at-air}) = [(R_{sample}/R_{standard}) - 1] \times 1000,$$

where R_{sample} is the isotope ratio ($^{13}C/^{12}C$) or ($^{15}N/^{14}N$) for the sample and $R_{standard}$ is the isotope ratio for the standard.

Statistical analyses

Separate contingency analyses were performed using Fisher's exact test to investigate the statistical association between the result of the interaction (fish evasion, fish dominance and no interaction) and the crayfish life stage (juvenile/adult), crayfish sex (male/female), and fish species. Cell standardised residuals were used to better understand the associations between variables, with an absolute value of this parameter above 1.5 indicating a lack of fit of H_0 (*i.e.* no association between variables) in that cell (Agresti 2007).

The diet overlap between *P. leniusculus* and native fishes was determined by using the Schoener overlap index (S) (Schoener 1970):

$$S = 1 - 0.5(\sum |p_{ia} - p_{ib}|),$$

where p_{ia} and p_{ib} are the relative proportions of each prey item *i* for species '*a*' and '*b,*' respectively. The index ranges from 0, which indicates no dietary overlap, to a maximum overlap of 1 when all prey items are found in equal proportions. Index values > 0.6 indicate significant dietary overlap (Wallace 1981). Data concerning the diets of the native fish species *B. quignardi*, *G. lozanoi*, *P. bigerri* and *S. trutta* were obtained from previous studies in the same study area (Oscoz *et al.* 2000, Oscoz *et al.* 2001, Oscoz *et al.* 2003, Oscoz *et al.* 2004, Oscoz *et al.* 2005, Oscoz *et al.* 2006a).

The statistical differences between the values of $\delta^{15}N$ and $\delta^{13}C$ obtained for *P. leniusculus* and native fishes at each site were tested with non-parametric analyses. The Kruskal-Wallis *H* test and *a posteriori* Mann-Whitney *U* tests were used to analyse these differences. Subsequently, the percentage of sites at which each fish species exhibited no significant differences from the values of $\delta^{15}N$ and $\delta^{13}C$ obtained for crayfish was calculated.

To calculate the niche space of each species and the overlap among the species, we used the packages "Stable Isotope Bayesian Ellipses in R" (SIBER, package for R version 2.10.1) and "Stable Isotope Analysis in R" (SIAR) to calculate the population metrics (Layman *et al.* 2007, Jackson *et al.* 2011). One model was developed per site with SIBER because the isotopic baseline differs among sites and it cannot be assumed that our target species have the same diet at all sites. The distribution of the isotopic signatures of individuals in isotopic space is used to quantify the niche size or isotopic niche. The isotope niche of a population integrates temporal and spatial population habitat variations and was represented as the corrected Standard Ellipses Areas (SEAc). These ellipses were estimated to minimise the bias caused by small sample sizes, using the variance and covariance of bivariate isotope data to contain approximately 40% of the data to represent a core isotopic niche for each population. Given the coordinates of two bivariate samples, the overlap function calculates computationally the area of overlap between two standard ellipses (see methodology in Jackson *et al.* 2011).

All statistical analyses were performed with SPSS 15.0 for Windows and R 3.1.3 (http://www.r-project.org). The level of significance was established at $P = 0.05$. The geographical information system software ArcGIS (version 10) was used for the elaboration of the maps.

Results

Habitat competition

The nocturnal behaviour of $n = 422$ individuals of *P. leniusculus* was studied in five localities along five Pyrenean rivers (Figure 1). A total of $n = 256$ encounters between *P. leniusculus* and native fishes were analysed in the video recordings (Figure 3). Most of the interactions were with native fishes (254 encounters: 99%). In particular, we analysed 103 interactions with *P. bigerri* (41%), 39 interactions with *B. quignardi* (15%), 28 interactions with *S. trutta* (11%), 5 interactions with *G. lozanoi* (2%) and 79 interactions with undetermined fish (31%). In addition, we observed 154 intraspecific interactions and two encounters with the common toad *Bufo bufo* (in one encounter, there was no interaction, and in the other encounter, the toad was the dominant). Most of the encounters between *P. leniusculus* and native fishes involved no interaction (168 encounters: 63%). Among the remaining encounters (98 encounters: 37%), *P. leniusculus* was dominant in 66%.

No association was observed between the sex of crayfish and the result of the interaction (F = 3.41, $P = 0.197$). However, the results of interactions differed significantly between juveniles and adult crayfish (F = 24.16, $P < 0.001$). Only in 4% (13% expected) of the encounters with adult crayfish was the fish dominant (typified residual = –3). However, in 25% (13% expected) of the encounters with juvenile crayfish, the fish was dominant (typified residual = 3.4).

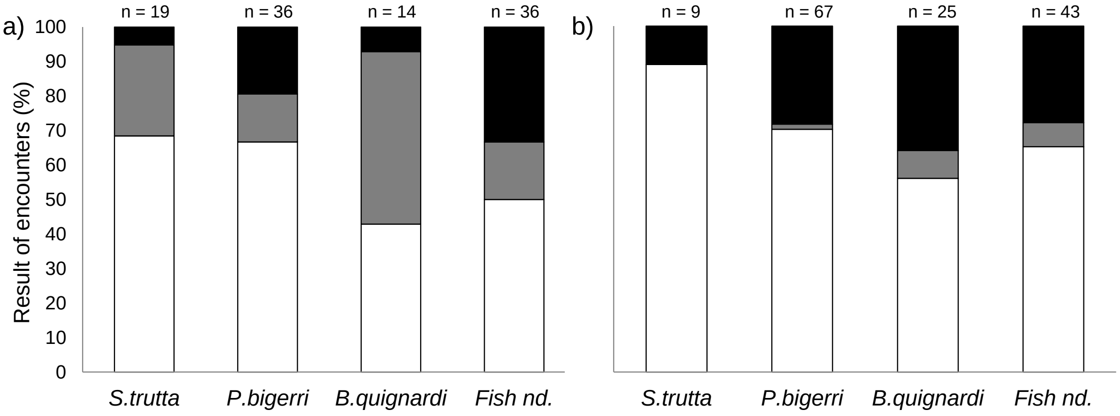


Figure 3. Results of the encounters (%) between native fishes and juveniles (a) or adults (b) of the signal crayfish (*Pacifastacus leniusculus*). No interaction (white), fish dominance (grey) and fish evasion (black) (n = number of encounters; Fish nd = fish species not determined).

In addition, a significant association between the fish species and the result of an interaction with a juvenile crayfish was observed (F = 21.85, *P* = 0.003), but no association was detected in encounters with adult crayfish (F = 9.30, *P* = 0.316) (Figure 3). All encounters (only 5) between *G. lozanoi* and juvenile crayfish involved an interaction (55% expected; typified residual = –1.5). In all encounters between juvenile crayfish and *G. lozanoi*, the fish was dominant (25% expected; typified residual = 3). In the unique encounter between an adult crayfish and *G. lozanoi*, the crayfish was dominant. In 50% of the encounters with juvenile crayfish, *B. quignardi* was dominant (25% expected; typified residual = 1.9). In 33% of encounters between undetermined fishes and juvenile crayfish, the fish escaped from crayfish (19% expected; typified residual = 1.9) (Figure 3).

The endangered species *C. calderoni* was visualized in recordings from the locality of Rípodas (Areta river), but due to the small size of the species and its low contrast in nocturnal recordings, it was difficult to see this species. The few times that this species was visible, no encounters with *P. leniusculus* were observed.

Table 1. Diet composition (%F$_i$: percentage of occurrence and %A$_i$: relative abundance) of the signal crayfish (*Pacifastacus leniusculus*, PL), the Pyrenean stone loach (*Barbatula quignardi,* BQ), the brown trout (*Salmo trutta,* ST), the Pyrenean gudgeon (*Gobio lozanoi,* GL) and the Pyrenean minnow (*Phoxinus bigerri,* PB). Fish diet composition data were obtained from previous studies in the same study area (Oscoz *et al.* 2000, Oscoz *et al.* 2004, Oscoz *et al.* 2005, Oscoz *et al.* 2006a). The dietary overlap between *P. leniusculus* and native fishes was determined by using the Schoener overlap index.

	F$_i$ (% occurrence)					A$_i$ (% abundance)				
Taxon	PL	BQ	ST	GL	PB	PL	BQ	ST	GL	PB
Turbellaria	-	-	1.05	-	-	-	-	0.05	-	-
Nematoda	-	-	38.95	6.67	2.04	-	-	7.30	1.29	0.81
Oligochaeta	-	-	8.42	-	-	-	-	0.49	-	-
Hydracarina	9.41	-	3.16	5.00	4.08	2.72	-	0.15	0.28	0.75
Ephemeroptera	27.06	64.00	70.53	15.00	23.13	12.76	10.32	25.73	0.92	5.33
Diptera	30.59	84.00	24.21	98.33	70.07	52.93	75.93	1.90	66.15	51.08
Plecoptera	1.18	4.00	12.63	1.67	0.34	0.42	0.26	0.58	0.09	0.05
Heteroptera	-	-	1.05	-	-	-	-	0.05	-	-
Megaloptera	-	-	1.05	-	-	-	-	0.05	-	-
Trichoptera	29.41	56.00	38.95	48.33	45.24	22.18	6.08	15.27	6.90	28.33
Coleoptera	2.35	-	4.21	1.67	2.38	0.63	-	0.19	0.09	0.40
Terrestrial insects*	8.24	16.00	22.11	63.33	35.03	1.46	1.32	1.90	6.53	9.16
Crustacea	11.76	32.00	38.95	35.00	9.52	5.86	6.08	45.57	16.93	1.96
crayfish	-	-	-	8.33	-	-	-	0.00	0.46	-
Gastropoda	3.53	-	8.42	6.67	6.46	1.05	-	0.68	0.37	2.11
fish	-	-	2.11	-	-	-	-	0.10	-	-
Schoener Index						-	0.77	0.39	0.69	0.84

*Adult winged insects, ants and spiders.

Trophic competition

For the study of stomach content and the analysis of stable isotopes, a total of 85 adult specimens of *P. leniusculus* were analysed (43 females and 42 males; 5 individuals per site). The stomach content exhibited some differences between males and females. Adult females had a higher frequency of algae (35%) and detritus (84%) than adult males (21% and 79%, respectively). In general, males consumed more benthic invertebrate individuals (271) than females (194). In addition, females had a higher abundance of Trichoptera (39%), Ephemeroptera (20%) and Gammaridae (11%) than males (10.3%, 6.3% and 2.6%, respectively). However, males had a higher abundance of Diptera (78%) than adult females (20%). Similarly, females had more occurrences of Trichoptera (35.7%), Ephemeroptera (29%) and Gammaridae (14%) than males (21%, 24% and 10%, respectively). However, in this case, occurrences of Diptera were more frequent in females (41%) than in males (19%). The occurrences and abundances of the remaining taxonomic groups (Gastropoda, Coleoptera, Hydracarina, Plecoptera and terrestrial insects) were similar between males and females.

To analyse the trophic overlap between the invasive crayfish and the native fishes, we compared the stomach contents of *P. leniusculus* (*n* = 85) with the published information for specimens of *B. quignardi* (*n* = 27), *P. bigerri* (*n* = 333), *S. trutta* (101) and *G. lozanoi* (*n* = 70) that were sampled in the Erro and Larraun rivers in previous studies between 1996 and 2002 (Oscoz *et al.* 2000, Oscoz *et al.* 2001, Oscoz *et al.* 2003, Oscoz *et al.* 2004, Oscoz *et al.* 2005, Oscoz *et al.* 2006a). The Schoener overlap index showed a high diet overlap between *P. leniusculus* and *B. quignardi*, *G. lozanoi* and *P. bigerri*. However, the overlap was lower with *S. trutta* (Table 1).

In contrast, the isotopic overlap (%) between *P. leniusculus* and native species was generally small based on the SEAc, which contains approximately 40% of the data. In this case, *S. trutta*, *G. lozanoi* and *B. quignardi* showed the largest areas of isotopic overlap (in decreasing order) whereas it was null for *P. bigerri* and *P. miegii*. The area of isotopic overlap was null with *P. bigerri* and *P. miegii*. Nevertheless, the isotopic values of the crayfish and the fish species were very close, and at many sites, no significant differences in isotopic signatures were observed (Annex 1, Figure 4 and Table 2).

Table 2. Average δ^{15}N and δ^{13}C of the species at the seventeen sampling sites in Navarra (Spain). Proportion of sites (%) without significant differences between the δ^{15}N and δ^{13}C values of the signal crayfish (*Pacifastacus leniusculus*) and those of native fishes in the non-parametric tests. The area of standard ellipses areas (SEAc) for each species and the area of overlap between *P. leniusculus* and native fishes are summarised.

				δ^{15}N	δ^{13}C	Area SEAc (‰^{2})	Area overlap (‰^{2})
	n	δ^{15}N ± SD	δ^{13}C ± SD	(% sites)		Mean ± SD	
P. leniusculus	85	8.3 ± 2.1	-26.7 ± 2.0	-	-	0.80 ± 0.49	-
B. quignardi	20	8.9 ± 2.7	-27.8 ± 3.3	25	50	0.68 ± 0.55	$(2.7 \pm 5.4)\text{x}10^{-4}$
G. lozanoi	25	10.5 ± 1.6	-26.1 ± 1.6	40	100	0.62 ± 0.25	$(1.3 \pm 2.6)\text{x}10^{-2}$
P. bigerri	85	10.0 ± 2.2	-26.8 ± 2.4	11.8	64.7	1.05 ± 0.67	$(2.8 \pm 3.2)\text{x}10^{-18}$
P. miegii	10	10.7 ± 2.6	-26.0 ± 1.3	50	50	0.55 ± 0.65	$(4.8 \pm 6.7)\text{x}10^{-18}$
S. trutta	60	9.3 ± 2.1	-27.3 ± 2.6	41.7	50	2.01 ± 2.00	0.1 ± 0.2

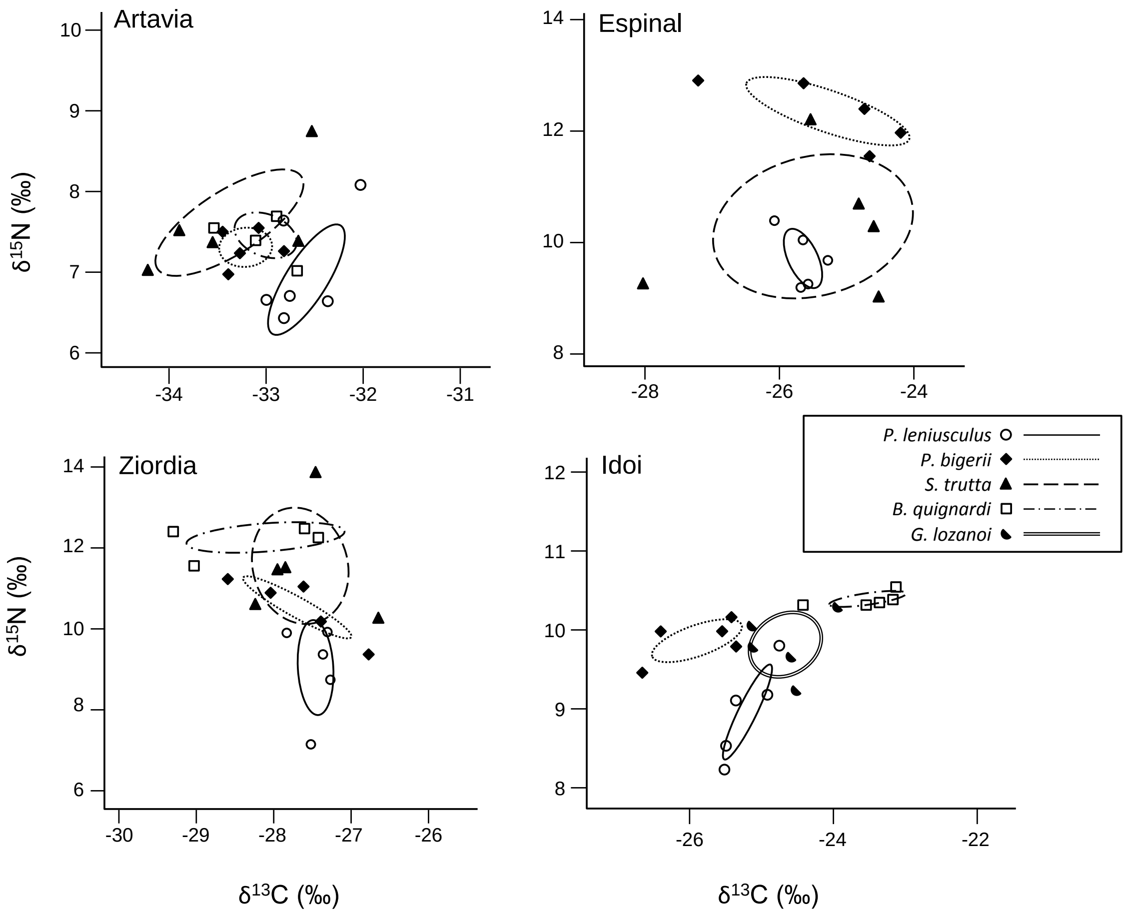


Figure 4. Stable isotope bi-plot illustrating the isotopic niches of the signal crayfish (*Pacifastacus leniusculus*), the Pyrenean minnow (*Phoxinus bigerri*), the brown trout (*Salmo trutta*), the Pyrenean stone loach (*Barbatula quignardi*) and the Pyrenean gudgeon (*Gobio lozanoi*) at four representative sampling stations. The circumferences are the standard ellipse areas (SEAc) that enclose 40% of the data for each species. The average isotopic overlap between *P. leniusculus* and native fish species at all sites is reported in Table 2.

Discussion

Habitat competition

The study of habitat competition in natural settings showed that *P. leniusculus* displaced native fishes to other areas. In addition, the result of an interaction between a native fish and *P. leniusculus* is strongly influenced by the life stage and the size of the crayfish. Fishes were mostly dominant when interacting with juvenile crayfish, whereas adults dominated most of their interactions with native fishes. Considering that most of the native fishes analysed (except *S. trutta*) have small sizes, we could only identify adult specimens to the species level in the nocturnal recordings. In general, *P. leniusculus* competed more for space and interacted more with the benthic species *B. quignardi* and *G. lozanoi* than with the pelagic species *S. trutta* and *P. bigerri*. This behaviour seems logical because *P. leniusculus* overlaps more spatially with benthic fish than pelagic fish in its use of habitat. This observation is similar to the results of Guan & Wiles (1997), which revealed intense interspecific competition between *P. leniusculus* and *C. gobio* in an artificial stream. Those authors observed that there was never more than a single occupant in a shelter and that a great reduction in shelter occupation by *C. gobio* and *B. barbatula* occurred when crayfish were present, as the benthic fish were ousted from the shelters as soon as a crayfish entered (Guan & Wiles 1997). In another study, the Paiute sculpin (*Cottus beldingi*) reduced its use of refuges and pools, shifted into higher velocity microhabitats, and spent more time fleeing in the presence of *P. leniusculus* (Light 2005). In the same way, shelter competition between *B. quignardi* and *P. leniusculus* is likely to be stronger during the day, when these species seek shelter, than at night, when they are outside the shelters.

Our results reinforce previous studies and prove that strong habitat and shelter competition occurs between benthic fish and crayfish. However, we must consider that the degree of interference and competition for shelters is likely to be strongly dependent on the overall availability of shelters. Previous studies suggested that shelter availability is sometimes limited under natural conditions because *P. leniusculus* may be found at densities higher than or similar to the number of potential shelters within an area (Light 2005).

The results of this study performed under natural conditions also confirm the idea that adult crayfish exclude small benthic fishes to other areas, although it is important to remark that the result of competition is strongly influenced by the relative sizes of both species. Previous field observations (via snorkelling) detected a competition for habitat/refuge between *P. leniusculus* and endemic Iberian loaches (*Cobitis calderoni* and *C. paludica*) in a Spanish stream, the Jarama river (Dr. Almeida, pers. observ.). This habitat competition may increase the vulnerability of excluded fishes to predation, but further studies are needed to evaluate how this exclusion affects fish populations (Degerman *et al*. 2006). Rahel & Stein (1988) showed that interactions among predators might also be important in aquatic ecosystems. In particular, those authors observed that interactions among a small prey fish, the Johnny darter (*Etheostoma nigrum*), and two predators, the rusty crayfish (*Orconectes rusticus*) and the smallmouth bass (*Micropterus dolomieu*), were most intense where both predators were common and shelter was limiting. In our study, *P. leniusculus* may increase the risk of predation by other predators for native fishes. This finding suggests that this crayfish and other alien crayfish species can increase the vulnerability of small fishes and juveniles to predators (*i.e.* piscivorous birds, *S. trutta*, and otters) by evicting them from shelters.

Finally, we found that most of the encounters between *P. leniusculus* and native fishes involved no interaction, especially encounters with pelagic fishes. On the other hand, fewer encounters with benthic fishes involved no interaction. Although previous studies under laboratory conditions suggested that *P. leniusculus* competes with pelagic fishes (Griffiths *et al.* 2004), our study has shown that such competition appears to be smaller in natural ecosystems, supporting our first hypothesis.

Trophic competition

The study of diet composition and the stable isotope analysis suggest that *P. leniusculus* and native fishes have similar diets and isotopic niches. The comparison of stomach content between *P. leniusculus* and native fishes suggests a large diet overlap with *P. bigerri*, *B. quignardi* and *G. lozanoi* (in decreasing order), and low with *S. trutta*.

Although the area of overlap between the isotopic niche of *P. leniusculus* and native fishes (using the SEAc) was generally small, the isotopic values were very close, and at many sites, there were no significant differences in isotopic signatures. At all sites, the difference in the average δ^{15}N between *P. leniusculus* and native fishes was lower than 3.4‰ so it is considered that both groups occupy the same trophic level (Post 2002). However, other studies showed that *P. leniusculus* occupied the same trophic level as Ephemeroptera, Odonata and *Notonecta* (Rosewarne *et al.* 2016). These authors analysed the gut-content of adult specimens of *P. leniusculus* in the River Stour (U.K.) which revealed a wide array of diet items, including macrophytes, algae, terrestrial detritus, benthic invertebrates and fish (Rosewarne *et al.* 2016). In this study we also found a wide diet items in the gut-content of *P. leniusculus*. The lower δ^{15}N values of *P. leniusculus* than native fishes might be caused by increased ingestion of algae and detritus (observed in the stomach contents), which have lower δ^{15}N.

The high isotopic overlap between *P. leniusculus* and *S. trutta* at some sites can be partially explained because juvenile and recently moulted crayfish may be a food resource for the fish. The reciprocal use of fish and crayfish may reflect such trophic interactions. Moreover, in our study, the isotopic niche width of *P. leniusculus* was smaller than the isotopic niches of *S. trutta* and *P. bigerri* and larger than the isotopic niches of *B. quignardi*, *G. lozanoi* and *P. miegii.* Invasive species are generally expected to have broader niches than their non-invasive counterparts because they are often generalists that take advantage of underexploited resources. Olsson *et al.* (2009) found that the introduced species *P. leniusculus* had a greater niche width than its ecological counterpart, the native noble crayfish (*Astacus astacus*), indicating that the introduced species used a wider range of habitats or food items than the native species. Regarding the trophic relationship with *S. trutta*, the wide isotopic niche of trout and the small trophic overlap (Schoener index) with *P. leniusculus* could be explained by a shift to the use of a wider range of available food resources. This possibility is confirmed by the variety of prey groups found in the stomachs of the animals (Oscoz *et al.* 2000, Oscoz *et al.* 2005). The cause for this varied diet may be the expansion of the trophic niches of top predators with increased population density due to high intraspecific competition, which leads to a differential diet specialisation among individuals (Svanbäck & Persson 2004). The null isotopic overlap between *P. leniusculus* and *P. miegii* may be explained by the high diet specialization of this fish species, which is known to feed primarily on diatoms, which have a different isotopic signature (Spanish Fish Catalogue 2014).

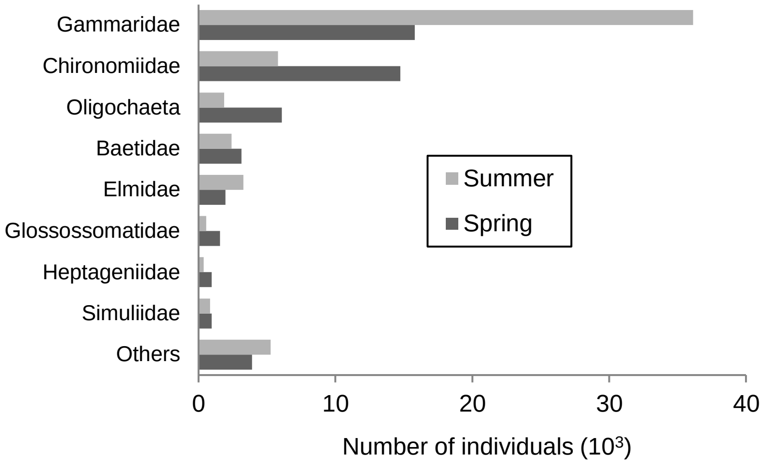


Figure 5. Field estimation of the abundances of benthic invertebrates in one square meter in the locality of Mugiro (Navarra, Spain) (modified from Oscoz *et al.* 2006b).

Considering the dietary and isotopic overlap between the invasive crayfish and native fishes, trophic competition may occur (primarily with *P. bigerri*, *B. quignardi*, *G. lozanoi* and *S. trutta*), especially when food resources are limiting in the aquatic system. Although the abundances of benthic invertebrates are often high in rivers with good quality water, in areas with high human pressure and during some periods of the year, the abundance of these food resources can be scarce (Figure 5). Considering that the same resources must be shared by several species, we suggest that trophic competition may be significant, especially in places with limited food resources. This trophic competition may be particularly relevant between *P. leniusculus* and *B. quignardi* or *G. lozanoi* because these species take shelter in the crevices between stones and feed primarily on the same benthic invertebrates. However, this competition is difficult to evaluate in the field, and the relationship between competition and niche overlap is complex (Holt 1987) because particular resources may not always limit populations and species may overlap with no competition.

It is very difficult to estimate the damage that habitat and trophic competition by the invasive *P. leniusculus* are causing in native populations of fish species. In fact, some scientists suggest that studies must last more than two years to detect a change in fish population densities, as inherent fluctuations occur in all fish populations over time (Degerman *et al.* 2006). This investigation is the first study in which spatial competition and trophic interactions between an introduced crayfish and native fishes have been studied under natural conditions. This field study confirms the assumptions of previous laboratory studies that introduced crayfish could have detrimental effects on fish populations, especially benthic fish species. Considering that endangered native fish species, such as the benthic fish *C. calderoni*, which is catalogued as endangered on the IUCN Red List (IUCN 2016), inhabit the distribution area of the non-native signal crayfish, conservation managers should take urgent measures to control populations of this crayfish.

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DISCUSIÓN GENERAL

GENERAL DISCUSSION

Existen multitud de estudios que hacen referencia a los daños medioambientales y económicos que originan las especies invasoras en los medios acuáticos (Gallardo *et al.* 2016, Rodríguez *et al.* 2005). Aunque muchos de ellos se han centrado en los impactos que producen el mejillón cebra *Dreissena polymorpha* (Pallas, 1771), el visón americano *Neovison vison* (Schreber, 1777) o el cangrejo rojo de las marismas *Procambarus clarkii* (Girard, 1852) (Durán *et al.* 2012, García-Díaz *et al.* 2013, Geiger *et al.* 2005), entre otras muchas especies, menos estudios se han centrado en los impactos que tiene el cangrejo señal *Pacifastacus leniusculus* (Dana, 1852) sobre los ecosistemas fluviales.

En España la investigación científica desarrollada sobre los impactos del cangrejo señal también es escasa. Esta especie se introdujo legalmente durante la década de los años 80 por algunas Comunidades Autónomas con el argumento de que era el sustituto ecológico ideal del casi desaparecido cangrejo de patas blancas *Austropotamobius italicus* (Faxon, 1914) (aunque oficialmente se lo conoce por *A. pallipes* (Lereboullet, 1858)). También se pensaba que actuaba como barrera ecológica entre el cangrejo rojo de las marismas y las poblaciones de cangrejo de patas blancas, sobre todo en tramos de cabecera. Sin embargo, ambos argumentos eran totalmente erróneos, entre otras cosas, porque el cangrejo señal también era portador del patógeno responsable de la afanomicosis, el cual fue el principal causante de la reducción drástica de las poblaciones del cangrejo de patas blancas. Además, en un estudio posterior se observó que el cangrejo rojo de las marismas era el dominante sobre el cangrejo señal (Alonso & Martínez 2006).

En el primer capítulo se hace una revisión bibliográfica sobre el estado de conocimiento de las especies de cangrejo de río presentes en la Península Ibérica a modo de introducción. Sin embargo, la revisión se publicó en el año 2013 y ya se ha quedado obsoleta porque en los últimos años se han publicado varios trabajos acerca de los cangrejos de río en la Península Ibérica. En dicha revisión no se incluyó el cangrejo de los canales *Orconectes limosus* (Rafinesque, 1817) porque en el momento del estudio desconocíamos si esa especie se iba a establecer definitivamente en los ecosistemas acuáticos peninsulares, algo que desgraciadamente se ha confirmado finalmente. El cangrejo de los canales es otra especie exótica originaria de Norteamérica y tolera un amplio rango de diferentes condiciones ambientales. Aunque generalmente vive en grandes ríos o ecosistemas lénticos con sustratos blandos y limosos (Holdich & Black 2007). Se introdujo de forma intencionada en Alemania en el año 1890 y, posteriormente se fue reintroduciendo ilegalmente por muchos países europeos (Souty-Grosset *et al.* 2006). En el año 2010 se detectó la primera población de esta especie en la Península Ibérica en el río Muga (Cataluña) (Benejam *et al*. 2011). Como las otras especies de cangrejos norteamericanas (*P. clarkii* y *P. leniusculus*), este crustáceo es vector del patógeno responsable de la afanomicosis, siendo otro problema añadido para el cangrejo de patas blancas.

Uno de los temas más controvertidos que se discuten en el primer capítulo es el estatus de las poblaciones ibéricas del cangrejo de patas blancas. Este tema ha provocado un debate muy intenso entre los investigadores durante los últimos años. Mientras que unos autores consideran que las poblaciones ibéricas son originarias de translocaciones humanas procedentes de Italia (Clavero *et al.* 2015, Clavero *et al*. 2016), otros autores consideran que son nativas de la Península Ibérica (Matallanas *et al*. 2016). Como se puede observar, estas investigaciones se han publicado recientemente, posteriores a la publicación de la revisión bibliográfica. Dada la relevancia de este asunto, es esencial clarificarlo lo antes posible y con carácter urgente para que las administraciones y los gestores puedan desarrollar y aplicar las medidas oportunas (capítulo 1).

En el mundo científico no parece haber un consenso claro y definido sobre los daños que el cangrejo señal produce sobre el medio natural. Además, algunos colectivos no consideran a esta especie como dañina para el medio natural y defienden su conservación y gestión como especie pescable. Por ello, en esta tesis doctoral se pretende resolver esta cuestión abordando todas las posibles relaciones entre el cangrejo señal y los factores abióticos (parámetros físicoquímicos del agua y hábitat) y bióticos (branquiobdélidos, invertebrados bentónicos y peces) en los ecosistemas fluviales navarros.

El éxito de la invasión del cangrejo señal en Europa es debido a su rápido crecimiento y madurez sexual, gran tolerancia a diferentes condiciones ambientales, comportamiento agresivo, gran tamaño y, sobre todo, a las múltiples translocaciones humanas (Souty-Grosset *et al.* 2006). En la Península Ibérica las poblaciones de cangrejo señal se encuentran presentes y estables mayoritariamente en los tramos medios y altos de la mitad norte peninsular. El área de distribución de esta especie en Navarra se sitúa entre los tramos de cabecera salmonícolas y los tramos bajos ciprinícolas. En particular, la abundancia del cangrejo señal esta positivamente relacionada con la cobertura vegetal y materia orgánica (entre otros), y negativamente relacionada con la presencia de grandes rocas (capítulo 2). En esta tesis doctoral se observó que este crustáceo exótico es incapaz de invadir los tramos más altos de cabecera probablemente por las bajas temperaturas del agua y las bajas cantidades de materia orgánica. De forma similar, otros estudios señalaron que en otros lugares con aguas frías, como el norte de Escandinavia y Austria, esta especie era incapaz de prosperar (Lowery & Holdich 1988, Weinländer & Füreder 2012). Esta limitación ambiental por parte del cangrejo invasor en tramos altos puede favorecer la conservación de estos ecosistemas acuáticos y la fauna nativa de tramos altos de cabecera (*e.g. Calotriton asper* (Dugès, 1852)). Actualmente, estos tramos altos son los únicos que mantienen un número reducido o nulo de especies exóticas invasoras, y deberían preservarse como refugios para la fauna autóctona.

En los capítulos 3, 4 y 5 se abordan las relaciones existentes entre el cangrejo señal y varios factores bióticos (branquiobdélidos y peces). Los branquiobdélidos son unos anélidos clitelados con una asociación ectosimbionte obligada con los cangrejos astacoideos. En esta tesis se descubre el primer registro a nivel mundial de un branquiobdélido del oeste de Norteamérica, *Xironogiton victoriensis* (Gelder & Hall, 1990), infestando un cangrejo del este de Norteamérica, el cangrejo rojo de las marismas, en dos cuencas hidrográficas diferentes de España (capítulo 3). Hasta entonces esta especie de anélido sólo se había encontrado sobre el cangrejo señal. Dado el amplio rango de distribución mundial no nativa del cangrejo señal y la observada capacidad de este ectosimbionte de infestar nuevas especies hospedadoras, se debe prestar atención a posibles futuras infestaciones de otras especies de cangrejo. Además, se desconocen los impactos que este branquiobdéllido exótico podría desencadenar sobre un nuevo hospedador en una ubicación diferente.

Dado este desconocimiento, se procedió a estudiar en detalle la relación biológica existente entre *X. victoriensis* y *P. leniusculus* (capítulo 4). Al igual que sugieren otros estudios similares con otras especies de branquiobdélidos, se observó que la abundancia de *X. victoriensis* fue mayor en los individuos de mayor tamaño y aquellos que mostraban mejor condición corporal (Keller 1992, Brown *et al.* 2012, DeWitt *et al.* 2013). Debido a que la condición corporal del cangrejo se incrementaba significativamente con la infestación de branquiobdélidos, se sugiere que el mutualismo podría ser la relación biológica existente entre *X. victoriensis* y *P. leniusculus*. De la misma forma que en esta tesis doctoral, otros estudios también vieron que los ejemplares recién mudados de cangrejo presentaban menores abundancias del anélido (Keller 1992). El hecho de que los ejemplares adultos y de mayor tamaño de cangrejo presenten más branquiobdéllidos se explica porque (1) ofrecen una mayor superficie para la alimentación y para la reproducción, y (2) la muda es un momento crítico para los branquiobdélidos (los cangrejos adultos mudan con menor frecuencia que los juveniles) (Young 1966).

También se analizó la evolución de la distribución de *X. victoriensis* y *P. leniusculus* entre los años 2005 y 2013 en la Cuenca del Ebro. Finalmente, se estudiaron los factores ambientales que afectaban a la abundancia del branquiobdélido. Tanto el cangrejo señal como su ectosimbionte branquiobdélido (*X. victoriensis*) tienen afinidad por zonas con abundante materia orgánica (capítulo 2 y capítulo 4). En concreto, la abundancia del ectosimbionte fue positivamente correlacionada con una elevada demanda biológica de oxígeno, fosfatos, coliformes y potasio (variables directamente relacionadas con la materia orgánica). Teniendo en cuenta que los branquiobdéllidos son omnívoros (y también su hospedador) y que se alimentan de los epibiontes y residuos orgánicos que crecen en el caparazón de su hospedador, es lógico que mayores cantidades de materia orgánica estén directamente relacionadas con mayores abundancias de alimento disponibles para los branquiobdéllidos (Brown *et al.* 2012, Thomas *et al.* 2013). Estas últimas relaciones encontradas entre los factores ambientales y la abundancias de branquiobdélidos son muy importantes, debido a que han sido poco estudiadas previamente (DeWitt *et al.* 2013).

En el capítulo 5 se estudian las interacciones entre el cangrejo señal y las especies nativas de peces. Los mecanismos de interacción entre los peces y cangrejos pueden ser directos, como la depredación o la competencia, o indirectos, mediante la alteración del hábitat. La competencia por el alimento y por refugio/espacio son los principales mecanismos de competencia (Reynolds 2011). Mientras que las interacciones interespecíficas entre cangrejos y peces han sido ampliamente estudiadas en condiciones de laboratorio (acuarios), pocos estudios han analizado estas interacciones en condiciones naturales.

Mediante grabaciones nocturnas en diferentes ríos navarros se estudió la competencia por espacio entre el cangrejo señal y las especies de peces nativas. Se observó que la mayoría de los encuentros entre el cangrejo señal y los peces nativos eran sin interacción (63%), especialmente con los peces pelágicos (70%). En cambio, sólo el 46% de los encuentros con peces bentónicos fue sin interacción. Estos resultados difieren de los obtenidos en otros estudios en condiciones artificiales (acuarios), que señalan una gran competencia entre cangrejos y especies pelágicas de peces (*e.g.* Griffiths *et al.* 2004). La competencia parece ser menor en condiciones naturales, debido a que en condiciones de laboratorio la restricción de espacio reduce la capacidad de escape de los peces, exagerándose los efectos de depredación y competencia. En el 66% de los encuentros con interacción el cangrejo señal fue el dominante de los mismos. El resultado de la interacción fue significativamente influenciado por el estadio del cangrejo (juvenil/adulto) y por las especies de peces, pero no por el sexo del cangrejo (capítulo 5). Otros estudios en condiciones artificiales también observaron que existe una competencia interespecífica por el espacio entre los cangrejos y los peces (Guan & Wiles 1997, Light 2005). En ocasiones, esta competencia por el espacio puede incrementar la vulnerabilidad de alevines y de especies pequeñas de peces a ser depredados por aves (*e.g.* garzas) o mamíferos ictiófagos (*e.g.* nutrias).

La competencia por alimento entre el cangrejo señal y las especies nativas se abordó con dos metodologías diferentes. En primer lugar, se comparó el contenido estomacal del cangrejo con el de los peces nativos. En segundo lugar, se analizaron los isótopos estables de carbono y nitrógeno del cangrejo y los peces para estudiar sus relaciones tróficas y su solapamiento isotópico. El solapamiento de dieta, calculado con el índice de Schoener, mostró un alto solapamiento con la chipa *Phoxinus bigerri* Kottelat, 2007, locha *Barbatula quignardi* (Bacescu-Mester, 1967) y el gobio *Gobio lozanoi* Doadrio & Madeira, 2004 (en orden decreciente), y un bajo solapamiento con la trucha común *Salmo trutta* Linnaeus, 1758. De la misma forma, se encontró un nicho isotópico similar entre el cangrejo y las especies nativas de peces, sugiriendo que puede existir una competencia trófica cuando los recursos son limitados.

Tanto el estudio comportamental (grabaciones) como el estudio trófico (contenido estomacal e isótopos estables) demostró que existe una competencia por alimento y por espacio entre el cangrejo señal y los peces nativos, especialmente con los peces bentónicos. Teniendo en cuenta que en el área de distribución del cangrejo señal habitan especies bentónicas de peces en peligro de extinción como la lamprehuela *Cobitis calderoni* Bacescu, 1962 (IUCN, 2016), se deberían tomar medidas urgentes para controlar las poblaciones de cangrejo señal.

Aunque los impactos de las especies exóticas de cangrejo presentes en la Península Ibérica –*P. clarkii*, *P. leniusculus*, *O. limosus* y *Cherax destructor* Clark, 1936– son ampliamente mencionados en la bibliografía (*e.g.* Geiger *et al.* 2005, Rodríguez *et al.* 2005), en muchas ocasiones son muy difíciles de evaluar debido a la complejidad de los ecosistemas fluviales. Sin embargo, son bastante conocidos algunos efectos negativos sobre las algas macrófitas (Geiger *et al.* 2005), los invertebrados bentónicos (Correia *et al.* 2005, 2007), las aves herbívoras (Rodríguez *et al.* 2005), los anfibios (Ilhéu *et al.* 2007) y los cultivos de arroz (Cano & Ocete 1994). A todos estos impactos, ahora hay que añadir los estudiados en esta tesis doctoral.

Por desgracia, durante muchos años las políticas de gestión de algunas especies exóticas de cangrejo (especialmente la gestión del cangrejo señal en algunas Comunidades Autónomas) iban orientadas hacia la utilización y conservación de sus poblaciones como un recurso pesquero. Progresivamente, las Comunidades Autónomas han ido mejorando estas políticas y unificando los criterios de gestión para todas las especies exóticas de cangrejo de rio. Dados los impactos medioambientales y económicos que tienen estas especies, las medidas de gestión deberían ir siempre orientadas hacia la erradicación de las mismas. Sin embargo, actualmente no existe ningún método eficaz para la erradicación de estas especies exóticas, excepto para aquellas especies que son sensibles al patógeno responsable de la afanomicosis (como por ejemplo el *C. destructor*). Aunque, en ocasiones, unos métodos adecuados de control y, sobre todo, una legislación adecuada, pueden frenar la expansión de estas especies y evitar la llegada de otras nuevas especies exóticas de cangrejo. La prevención debería ser algo primordial si tenemos en cuenta que actualmente una vez que se establece una especie exótica en el medio natural, su erradicación es prácticamente imposible. Finalmente, la divulgación y educación de la población (especialmente la que está directamente vinculada al medio natural: pescadores, cazadores, agricultores y ganaderos) sobre los daños que las especies invasoras de cangrejo desencadenan sobre los ecosistemas naturales y su fauna nativa, debería considerarse como un mecanismo eficaz para evitar su expansión y la introducción de nuevas especies exóticas (Kozák *et al.* 2015).

Como se ha comentado anteriormente no parece haber un consenso claro y definido sobre los impactos que el cangrejo señal produce sobre los ecosistemas naturales y su fauna nativa. Sin embargo, en esta tesis doctoral quedan patentes los daños medioambientales que esta especie exótica invasora desencadena en aquellos lugares donde está introducida. En primer lugar, se ha observado que los organismos ectosimbiontes de este cangrejo invasor pueden infestar a otras especies, pero se desconocen los efectos que pueden desencadenar estas nuevas asociaciones biológicas. En segundo y último lugar, ha quedado claro que esta especie invasora de cangrejo compite por los recursos tróficos y por el uso del hábitat con especies endémicas de peces, algunas de ellas catalogadas por la Unión Internacional para la Conservación de la Naturaleza (UICN) como en peligro de extinción.

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CONCLUSIONES GENERALES

GENERAL CONCLUSIONS

En la presente tesis doctoral se abordan las relaciones entre el cangrejo señal *Pacifastacus leniusculus* (Dana, 1852) y los factores abióticos (parámetros físicoquímicos del agua y características físicas) y bióticos (branquiobdélidos, peces) en los ecosistemas fluviales navarros. A continuación se exponen las principales conclusiones:

1. Dada la actual dificultad técnica de erradicar las especies de cangrejo exóticas y los daños ambientales que producen en los ecosistemas acuáticos, una adecuada gestión y legislación para prevenir la entrada de nuevas especies y controlar las que ya están establecidas debería ser primordial. La disparidad entre los criterios de gestión de las distintas administraciones y el conflicto conceptual entre la consideración del cangrejo señal como una especie exótica invasora y, a la vez, una especie pescable, son importantes escollos en la gestión global de esta especie.

2. El hábitat óptimo del cangrejo señal se sitúa entre los tramos salmonícolas de cabecera, con aguas frías y baja proporción de materia orgánica, y los tramos ciprinícolas (tramos bajos), con aguas más cálidas, donde cohabita con otra especie invasora de cangrejo, el cangrejo rojo de las marismas *Procambarus clarkii* (Girard, 1852).

3. La presencia del cangrejo señal está limitada en los tramos de cabecera porque las temperaturas son extremadamente bajas y hay poca materia orgánica. La existencia de factores ambientales limitantes en tramos altos favorece la conservación de estos ecosistemas acuáticos y su fauna nativa.

4. Aunque la especie de branquiobdélido ectosimbionte exótica, *Xironogiton victoriensis* (Gelder & Hall, 1990), se pensaba que era específica del cangrejo señal (su hospedador habitual), se ha observado que también puede infestar a otras especies de cangrejo, como el cangrejo rojo de las marismas. Las consecuencias de estas nuevas relaciones biológicas en otros lugares y con diferentes especies se desconocen.

5. Los ejemplares de cangrejo señal adultos con mayor tamaño y con mejor condición corporal presentan mayores abundancias del ectosimbionte. El mutualismo es el mejor tipo de relación biológica que explica la relación existente entre *X. victoriensis* (ectosimbionte) y el cangrejo señal (hospedador).

6. La abundancia del branquiobdélido *X. victoriensis* sobre su hospedador, el cangrejo señal, está directamente relacionada con una elevada demanda biológica de oxígeno, fosfatos, coliformes y potasio.

7. La mayor parte de los encuentros entre cangrejos y peces en el medio natural son sin interacción, especialmente aquellos con especies de peces pelágicas. Sin embargo, en los encuentros con interacción, el cangrejo señal es el dominante de los mismos en la mayor parte de ellos.

8. El estudio del contenido estomacal demuestra que existe un gran solapamiento entre la dieta del cangrejo señal y la dieta de la chipa *Phoxinus bigerri* Kottelat, 2007, la locha *Barbatula quignardi* (Bacescu-Mester, 1967) y el gobio *Gobio lozanoi* Doadrio & Madeira, 2004 (en orden decreciente), y un bajo solapamiento con la trucha común *Salmo trutta* Linnaeus, 1758.

9. Existe una competencia por el alimento y por el hábitat/refugio entre el cangrejo señal y las especies nativas de peces, en particular con las especies de peces bentónicas, como la locha o la lamprehuela *Cobitis calderoni* Bacescu, 1962.

ANEXO 1st *ANNEX*

LEGISLACIÓN Y NORMATIVA PESQUERA

Legislation and fisheries regulation

Anexo 1 de Vedia & Miranda (2013)
(Capítulo 1)

Legislation

Global, Spanish and Portuguese legislation and protection status of crayfish species present in the Iberian Peninsula is explained in detail below and in Table 1.1:

Table 1.1. *Austrapotamobius italicus* conservation status in the Regional Endangered Species Catalogues. Protection levels are equivalent to those established by the Law 4/1989 and by the IUCN (EN: endangered; VU: vulnerable).

Estado de conservación de Austrapotamobius italicus *de los Catálogos de Especies Amenazadas Regionales. Los niveles de protección son análogos a los establecidos en la Ley 4/1989 y por la UICN (EN: en peligro; VU: vulnerable).*

Autonomous Community	Regional Endangered Species Catalogues	Protection Status	Recovery plan
Andalucía	Law 8/2003	EN	Conservation program
Aragón	Ordinance 181/2005	EN	Ordinance 127/2006
Asturias	Ordinance 32/1990	VU	No
Cantabria	Ordinance 120/2008	VU	No
Castilla-La Mancha	Ordinance 33/1998	VU	In preparation
Castilla y León	No	VU	No
Cataluña	Ordinance 2/2008	EN	In preparation
Comunidad Valenciana	Ordinance 32/2004	EN	Under implementation
Extremadura	Ordinance 37/2001	EN	No
Galicia	Ordinance 88/2007	EN	No
La Rioja	Ordinance 59/1998	EN	Ordinance 47/2000
Madrid	Ordinance 18/1992	VU	No
Murcia	Ley 7/1995	VU	No
Navarra	Foral Ordinance 142/1996	EN	Ordinance Foral 143/1996
País Vasco	Ordinance 10 of July 1998	EN	No

Spain

In the Real Ordinance 139/2011 of the 4th of February, for the development of the List of Wildlife Species in Special Protection Regime and the Spanish Catalogue of Endangered Species, the crayfish *Austropotamobius italicus italicus* is catalogued as vulnerable (BOE 2011b).

In the Real Ordinance 1628/2011 of the 14th of November, that regulates the Spanish Catalogue of Invasive Alien Species, the following species of crayfishes are included: *Procambarus clarkii*, *Pacifastacus leniusculus* and *Cherax destructor* (BOE 2011a). *P. clarkii* and *P. leniusculus* were legally introduced for fishing purposes before

Law 42/2007 came into effect. This law includes the second transitional provision whose objectives involving invasive crayfish species are 1) to prevent the spread beyond its current range and 2) the management, control and eradication through hunting, fishing and forestry (BOE 2011a).

Portugal

In the Resolution nº 741/2000 of the 8th of September (Diário da República 2000) the fishing of *A. i. italicus* (referring to *A. pallipes*) is forbidden in all water bodies in Portugal due to the drastic reduction of its populations. The Ordinance-Law nº 49/2005 of the 24th of February (Diário da República 2005), modified by the Ordinance-Law nº 140/99 of the 24th of April (transposition of the Birds Directive and Habitats), including *A. i. italicus* in the Annex B-II (animal and plant species of Community interest whose conservation requires the creation of special areas of conservation) and Annex B-V (animal and plant species of Community interest whose capture, recollection and exploitation may be subject of management).

Red swamp crayfish is considered by the Portuguese legislation very prolific and aggressive species. In the Resolution nº 1054/90 of the 13th of October (Diário da República 1990) *P. clarkii* fisheries is considered as a mechanism to control their proliferation. This species is included in Annex I (alien species introduced in Portugal) and Annex III (exotic species representing an ecological risk) of the Ordinance-Law nº 565/99 of the 21th of December (Diário da República 1999). The third point of the eighth article of this law prohibits the possession, sale, purchase, transportation, economic exploitation and use of *P. clarkii* as a pet.

Signal crayfish is included in the Annex I (alien species introduced in Portugal) of the Ordinance-Law nº 565/99 of the 21th of December (Diário da República 1999). However, this species has not been included in the Annex III (alien species representing an ecological risk).

Fisheries regulation

Iberian crayfish fisheries regulation has been synthesized in the Table 1.2. It is important to know that this table is simpler than reality in order to reduce the real complexity.

Table 1.2. Fishing authorization, size (cm: centimetres), number of catches (u: units) and fishing period of the two more abundant crayfish species of the Iberian Peninsula: the red swamp crayfish *Procambarus clarkii* and signal crayfish *Pacifastacus leniusculus*. "-": no mention.

Autorización de pesca, talla (cm: centímetros), número de capturas (u: unidades) y periodo hábil de las dos especies de cangrejo de río más abundantes en la Península Ibérica: el cangrejo rojo de las marismas Procambarus clarkii *y el cangrejo señal* Pacifastacus leniusculus. *"-": ninguna mención.*

	Procambarus clarkii		***Pacifastacus leniusculus***	
	Size (cm), Catches (u)	Fishing period	Size (cm), Catches (u)	Fishing period
Álava	Free	Jun.-Sep.	Free	Jul.-Sep.
Andalucía	Free	All year	Forbidden	
Aragón	Free	All year	-	-
Asturias	Free	Mar.-Nov.	-	-
Bizkaia	-	-	9cm/15u	Jul.
Cantabria	-	-	Free	Jul.-Sep.
Castilla-La Mancha	Free	Jun.-Ene.	Forbidden	
Castilla y León	Free	Jun.-Dec.	Free	Jun.-Oct.
Cataluña	Free	All year	Free	All year
Comunidad Valenciana	Free	All year	Forbidden	
Extremadura	Free	All year	Forbidden	
Galicia	Free	All year	Free	All year[1]
Guipúzcoa	-	-	Forbidden[2]	
La Rioja	Free	All year	Free	All year[3]
Madrid	Free	Jul.-Oct.	Free	Jul.-Oct.
Murcia	Free	All year	Forbidden	
Navarra	Free	All year	Free	All year[4]
PORTUGAL	Free	All year	-	-

1 From 17th March to 31th July in Xallas River.
2 Only can be fished in the fishing stretch of the Leizarán River.
3 From 15th July to 30th September in Iregua River.
4 From 1st June to 30th September in fishing stretch.

Pacifastacus leniusculus can be fished together with *Procambarus clarkii* without any restrictions in Cataluña, Galicia, La Rioja and Navarra. In the regional administrations of Álava, Cantabria, Castilla y León and Madrid signal crayfish can be fished with limitations exclusively in the fishing period. In Bizkaia *P. leniusculus* can be fished with size, number of catches and fishing period limitations. Fisheries is forbidden in Andalucía, Castilla-La Mancha, Comunidad Valenciana, Extremadura and

Murcia. This species cannot be fished in Guipúzcoa except in Leizarán River where there are size, number of catches and fishing period limitations.

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ANEXO 2^{nd} *ANNEX*

REGISTROS ADICIONALES DE *XIRONOGITON VICTORIENSIS* EN LA CUENCA DEL EBRO (ESPAÑA)

Additional records of Xironogiton victoriensis *in the Ebro River Basin (Spain)*

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(Capítulo 4)

Annex 2. Additional records of *Xironogiton victoriensis* in the Ebro River Basin (Spain).

River	Sample Station	Record coordinates		Record date
		x	y	
Arakil	Iturmendi	571647	4749812	15/07/2009
Arakil	Asiain	599187	4742893	25/08/2011
Arakil	Oskia	596101	4749128	05/08/2013
Arakil	Ziordia	562529	4746713	05/08/2013
Arga	Huarte	615628	4743366	23/07/2009
Arga	Huarte	615628	4743366	19/07/2010
Arga	Landaben -Pamplona	606136	4739760	20/09/2010
Arga	Ororbia	602376	4741114	22/08/2012
Arga	Ororbia	602373	4741117	01/08/2013
Arga	Idoi	618011	4749877	09/07/2013
Arga	Urtasun	621320	4757120	17/08/2013
Arrañosin	Burguete	636158	4761043	03/07/2013
Basaburua	Udabe	596584	4757983	01/08/2013
Ebro	Logroño - Varea	551138	4702438	12/09/2011
Ebro	Frías	476016	4735468	20/08/2013
Ega	Zubielki	576918	4725576	14/07/2009
Ega	Aguas Abajo de Estella	580290	4724288	25/07/2013
Ega	Legaria	567232	4722978	19/07/2013
Ega	Zúñiga	561825	4726337	26/08/2013
Erro	Zunzarren	626101	4745990	17/08/2013
Esca	Burgui	663516	4731726	20/08/2009
Esca	Burgui	663516	4731726	21/09/2010
Esca	Burgui	663516	4731726	23/08/2011
Esca	Burgui	663516	4731726	21/08/2012
Esca	Burgui	665384	4733549	23/07/2013
Esca	Roncal	666611	4737110	23/07/2013
Irati	Oroz - Betelu (Olaldea)	639658	4753120	16/07/2009
Irati	Aribe	642676	4757204	24/07/2013
Irati	Olaldea	639929	4754019	24/07/2013
Irati	Orbaitzeta	644320	4759440	29/08/2013

Izki	Antoñanza	549870	4725563	30/07/2013
Jerea	Palazuelos de Cuesta Urria	470490	4737548	18/09/2012
Juslapeña	Ollacarizqueta	606532	4748583	06/03/2013
Larraun	Urritza	594965	4757806	28/07/2010
Larraun	Urritza	594965	4757806	27/09/2011
Larraun	Urritza	594965	4757806	13/09/2012
Larraun	Latasa	595726	4755961	15/07/2013
Larraun	Mugiro	593640	4758980	01/08/2013
Lizarrusti	Lizarragabengoa	577126	4753550	05/08/2013
Mayor	Aguas Abajo Villoslada de Cameros	524423	4661345	13/09/2011
Mediano	Olagüe	612117	4758758	07/08/2013
Nela	Puentedey	444029	4758603	19/09/2012
Nela	Paralacuesta	459888	4749498	20/08/2013
Oja	Casalarreina	507133	4710934	14/07/2007
Oja	Casalarreina	507133	4710934	15/09/2011
Oroncillo	Orón	501770	4724480	28/07/2010
Oroncillo	Orón	501066	4722003	21/08/2013
Piedra	Cimballa	602857	4550825	16/07/2013
Salazar	Aspurz	650031	4730080	22/07/2013
Salazar	Ezcároz	655646	4751070	18/07/2013
Salazar	Güesa	656136	4739799	18/07/2013
Santa Engracia	Ollerias	526398	4761355	21/07/2009
Subialde o Zayas	Murua	521821	4759702	21/07/2009
Tirón	Haro	512206	4714251	26/09/2012
Ultzama	Iraizotz	608730	4761421	30/07/2013
Ultzama	Latasa	609167	4756770	30/07/2013
Ultzama	Olave	613973	4749060	07/08/2013
Ulzama	Olave E.A.	613883	4749805	18/08/2008
Ulzama	Olave	613883	4749805	23/07/2009
Ulzama	Olave	613883	4749805	24/08/2011
Ulzama	Oricain	612236	4744756	09/07/2013
Urederra	Artavia	575023	4732235	19/07/2013

Urrobi	Puente carretera Garralda	635417	4759471	20/07/2010
Urrobi	Puente carretera Garralda	635417	4759471	24/08/2011
Urrobi	Puente carretera Garralda	635417	4759471	22/08/2012
Urrobi	Puente carretera Garralda	635470	4759490	24/07/2013
Urrobi	Espinal	633720	4758020	03/07/2013
Urrobi	Uriz	632583	4749396	29/08/2013
Zadorra	Mendibil-Durana	529775	4749946	01/10/2008
Zadorra	Entre Mendivil y Durana	529775	4749946	22/07/2009
Zadorra	Mendibil	529775	4749946	15/07/2010
Zadorra	Arce - Miranda de Ebro	508579	4725151	03/10/2011
Zadorra	Mendibil-Durana	529775	4749946	04/10/2011
Zadorra	Mendibil (aguas abajo)	529775	4749946	11/09/2012
Zadorra	Entre Mendivil y Durana	529764	4749927	06/08/2013
Zayas	Martiova	520224	4746970	07/08/2013
Zidacos	Barasoain	610823	4717501	06/07/2009
Zidacos	Barasoain	610823	4717501	19/07/2010

ANEXO 3rd *ANNEX*

TABLA RESUMEN DE LAS 43 LOCALIDADES MUESTREADAS EN NAVARRA (ESPAÑA)

Summary table of the 43 localities sampled in Navarra (Spain)

Apéndice 2 de Vedia et al. *(2016)*
(Capítulo 4)

Annex 3. Summary table of the 43 localities sampled in Navarra (Spain). The values of hydrochemical variables are the average of the measures taken *in situ* between the years 2013 and 2014. The 18 sampling points with 26 additional environmental variables are marked by asterisks (see methods). NC 2013: number of crayfish sampled in 2013 NC 2014: number of crayfish sampled in 2014.

Locality	Conductivity	Tª (ºC)	pH	Oxygen (mg/l)	Prevalence	NC 2013	NC 2014
Aizarotz	312.50	12.26	8.27	10.27	-	0	0
Aribe*	176.50	17.34	8.49	9.69	90	14	15
Artavia*	468.17	13.56	8.21	8.79	100	14	18
Aspurz*	350.50	20.93	8.29	9.13	33	112	46
Artaza	453.67	11.50	8.36	9.94	-	0	0
Belagua	339.33	13.88	8.44	9.14	-	0	0
Burguete	158.03	13.74	8.07	8.98	100	2	0
Burgui*	326.83	16.18	8.18	8.31	100	2	8
Erro	370.50	11.23	7.82	10.19	-	0	0
Espinal*	196.25	15.03	8.21	8.83	100	22	23
Ezcároz*	345.67	15.78	8.36	10.05	95	60	9
Fabrica orbaiceta	98.68	13.71	8.03	9.24	-	0	0
Güesa*	354.83	18.01	8.40	8.91	66	90	16
Idoi*	250.75	19.61	8.47	7.52	88	149	79
Iraizotz*	183.00	15.94	7.79	9.46	100	74	60
Isaba	326.17	13.47	8.37	9.06	-	0	0
Latasa (larraun)*	500.50	18.35	8.61	9.01	96	133	81
Latasa (ultzama)*	356.00	17.18	8.21	8.92	98	162	89
Legaria	758.17	16.62	7.79	9.01	0	1	1
Lizarragabengoa	312.75	16.51	8.33	9.20	51	92	87
Mugiro	391.75	15.23	8.30	9.07	70	132	43
Ochagavia	353.17	13.51	8.45	8.65	-	0	0
Olaldea*	222.00	17.69	8.27	9.08	100	2	2
Olagüe	268.50	15.58	8.08	9.18	100	18	8
Olave	410.25	19.54	8.00	6.99	100	56	57
Ongoz	390.83	15.78	8.31	9.86	0	4	2
Oobaiceta	185.90	15.96	8.53	9.36	100	20	5

Oroquieta	267.50	12.33	8.30	10.17	-	0	0
Oskia*	426.25	20.01	8.39	7.73	100	49	78
Quinto Real	165.50	12.88	8.45	9.78	-	0	0
Riezu	493.17	11.45	7.95	9.41	-	0	0
Roncal	327.33	15.90	8.24	8.52	100	2	18
Roncal Pueblo	322.83	15.38	8.30	8.64	-	0	1
Sorogain	248.75	12.56	8.39	10.57	-	0	0
Udabe*	341.75	16.05	8.40	9.38	86	132	28
Uriz*	254.17	16.05	8.28	9.62	98	80	32
Urtasun*	194.75	18.25	8.32	7.68	82	99	119
Uztarroz	338.83	14.65	8.41	8.98	-	0	0
Virgen de las Nieves	140.20	13.59	8.50	8.99	-	0	0
Ziordia*	398.75	15.01	7.92	9.10	89	143	80
Zudaire	392.50	13.66	8.43	9.24	-	0	0
Zunzarren	400.00	16.36	8.43	9.53	83	68	16
Zuñiga*	826.83	17.72	8.20	9.08	45	93	66

ANEXO 4th *ANNEX*

VALORES DE δ13C Y δ^{15}N Y PROPORCIÓN C/N DEL CANGREJO SEÑAL Y PECES NATIVOS

δ^{13}C and δ^{15}N values and C/N ratio of the signal crayfish and native fishes

Anexo 1 de Vedia et al. *(en revisión)*
(Capítulo 5)

Annex 4. δ^{13}C and δ^{15}N values and C/N ratio of the signal crayfish (*Pacifastacus leniusculus*) and native fishes at the seventeen sampling sites in Navarra (Spain). The numbers in brackets indicate the sampling points represented in Figure 1. Significant differences (Mann-Whitney *U* tests) between the δ^{15}N and δ^{13}C values of *P. leniusculus* and the values of native fishes within each site are marked with an asterisk (*). In sites 10, 13 and 17 only night recordings were made.

Locality	River	Species	n	δ^{13}C ± SD	δ^{15}N ± SD	C/N mass ratio
Zuñiga (1)	Ega	*P. leniusculus*	5	-28.9 ± 0.3	9.9 ± 0.6	3.4 ± 0.1
		P. bigerri	5	-29.7 ± 0.3*	10.5 ± 0.5*	3.5 ± 0.1
		S. trutta	5	-29.7 ± 0.7	11.5 ± 0.7*	3.3 ± 0.1
		G. lozanoi	5	-28.9 ± 0.7	11.1 ± 0.2*	3.3 ± 0.1
Artavia (2)	Urederra	*P. leniusculus*	5	-32.6 ± 0.4	6.9 ± 0.7	3.4 ± 0.1
		P. bigerri	5	-33.2 ± 0.3*	7.3 ± 0.2	3.4 ± 0.1
		S. trutta	5	-33.4 ± 0.7	7.6 ± 0.7	3.3 ± 0.1
		B. quignardi	5	-33.0 ± 0.3	7.5 ± 0.3	3.5 ± 0.1
Ziordia (3)	Ega	*P. leniusculus*	5	-27.5 ± 0.2	9.0 ± 1.1	3.3 ± 0.0
		P. bigerri	5	-27.7 ± 0.7	10.5 ± 0.8*	3.4 ± 0.1
		S. trutta	5	-27.6 ± 0.6	11.5 ± 1.4*	3.4 ± 0.1
		B. quignardi	5	-28.1 ± 1.0	12.2 ± 0.4*	3.7 ± 0.1
Lizarrag.[1] (4)	Lizarrusti	*P. leniusculus*	5	-26.6 ± 0.3	3.4 ± 1.0	3.5 ± 0.1
		P. bigerri	5	-27.4 ± 0.8	6.0 ± 1.4*	3.6 ± 0.1
		S. trutta	5	-29.2 ± 0.7*	5.4 ± 0.5*	3.4 ± 0.2
Udabe (5)	Basaburua	*P. leniusculus*	5	-26.6 ± 0.5	7.5 ± 0.5	3.3 ± 0.0
		P. bigerri	5	-27.9 ± 0.7*	8.7 ± 0.5*	3.6 ± 0.1
		S. trutta	5	-27.9 ± 0.6*	8.8 ± 0.5*	3.4 ± 0.1
Latasa (6)	Larraun	*P. leniusculus*	5	-26.0 ± 0.1	9.8 ± 0.5	3.5 ± 0.1
		P. bigerri	5	-26.2 ± 0.6	10.8 ± 0.4*	3.6 ± 0.2
		G. lozanoi	5	-25.6 ± 0.5	10.4 ± 0.1	3.5 ± 0.1
		S. trutta	5	-26.3 ± 0.5	10.6 ± 0.2*	3.2 ± 0.1
		P. miegii	5	-24.8 ± 0.5*	9.4 ± 0.1	3.6 ± 0.3
Oskia (7)	Arakil	*P. leniusculus*	5	-26.8 ± 0.3	12.0 ± 0.7	3.3 ± 0.1
		P. bigerri	5	-28.2 ± 0.9*	13.9 ± 0.7*	4.2 ± 0.6
		P. miegii	5	-26.7 ± 1.1	13.2 ± 0.3*	3.8 ± 0.2
Iraizoz (8)	Ulzama	*P. leniusculus*	5	-24.0 ± 0.3	8.0 ± 0.4	3.4 ± 0.1
		P. bigerri	5	-24.0 ± 0.6	10.4 ± 0.4*	3.7 ± 0.2
		S. trutta	5	-23.7 ± 0.5	10.4 ± 0.7*	3.4 ± 0.2
Latasa (9)	Ulzama	*P. leniusculus*	5	-25.0 ± 0.1	10.6 ± 0.5	3.4 ± 0.2
		P. bigerri	5	-25.2 ± 0.4	13.8 ± 1.6*	4.1 ± 0.3
		S. trutta	5	-25.7 ± 0.3*	10.7 ± 1.0	3.4 ± 0.1
Olave (10)	Ulzama					
Urtasun (11)	Arga	*P. leniusculus*	5	-27.8 ± 0.4	7.1 ± 1.0	3.4 ± 0.1
		P. bigerri	5	-23.9 ± 0.3*	8.1 ± 0.3	3.8 ± 0.1
		S. trutta	5	-24.8 ± 1.7*	7.5 ± 0.2	3.3 ± 0.1
Idoi (12)	Arga	*P. leniusculus*	5	-25.2 ± 0.3	8.9 ± 0.6	3.3 ± 0.0
		P. bigerri	5	-25.9 ± 0.6	9.8 ± 0.3*	3.8 ± 0.4
		G. lozanoi	5	-24.7 ± 0.5	9.8 ± 0.4	3.6 ± 0.3
		B. quignardi	5	-23.5 ± 0.5*	10.4 ± 0.1*	3.5 ± 0.1
Lizoáin (13)	Erro					
Espinal (14)	Urrobi	*P. leniusculus*	5	-25.7 ± 0.3	9.7 ± 0.5	3.4 ± 0.1
		P. bigerri	5	-25.3 ± 1.2	12.3 ± 0.6*	4.2 ± 0.4

		S. trutta	5	-25.5 ± 1.5	10.3 ± 1.3	3.4 ± 0.1
Uriz (15)	Urrobi	*P. leniusculus*	5	-25.2 ± 0.6	6.9 ± 0.4	3.4 ± 0.1
		P. bigerri	5	-23.8 ± 1.1*	10.3 ± 0.7*	3.9 ± 0.3
Aribe (16)	Irati	*P. leniusculus*	5	-25.6 ± 0.3	5.6 ± 0.5	3.5 ± 0.1
		P. bigerri	5	-25.3 ± 1.0	6.8 ± 0.3*	3.8 ± 0.1
		S. trutta	5	-27.4 ± 1.3*	6.3 ± 0.5	3.3 ± 0.1
		B. quignardi	5	-26.5 ± 0.5*	6.5 ± 0.4*	3.6 ± 0.2
Rípodas (17)	Areta					
Ezcaroz (18)	Salazar	*P. leniusculus*	5	-25.8 ± 0.2	8.5 ± 0.4	3.4 ± 0.1
		P. bigerri	5	-26.5 ± 0.7	10.8 ± 0.3*	3.6 ± 0.2
		S. trutta	5	-26.9 ± 0.7*	10.8 ± 1.0*	3.3 ± 0.2
Guesa (19)	Salazar	*P. leniusculus*	5	-26.3 ± 0.7	9.0 ± 0.8	3.3 ± 0.1
		P. bigerri	5	-27.5 ± 0.7	10.2 ± 0.5*	3.5 ± 0.4
		G. lozanoi	5	-25.9 ± 0.8	10.7 ± 0.4*	3.3 ± 0.1
Aspurz (20)	Salazar	*P. leniusculus*	5	-28.4 ± 1.0	8.2 ± 0.8	3.4 ± 0.2
		P. bigerri	5	-28.3 ± 0.5	9.4 ± 0.4*	3.3 ± 0.1
		G. lozanoi	5	-27.3 ± 0.5	9.6 ± 0.4*	3.3 ± 0.1

[1] Lizarragabengoa