DEPARTAMENTO DE ZOOLOGÍA

UNIVERSIDAD DE CÓRDOBA

LAS COMUNIDADES DE PECES DEL RÍO GUADIAMAR

Y

EL ACCIDENTE MINERO DE AZNALCÓLLAR

TESIS DOCTORAL

Ramón José De Miguel Rubio

Córdoba, 2013

TITULO: *lAS COMUNIDADES DE PECES DEL RIO GUADIAMAR Y EL ACCIDENTE MINERO DE AZNALCÓLLAR.*

AUTOR: *RAMÓN JOSÉ DE MIGUEL RUBIO*

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LAS COMUNIDADES DE PECES DEL RÍO GUADIAMAR

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EL ACCIDENTE MINERO DE AZNALCÓLLAR

Memoria presentada para optar al

Grado de Doctor en Ciencias Ambientales por la Universidad de Córdoba

Fdo: Ramón José De Miguel Rubio

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Córdoba, 2013

CARLOS FERNÁNDEZ DELGADO, FRANCISCO JOSÉ OLIVA PATERNA Y LUCÍA GALVEZ BRAVO

INFORMAN:

Que La Tesis titulada “LAS COMUNIDADES DE PECES DEL RÍO GUADIAMAR Y EL ACCIDENTE MINERO DE AZNALCÓLLAR” de la que es autor el licenciado en Ciencias Ambientales Don RAMÓN JOSÉ DE MIGUEL RUBIO, ha sido realizada bajo nuestra dirección y asesoramiento en el Departamento de Zoología de la Universidad de Córdoba, en el Departamento de Zoología y Antropología Física de la Universidad de Murcia y en el Instituto de Investigación en Recursos Cinegéticos (IREC) de la Universidad de Castilla-La Mancha – CSIC, y reúne las condiciones científicas necesarias para ser presentada ante el tribunal correspondiente con el fin de obtener el Grado de Doctor en Ciencias Ambientales por la Universidad de Córdoba

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TITULO DE LA TESIS: *LAS COMUNIDADES DE PECES DEL RÍO GUADIAMAR Y EL ACCIDENTE MINERO DE AZNALCÓLLAR*

DOCTORANDO: RAMÓN JOSÉ DE MIGUEL RUBIO

INFORME RAZONADO DE LOS DIRECTORES DE LA TESIS

El doctorando ha llevado a cabo un amplio trabajo de investigación, obteniendo resultados muy importantes en el ámbito de la Ictiología y la Ecología aplicada.

La tesis doctoral se ha desarrollado favorablemente, dando lugar a un trabajo de investigación que se ha publicado en una revista internacional:

De Miguel, R.J., F.J. Oliva-Paterna, L. Gálvez-Bravo, C. Fernández-Delgado. (2013). Habitat quality affects the condition of Luciobarbus sclateri in the Guadiamar River (SW Iberian Peninsula): Effects of disturbances by the toxic spill of the Aznalcóllar mine. *Hydrobiologia* 700: 85–97.

Y a dos comunicaciones a congresos:

De Miguel, R.J., Oliva-Paterna, F.J., Aranda, F., Moreno –Valcarcel, R., Gálvez-Bravo, L.I. and Fernández-Delgado, C. Condition of *Luciobarbus* *sclateri* as an indicator to assess the recovery status of fish populations on Guadiamar river (SW, Iberian Peninsula) after the aznalcollar mine spill. *9th International Congress of the Biology of Fish (American Fisheries Society).* Barcelona. 5-9 de julio de 2010.

De Miguel Rubio, R.J., L. Gálvez-Bravo, F.J. Oliva-Paterna, C. Fernández-Delgado. The relevance of the toxic spill for fish diversity in the Guadiamar River basin 8 years after the Aznalcollar accident. *SIBIC2012 – IV JORNADAS IBÉRICAS DE ICTIOLOGÍA.* 17-19 de julio, Girona.

Por todo ello, se autoriza la presentación de la tesis doctoral.

Córdoba, 15 de enero 2013

Firma de los directores

F.J. Oliva

Lucía Gálvez

Carlos Fernández Delgado

Francisco José Oliva Paterna

Lucía Gálvez Bravo

A mi Madre,

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RESUMEN

La presente memoria de Tesis analiza el impacto del vertido de las minas de Aznalcóllar (Sevilla) sobre la comunidad de peces del tramo afectado del río Guadiamar a corto, medio y largo plazo. Tras la completa desaparición de la ictiofauna perteneciente al tramo afectado en 1998, dos años después, concluidas las labores de limpieza más importantes, empieza la recolonización desde los tramos no afectados del cauce principal y en menor medida desde los tributarios. En esta primera fase del proceso de recolonización la comunidad tiende a parecerse a la de referencia localizada aguas arriba. Sin embargo aún en 2002 se observa como la población de la especie más dominante en el tramo, *Luciobarbus sclateri*, sigue afectada por los restos del vertido reflejado en su baja condición corporal. A partir de 2005, la estructura de la comunidad empieza a diferenciarse de aquella de referencia. Este hecho se justifica con un análisis de relación entre la riqueza y diversidad de especies y las variables ambientales a diferentes escalas. Éste muestra como las especies autóctonas prefieren los tramos bien conservados, fuera del tramo afectado tanto por el vertido minero como por el embalse del Agrio, mientras que es en éste tramo donde las exóticas proliferan. No obstante, el efecto que los restos del vertido producen en 2006 no es superior al resto de perturbaciones antrópicas que se producen en el resto de las subcuencas del Guadalquivir. Finalmente, 13 años después del vertido, la proliferación de las especies exóticas y el descenso de las autóctonas por la suma de perturbaciones antrópicas, hacen que la comunidad de peces del Guadiamar no se pueda considerar recuperada.

ABSTRACT

This PhD Thesis analyzes the impact of the toxic spill from the Aznalcóllar mines (Sevilla) on the fish assemblage of the affected section of the Guadiamar River in the short, medium and long term. After the complete disappearance of fish in the affected section in 1998, two years later, once major cleaning works ended, recolonization from unaffected reaches of the main channel began, and to a lesser extent from tributaries. During this first phase of the recolonization process, fish assemblages in the affected area bore some resemblance to those of the reference upstream site. However, even as late as 2002 the population of the most dominant species in the stretch, *Luciobarbus sclateri*, was still affected by the spill remains, as reflected by their low somatic condition. From 2005, fish assemblage structure began to differentiate from that of the reference site. This was revealed by analyzing the relationship between species richness and diversity and environmental variables at different scales. This analysis revealed how native species preferred well-preserved stretches outside the reach affected by both the mining spill and the Agrio reservoir, while it was in this reach where exotics thrived. However, the remaining spill effect in 2006 was not greater than other anthropogenic disturbances common to other sub-basins of the Guadalquivir River. Finally, 13 years after the spill, an increase of exotic species and decrease of natives due to the accumulation of human disturbances hinder/stop the Guadiamar fish community from full recovery.

INTRODUCCIÓN GENERAL y OBJETIVOS

El vertido de las minas de Aznalcóllar

El 25 de abril de 1998 se produjo la rotura del muro exterior de contención de la balsa de estériles mineros procedentes de las minas de Aznalcóllar (Sevilla) propiedad de la empresa sueco-canadiense Boliden-Apirsa S.L., produciéndose una de las mayores catástrofes ambientales de las últimas décadas en Europa (Arenas *et al*., 2008). La grieta abierta provocó un vertido de aproximadamente 6 hm^3, un tercio de los cuales estuvo conformado por lodos tóxicos procedentes del proceso de flotación de la pirita y el resto por aguas ácidas cargadas de metales pesados en disolución (Fig. 1). La consecuencia inicial fue una afección sobre un tramo fluvial de unos 67 km desde la propia mina hasta el límite con el Parque Nacional de Doñana, contaminando un anchura variable de un mínimo de 500 m en los márgenes del cauce principal del río Guadiamar y una superficie total aproximada de 4.634 ha pertenecientes a nueve municipios de la provincia de Sevilla (Arenas, 2003) (Fig. 1).

En su conjunto, la composición principal del vertido minero presentaba un alto contenido de elementos con alta toxicidad, entro los que podemos destacar el Azufre (35-40%), Hierro (34-37%), Zinc (0.8%), Plomo (0.8%), Arsénico (0.5%), Cobre (0.2%), Antimonio (0.05%), Cobalto (0.006%), Talio (0.005%), Bismuto (0.005%), Cadmio (0.0025%), Plata (0.0025%), Mercurio (0.001%) y Selenio (0.001%) (Grimalt y Macpherson, 1999). Los lodos tóxicos sedimentaron mayormente en los primeros 40 km del recorrido desde la balsa minera establecida en el río Agrio, alcanzando un espesor de 3 m en la zona cercana a dicha balsa y menos de 1 m al llegar a la zona de Entremuros próxima a Doñana. Las aguas contaminadas en cambio, prosiguieron atravesando toda la zona de Entremuros y siendo retenidas por diques de tierra construidos en la zona del Lucio del Cangrejo, en el límite Norte del Parque Nacional de Doñana (López-Pamo *et al*., 1999).

Figura 1. Fotografías que muestran la rotura del muro de la balsa de estériles que almacenaba la empresa Boliden-Apirsa S.L. en Aznalcóllar (arriba) y la extensión de los lodos tóxicos y aguas ácidas por el río Guadiamar (abajo). © Consejería de Medio Ambiente, Junta de Andalucía.

En las primeras fases de la catástrofe, el desbordamiento del cauce principal del río Guadiamar, la acidez del agua y la fina granulometría de los lodos, debieron provocar la asfixia de prácticamente la totalidad de la fauna piscícola. Entre otros, esto se tradujo en la recogida, principalmente en la zona próxima a la marisma, de aproximadamente de 37.4 t de peces muertos. Los grupos de peces identificados en el montante de estos especímenes y su composición relativa fue la siguiente (Valls y Blasco, 2005): carpas (75-80%), mugílidos (10-16%), barbos (6-8%), anguilas (4%) y otras especies (5%).

Múltiples fueron las actuaciones realizadas entre las primeras acciones de control y remediación de la contaminación producida. A la semana del accidente empezaron las labores de retirada mecánica de los lodos y, como medida de urgencia, se construyeron 15 diques de retención en la zona afectada del cauce principal del río Guadiamar para evitar que las lluvias otoñales arrastrasen la fracción de lodos aún sin retirar. Estos trabajos duraron aproximadamente siete meses (Fig. 2). Sin embargo, la limpieza del cauce incluyendo procesos de drenado en las zonas más profundas, tuvo que posponerse hasta el verano de 1999, cuando el caudal mínimo facilitó el acceso a la maquinaria de limpieza (Arenas *et al*., 2008) (Fig. 2). El movimiento de tierras ocasionado durante las tareas de limpieza de la llanura de inundación, las orillas y el propio cauce (Fig. 2), provocó una importante destrucción de la protección natural contra la erosión de las orillas (Gallart *et al*., 1999). Para remediar este efecto, se llevaron a cabo labores de estabilización de taludes con empalizadas y reforestación de las márgenes (Arenas *et al*., 2008) (Fig. 3). En determinadas zonas se realizó un segundo proceso de limpieza, por lo que las tareas se prolongaron hasta el año 2002 (Fernández-Delgado *et al*., 2006). El agua embalsada en la zona de Entremuros fue depurada y tratada durante tres meses con carbonatos y sosa para elevar su pH (3.5-5.5), y posteriormente fue vertida en la zona de marisma (Arenas *et al*., 2008). Una vez retirados los lodos, aquellos terrenos anegados por el vertido fueron químicamente tratados con medidas correctoras encaminadas a estabilizar su pH, para incrementar su materia orgánica y aumentar la capacidad de retención de los metales pesados con carbonatos y óxidos de hierro (Arenas *et al*., 2008; Cabrera *et al*., 2008).

Figura 2. Labores de retirada mecánica de los lodos en la llanura de inundación (izquierda) y posteriormente del cauce (derecha). © Consejería de Medio Ambiente, Junta de Andalucía.

En su conjunto, todas estas medidas probablemente agravaron los efectos del vertido minero, alterando significativamente las características geomorfológicas, hidrológicas y geoquímicas del sistema fluvial (Gallart *et al*., 1999; Garralón *et al*., 1999). En parte, algunas de las medidas de actuación inmediata pudieron prolongar, a escala local, ciertos efectos ambientales del vertido generando impactos adicionales en la recuperación de ciertos componentes faunísticos (Macklin *et al*., 1999; Hudson-Edwards *et al*., 2003; Turner, 2003). Por ejemplo, la fauna y flora colonizadora del río se encontró con un hábitat profundamente alterado y una elevada carga contaminante residual (Pérez-Alejandre, 2009). En este contexto, la recuperación de la comunidad de peces va a depender, no únicamente de la restitución de un mínima calidad ambiental, sino también de la evolución en el hábitat disponible para su establecimiento.

Figura 3. Labores de acondicionamiento de taludes (izquierda) y trabajos de reforestación (derecha). © Consejería de Medio Ambiente, Junta de Andalucía.

Con el fin de que el río Guadiamar y su llanura de inundación recuperasen las condiciones previas al vertido o incluso anteriores a las importantes transformaciones que ha sufrido a lo largo de su historia, la Consejería de Medio Ambiente de la Junta de Andalucía puso en marcha una última medida de restauración a largo plazo que consistiría en diseñar un plan de acción denominado *La Estrategia del Corredor Verde del Guadiamar* (Arenas *et al*., 2008). Este documento marco desarrollaría el Proyecto del Corredor Verde cuyos objetivos fueron: evitar el cultivo de los terrenos afectados por el vertido, establecer un espacio de uso público que pusiese en valor la naturaleza y, quizás el más importante, crear un espacio natural protegido en el pasillo ecológico o eje de conexión entre dos reservas de la biosfera, las Dehesas de Sierra Morena al norte y el área de Doñana al sur (Arenas *et al*., 2008; Pinto *et al*., 2008).

La investigación ictiológica en el proceso de restauración

El asesoramiento científico ha estado presente desde el principio, tanto en las acciones urgentes iniciales como en el establecimiento de actuaciones continuadas una vez finalizado el periodo crítico inicial (Arenas y Montes, 2008). Dada la compleja situación creada tras el accidente minero, además del Proyecto del Corredor Verde, se estableció y desarrolló el Programa de Investigación del Corredor Verde del Guadiamar (PICOVER), como órgano de asesoramiento en el plan de acción desarrollado. De este modo, la investigación se convirtió en un factor fundamental en el desarrollo del corredor verde y, en consecuencia, en el seguimiento ambiental del proceso de recuperación tras el vertido (Montes, 2003).

Entre los objetivos específicos de PICOVER también se encontraba el fomentar la investigación básica y ayudar a la formación de jóvenes investigadores. Entre los restos científicos se estableció el diseño de un Plan de Seguimiento que permitiese la identificación de los cambios e interacciones producidas, pasando de una fase inicial eminentemente descriptiva a otra cuantitativa. El presente trabajo de tesis doctoral se encuadra en este contexto.

La estructura y composición de la comunidad de peces, al igual que el resto de componentes faunísticos, está controlada por los factores bióticos y abióticos del sistema (Matthews, 1998; Wootton, 1998; entre otros). Sin embargo, los peces fluviales poseen una serie de características que los hacen especialmente interesantes para su uso como indicadores de calidad ambiental con variaciones significativas en su tolerancia a las perturbaciones ambientales (Hermoso *et al*. 2009 y 2010). Así por ejemplo, se encuentran en posiciones elevadas en las redes tróficas pero con un rango amplio de especies en los distintos niveles, de este modo son buenos indicadores de procesos ecológico globales (Hynes, 1995). Son organismos relativamente longevos y móviles, por lo que reflejan en buena medida los efectos, tanto a escalas espaciales amplias como a largo plazo, de los factores de estrés ambiental (Harris, 1995). Las especies suelen presentar unos requerimientos de hábitat particulares (bentónicos, reófilos, limnófilos, etc.), convirtiéndose así en buenos indicadores de la alteración de dichos hábitats. Muchas especies han desarrollado complejos mecanismos migratorios haciéndoles muy sensibles a las interrupciones del continuo fluvial (Lucas and Baras, 2000). Finalmente, en general son relativamente fáciles de capturar e identificar, en comparación con otros componentes bióticos presentes en sistemas fluviales, presentando la mayoría de especímenes capturados una gran facilidad de procesado en campo y la posibilidad de su liberación posterior.

Las características especiales de la situación creada en la cuenca del río Guadiamar tras el vertido minero, convertían el seguimiento y estudio del efecto del vertido minero sobre la comunidad de peces en un objeto muy interesante de estudio, además de ser una necesidad propia del programa de restauración. No únicamente por las oportunidades de conocimiento científico que ofrecía, sino también por el interés aplicado que pudiera derivar de los resultados obtenidos. De este modo, los objetivos principales del presente trabajo fueron encuadrados en una serie de estudios o trabajos cuya finalidad conjunta ha sido aportar información básica sobre la comunidad de peces del sistema fluvial de la cuenca del río Guadiamar durante el proceso de restauración; información indispensable para la evaluación del proceso y restablecimiento de los criterios científicos aplicados.

Objetivos

I. Caracterizar de forma específica la ictiofauna actual presente en la cuenca del río Guadiamar después del vertido minero y el proceso de recolonización.

I.1 Descripción mediante listado actualizado de las especies del la cuenca del río Guadiamar.

I.2. Evaluación del efecto a largo plazo del vertido minero mediante análisis descriptivo de los grupos de especies en función de los sectores fluviales de estudio.

II. Evaluar el efecto a corto y medio plazo de las perturbaciones derivadas del vertido minero mediante estudio comparado de parámetros descriptores de las poblaciones de *Luciobarbus sclateri* (Günther, 1868) y de la comunidad de peces en su conjunto.

II.1 Determinación y evaluación de los cambios espaciales en la condición somática de individuos como indicador del estado poblacional de *L. sclateri* en tramos fluviales afectados y no afectados de la cuenca.

II.2 Análisis de las relaciones entre la condición poblacional de *L. sclateri*, a escala de tramo fluvial, y variables ambientales relativas a la calidad del hábitat, del agua y nivel de interacciones biológicas con poblaciones de peces.

II.3 Identificación de las principales variables ambientales, a escalas espaciales diferentes, que determinan la riqueza y diversidad de peces autóctonos y exóticos en la totalidad de la cuenca.

II.4 Evaluación del grado de influencia de las alteraciones provocadas por el vertido minero sobre la riqueza y diversidad de peces del río Guadiamar 8 años después del mismo.

III. Evaluar la evolución temporal del proceso de recolonización y de la comunidad de peces del tramo afectado por el vertido minero en el cauce principal del río Guadiamar.

III.1 Caracterización del proceso de recolonización observado durante los 13 años posteriores al vertido minero, indicando las principales fuentes de recolonización y evolución de la estructura y especies dominantes en la comunidad.

III.2 Evaluación del nivel de recuperación de la comunidad de peces en el tramo afectado y, por lo tanto, análisis de la eficacia del programa de restauración en el proceso de recolonización de la ictiofauna.

ÁREA DE ESTUDIO

Cuenca del río Guadiamar

El río Guadiamar es el último de los grandes afluentes que recibe el río Guadalquivir por su margen derecha antes de su desembocadura. Discurre entre las dos provincias más occidentales de la Comunidad Autónoma de Andalucía, Sevilla y Huelva, formando parte de un sistema hidrológico natural que sirve de nexo entre los sistemas morfoestructurales de Sierra Morena Occidental y el litoral del Parque Nacional de Doñana.

En el presente trabajo se extraen datos sobre la ictiofauna perteneciente a los cursos fluviales que constituyen la cuenca del río Guadiamar a excepción de la parte canalizada que discurre por la marisma, denominada zona de entremuros (Fig. 1). El punto más septentrional de la cuenca coincide con el nacimiento del río Guadiamar, localizado en el término municipal de Castillo de las Guardas (37º45´ N, 6º22´ W) y su vértice inferior, donde comienza la zona canalizada hasta la desembocadura en las marismas del Guadalquivir, se halla en el término de Aznalcázar (37º10´ N, 6º12´ W). En origen la cuenca drenaba una superficie de 1880 Km^2 que, debido al encauzamiento artificial en la zona de entremuros, se ha reducido hasta los 1325 Km^2 (Borja *et al*. 2001), recorriendo su cauce principal una distancia próxima a los 80 Km con un desnivel de 320 m.

La hidrografía de la cuenca presenta una disposición asimétrica. Así, mientras que los afluentes de la margen derecha son abundantes con una red ampliamente desarrollada (alcanzando el orden 4) (Crispinejo, Cañaveroso, Alcarayón, etc.), los de la margen izquierda son escasos, cortos y de escasa entidad (no superan el orden 2) (Fig. 1).

Desde el punto de vista climático, la cuenca queda encuadrada dentro del ámbito mediterráneo subhúmedo de rasgos oceánicos, caracterizado por inviernos suaves y relativamente lluviosos, en contraste con períodos estivales muy marcados por la sequía (Aguilar *et al*., 2003). El régimen térmico anual es suave con una temperatura media de

18,2ºC, valores promedios máximos en julio (29ºC, máximos diarios comunes de hasta 40ºC) y mínimos en enero (9ºC). La precipitación media anual es de 624 mm, si bien, su dinámica temporal muestra un claro carácter torrencial entre otoño y primavera, así como una elevada variación interanual. Dichas precipitaciones se reparten de forma heterogénea por la totalidad de la cuenca, oscilando entre ombroclimas secos en la zona de marisma (precipitación anual inferior a 600 mm) y subhúmedos en la zona alta (precipitación entre 700 y 900 mm) (Cabezudo *et al.*, 2002; SAIH, 2012). Los obstáculos orográficos de Sierra Morena en la zona de cabecera y del farallón del Aljarafe en la zona oriental, provocan un aumento de la precipitación de sur a norte y de oeste a este, respectivamente (Borja *et al.*, 2001). El río Guadiamar muestra un periodo de flujo máximo entre los meses de Enero a Marzo, con unos caudales medios de 13 m^3/s; los caudales mínimos se producen entre Junio y Octubre, en los que no se suelen superar los 3 m^3/s (Gallard *et al.*, 1999; SAIH, 2012).

Atendiendo a características hidrológicas, climáticas y geológicas la cuenca presenta una clara división en tres ámbitos: la sierra, la campiña y la marisma (Aguilar *et al.*, 2003). Éstos coinciden aproximadamente con los tramos alto, medio y bajo establecidos en el cauce principal del río Guadiamar en el contexto del presente estudio (Fig. 1).

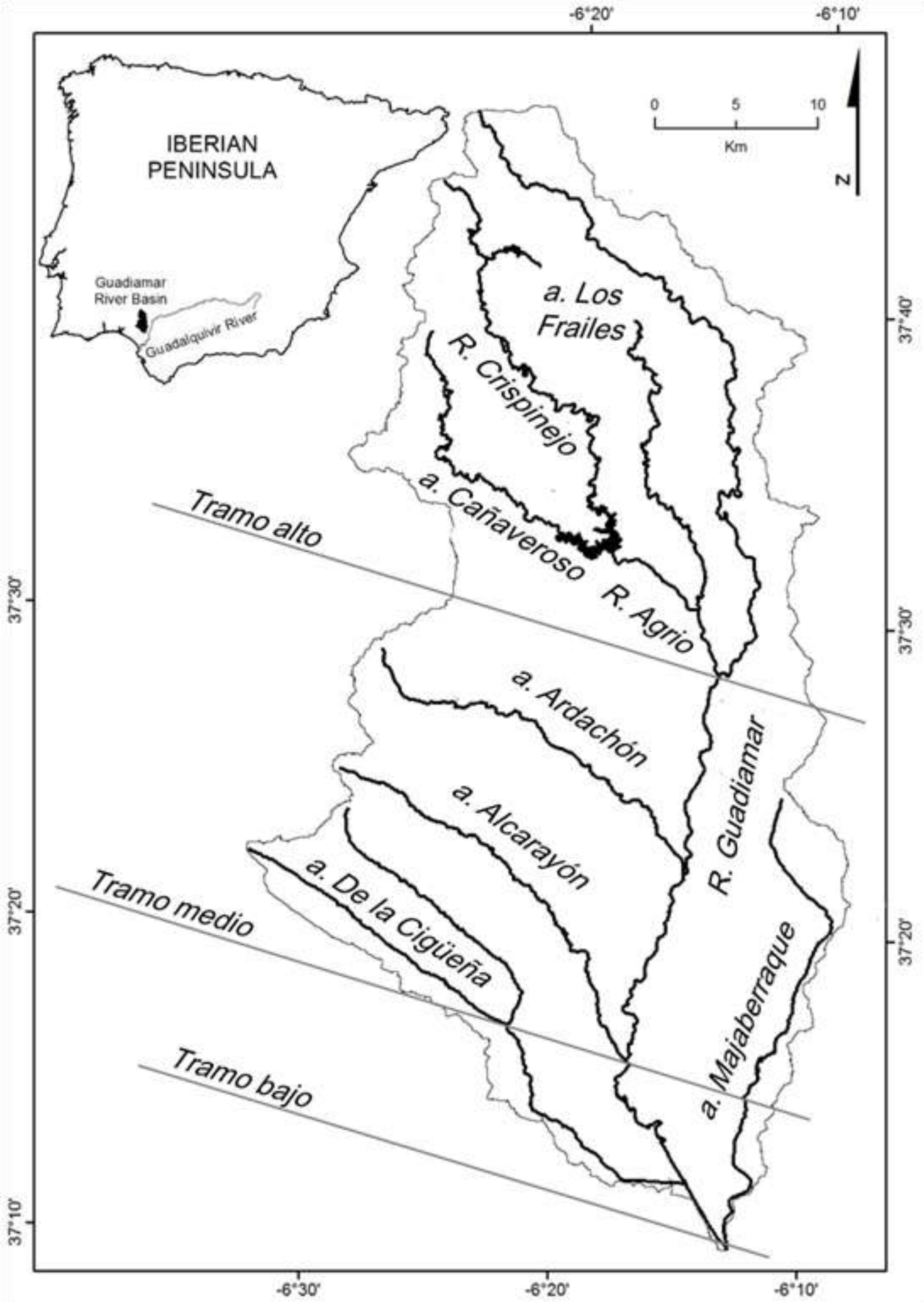

Figura 1. Cuenca del río Guadiamar, principales cursos fluviales y la sectorización utilizada en el presente estudio.

Como tramo alto se asigna la zona comprendida entre el nacimiento del río Guadiamar y su confluencia con el río Agrio. Éste queda asociado a la Unidad Occidental o Surportuguesa-Onubense de Sierra Morena, por donde abundan las Pizarras del Paleozoico y se extiende el horizonte geológico que contiene las mineralizaciones de sulfuro (*Faja Pirítica Ibérica*). Su modelado presenta superficies de aplanamiento ocasionalmente rotas por crestas de cuarcitas y valles muy acusados. La vegetación dominante es el bosque mediterráneo de montaña media andaluza no caliza (Cabezudo *et al*. 2002; Pérez Latorre *et al*., 2002), que constantemente se convierte en dehesas o en zonas modificadas con plantaciones de diferentes especies de pino y eucaliptos (Fig. 2).

Figura 2. Entorno del río Guadiamar en el tramo alto de la cuenca.

El tramo medio comprendería desde la confluencia del río Agrio en el Guadiamar, hasta la confluencia del arroyo Alcarayón. Se asienta tanto sobre materiales constituyentes de la margen sur o depresión periférica del Macizo Hespérico con calizas de borde, margas y arenas, como sobre depósitos aluviales del cuaternario. En una aproximación geomorfológica, predominan las lomas suaves típicas de campiña, interrumpidas únicamente por los escalones de las terrazas del cauce principal y el farallón del Aljarafe. La vegetación natural ha sido prácticamente eliminada debido a la fuerte presión agrícola de la zona (Fernádez-Delgado *et al*., 2002), a excepción de contadas áreas o zonas de ribera en las que se reconoce algo de vegetación arbórea natural (Fig. 3). La actividad agrícola ha ido socavando el matorral y el bosque de galería, reduciéndolo a una pequeña franja en a las riberas del cauce. Entre los restos de vegetación aparecen ejemplares arbóreos y arbustivos de especies rupícolas autóctonas tales como fresno (*Fraxinus angustifolia*), alamo (*Populus alba),* chopo (*Populus nigra*), olmo (*Ulmus minor*) y sauce (*Salíx purpurea*, S. *atrocinerea*) (Aguilar *et al.*, 2003).

Figura 3. Entorno del río Guadiamar en el tramo medio de la cuenca.

Por último, el tramo bajo sería todo el cauce que, aproximadamente a partir de la confluencia del arroyo Alcarayón, discurre canalizado por zona de entremuros. Esta planicie con escaso desnivel se constituye de materiales finos típicos de marisma como arcillas y limos del Holoceno. Aquí, las constantes anegaciones del terreno han propiciado que se mantenga la vegetación original, la cual, dependiendo del grado de inundación puede presentarse con distintas fisonomías: una marisma alta con almajanos (*Salicornia spp.*); pastizales de *Sylibus marianum*, *Hordeum murinum* y *Plantago* *coronopus*, en zonas más altas sin estar sometidas a continuas inundaciones; o bien como área completamente deprimida donde se mantiene la humedad y alberga *Typha*, *Phragmites* y *Scirpus* (Fig. 4).

Figura 4. Entorno del río Guadiamar en el tramo bajo de la cuenca.

Al ser el Guadiamar el último gran afluente que recibe el Guadalquivir por su margen derecha, se convierte en el único nexo de unión entre Sierra Morena y la marisma del Guadalquivir, lo que a efectos de conservación, le confiere carácter de corredor ecológico natural entre la sierra y el litoral (Montes, 1999).

Principales impactos sobre la red fluvial

Las perturbaciones presentes en la cuenca del río Guadiamar pueden clasificarse como impactos directos o indirectos sobre la red fluvial (Fernández-Delgado y Drake, 2005). Los directos son entendidos como actuaciones sobre el cauce o su llanura de inundación (ejemplos: embalses, presas, azudes, drenajes, canalizaciones, etc.). Los impactos indirectos ejercen su efecto a nivel de cuenca o fracciones de la misma (ejemplos: contaminación agrícola difusa, deforestación, infraestructuras viarias, actividades mineras, poblaciones y urbanizaciones, etc.) (Cowx and Welcomme, 1998).

En el contexto del presente trabajo de Tesis Doctoral, y con la finalidad de interpretar varios de los resultados obtenidos en nuestros trabajos, podemos destacar algunos de los impactos principales en función de los tramos establecidos en la cuenca de estudio (Fig. 5). Así, en el tramo alto de la cuenca, el principal impacto sobre la red fluvial está representado por la mina de Aznalcóllar-Los Frailes. Ésta ha supuesto la construcción del embalse del río Agrio y su canalización bajo tierra para abastecer las necesidades de la explotación minera (Borja *et al.*, 2001). Además, los lixiviados procedentes del yacimiento son recogidos por el río Agrio aguas abajo de la localización de la mina.

Los impactos destacables en el tramo medio están relacionados con la suavización del relieve y la presencia de suelos más fértiles. Estas características han propiciado el desarrollo de la agricultura y el asentamiento de numerosos núcleos de población (Fig. 5), lo que supone un importante aumento en la captación de agua, vertidos urbanos e industriales, contaminación difusa de productos fitosanitarios y la elevada deforestación.

Por último, el tramo bajo sufre, además de la acumulación de los impactos acaecidos aguas arriba, la canalización de su red fluvial tanto para el cauce principal en la zona de entremuros, así como para la mayoría de tributarios convertidos en caños y regulados mediante compuertas para controlar el nivel de la marisma.

De entre todas estas perturbaciones, aquella que produjo el impacto más extenso y en menor tiempo fue el vertido de lodos tóxicos de 1998, el cual se produjo en el tramo alto y defaunó por completo el tramo medio y parte del tramo bajo (vide supra) (Fig. 5).

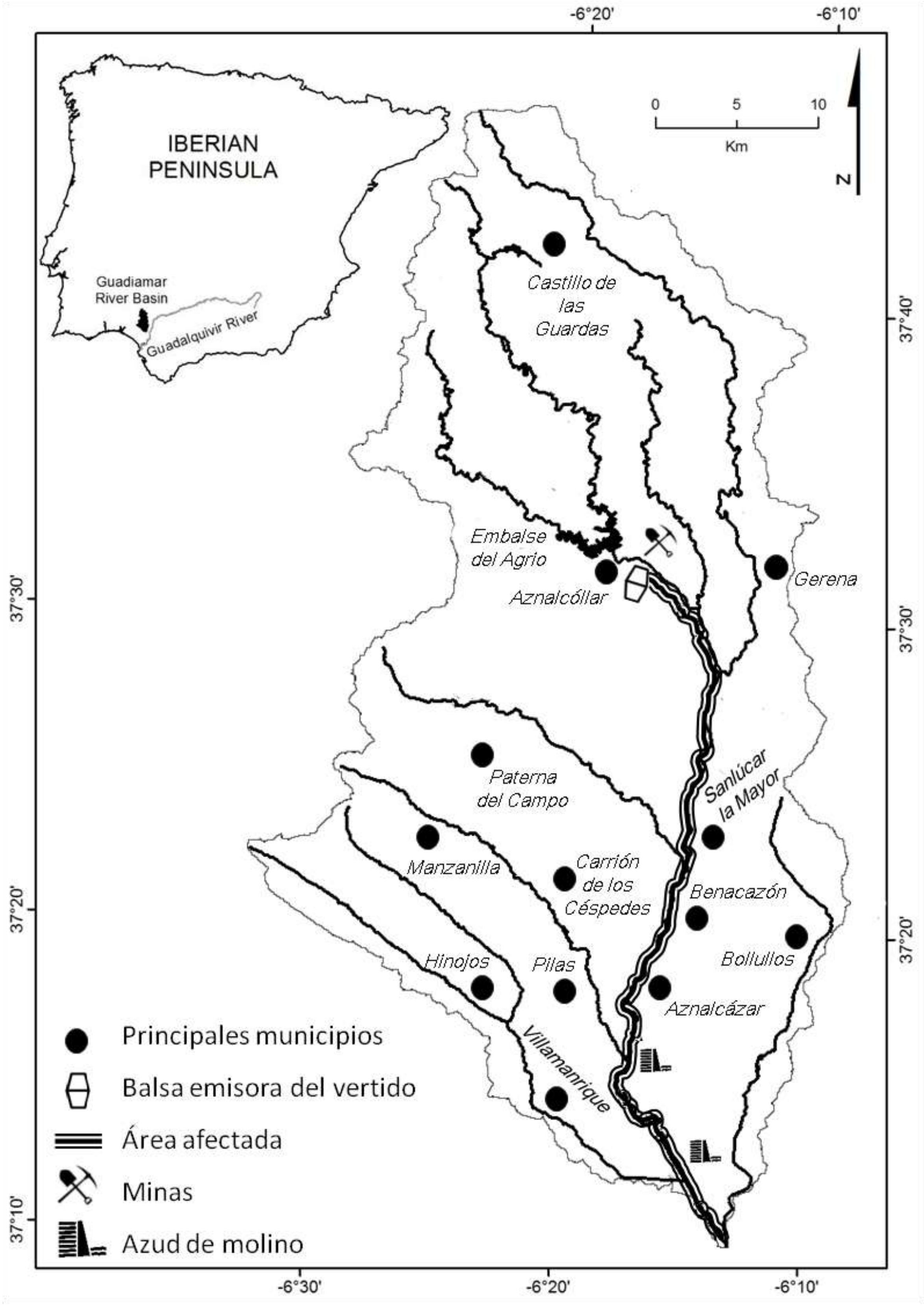

Figura 5. Potenciales impactos tenidos en cuenta para la comunidad de peces en los cursos fluviales de la cuenca del río Guadiamar.

PARTE I:

Ictiofauna de la cuenca del Guadiamar 13 años después del vertido de las minas de Aznalcóllar.

I.1- Long-term fish species composition after one of the worst toxic spills in Europe.

Introduction

The Iberian ichthyofauna includes only a few families, with a high degree of diversification at the species level and numerous endemisms. These endemisms compound the 80% of the species that belong to the families Cyprinidae, Cobitidae and Cyprinodontidae present in the Iberian Peninsula, which reaches the highest endemism proportion in Europe (Doadrio, 2001; Kottelat and Freyhof, 2007). The isolation from other European basins, the relatively small size of Iberian drainages and climatic factors are the main causes of differentiation of many independent and isolated populations (Clavero *et al*., 2004). Southern Iberian basins endure long droughts and abrupt floods, common in the Mediterranean climate (Gasith and Resh, 1999). This variability is the key to fish assemblage structure (Pires *et al*., 1999; Magalhaes *et al*., 2002a, 2002b), where the watercourse position within the river basin network is determinant (Filipe *et al*., 2002). Thereby, the extreme dry conditions in summer cause significant loss of habitat and connectivity (Matthews and Marsh-Matthews, 2003) and consequently, a mighty decrease in fish populations. In addition to this naturally fragile balance, water extraction by humans increase in this season mainly in areas with agricultural and touristic land uses (Clavero and Hermoso, 2011; Ribeiro and Leunda, 2012), which cause habitat degradation or destruction by pollution, hydraulic engineering (reservoirs, channels, etc.), land use changes and exotic species introductions. The sum of these effects is causing a progressive loss of fish communities in the Iberian drainages (García-Berthou and Moreno-Amich, 2000, Corbacho and Sánchez, 2001, Clavero *et al*. 2004; Ribeiro *et al*., 2008).

Nevertheless, the above pressures can be overcome by a pulse disturbance such as a large toxic spill. One of the most damaging discharges in Europe occurred in the Guadiamar River basin, south-western Iberian Peninsula. On 25 April 1998, 6 hm^3 of metallic sludge were released from a tailing pond belong to the Aznalcóllar-los Frailes mine in the province of Seville, Spain (Aguilar *et al.*, 2003). The spill defaunated the Agrio River and 67 km of the Guadiamar River fluvial section, the main watercourse in the basin (Grimalt and Macpherson, 1999). Fish were probably the most affected vertebrates during the weeks following the accident. According to different estimates more than 35 t of dead specimens were collected from the affected area (Valls and Blasco, 2005). Immediately, mud withdrawal commenced and a recovery plan consisting in chemical stabilisation, bank restoration and reforestation was undertaken, not only to repair the damaged ecosystems, but in order to transform the affected area into a green corridor between two well conserved ecosystems: Sierra Morena in the north and Doñana National Park in the south (Arenas *et al.*, 2008). Most major effects related to the spill were mitigated with the recovery works; however, mining leachates, insufficiently treated sewage, industrial spills, agricultural diffuse pollution, water captations and numerous watercourse obstacles present in the watershed before the accident have not been alleviated yet (personal observation). Therefore, because these pressures were mightily acting before 1998, it is likely that the fish community affected by the spill was already deteriorated with respect to that early Guadiamar River fish community structure, thus, the current fish community is the result of both pulse and press disturbances (Lake, 2000) acting together.

Despite this outstanding recovery process, no information has so far been published about fish community composition in the Guadiamar River basin after the spill. Accordingly, this work aimed to provide a current status list for Guadiamar basin fish species that can be used as a comparative document at in future studies where further changes in community structure are anticipated.

Material and Methods

Study area

The Guadiamar River basin drains an area of 1.880 Km^2 in the South-western Iberian Peninsula near the Guadalquivir River mouth (Fig. 1). Climate is sub-humid Mediterranean with oceanic influences and average temperatures range from 9ºC in winter to 29ºC in summer. The river network flow in this climate is subject to an irregular hydrological regime on both intra- and inter-annual scale, with natural disturbances such as major droughts and floods. The severe droughts cause the drying of most small streams and the creation of isolated pools during summer (Gasith and Resh 1999). The catchment shows an environmental transition linked to river section type. Pine forests (*Pinus* spp.) and dehesas (*Quercus* spp., xeric Mediterranean forest) are the predominant vegetation in the upper section; olive groves, cereal fields and vineyards form a mosaic in the middle section; and close to the Doñana Natural Park, in the lower section, the marsh starts, dominated by *Salicornia* spp. shrubs, and pastures with *Sylibus marianum*, *Hordeum murinum* and *Plantago coronopus*. The hydrological network is interrupted by three dams (Fig. 1). Two of them collect less than 4 hm^3 in the source area, and there is one large reservoir (20 hm^3) in the Agrio River, slightly upstream from the spill point (Borja *et al*. 2001).

Data sources and sampling protocols

Guadiamar River basin fish composition was obtained from a wider study that assessed the long-term effects caused by the spill on fish communities. This monitoring focused on the affected area, which occupied both the fluvial sector and marshland. Fish were caught between 1999 and 2011 (intensive campaigns in 2002-2004; 2006-2007 and 2011). The inclusion of species in the list drawn for this work (Table 1) derived from different information sources obtained in several projects. Data about Guadiamar River basin fishes available in the literature was also included, however, pre-spill cites have not been included in the up-dated list.

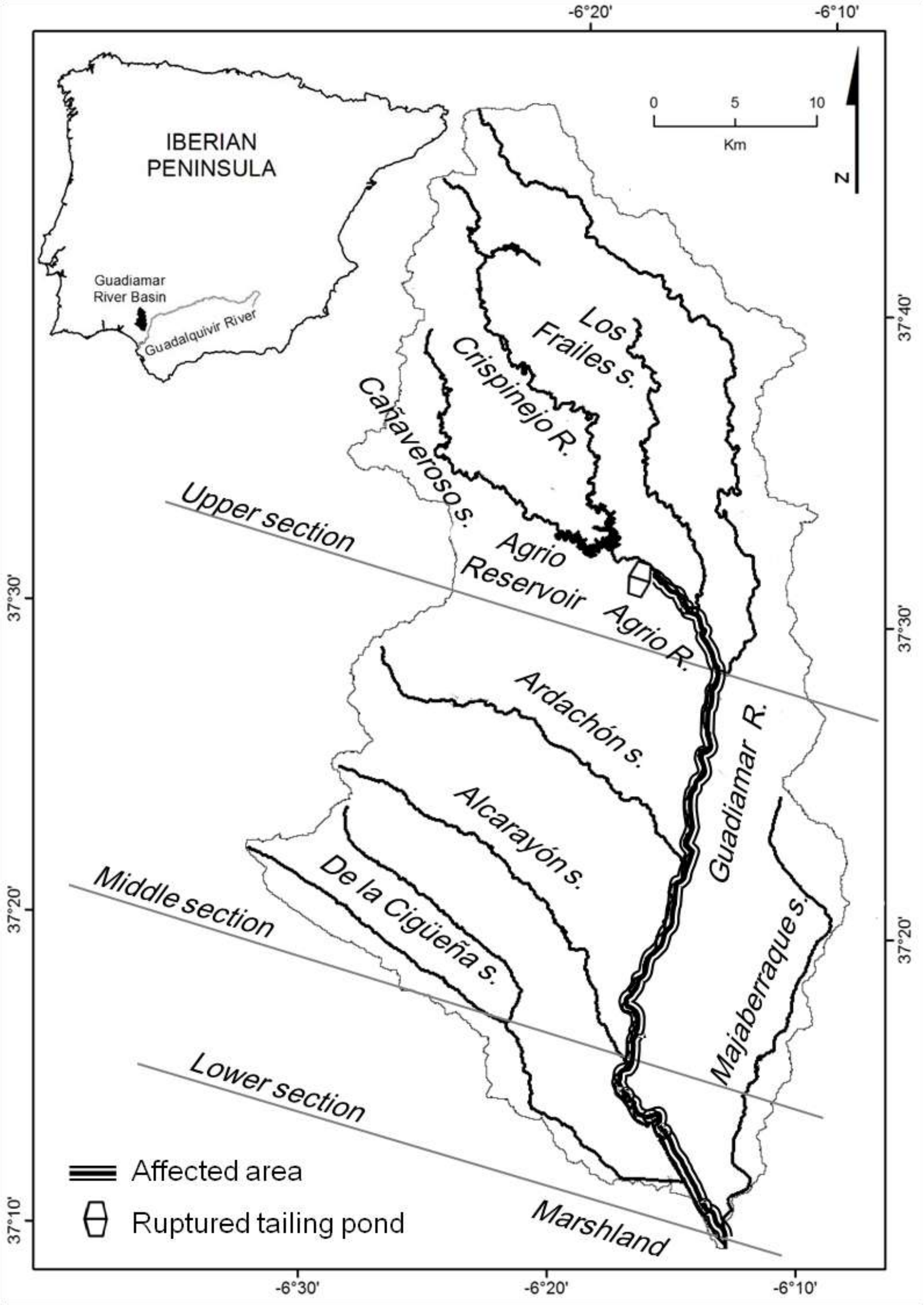

Figure 1. Location of the Guadiamar River basin where different sections, river network and the area affected by the spill are shown.

A total of 78 sampling sites located on the fluvial sector of the watershed were surveyed. Although sampling effort in the main watercourse was higher, the permanent streams were split into hydrological fragments that allowed us to cover the whole network. The minimum stretch covered at each sampling site was approximately 100 m long. An attempt was made to sample all different habitats in order to detect the complete species richness at a given sampling site. Captures were collected using different types of sampling methods depending on site characteristics: (1) electrofishing following the CEN standard protocol (CEN, 2003); (2) sampling of fish larvae (only in 2003 and 2004) using light traps similar to those described in Floyds *et al*. (1984) (Pérez-Alejandre, 2009); (3) passive sampling using minnow-traps (Harrison *et al.*, 1986; 0.5 m length, 0.03 m diameter entrance) left for roughly 22-24 hours; and (4) sampling with multi-mesh gillnets (30 m long and 1.5 m deep) placed transversely from the edges in deep zones, with a soaking time longer than 12 hours.

Most specimens were identified *in situ* up to species level and released thereafter. Young-of-the-year and larvae were identified in the laboratory while fresh (non-preserved specimens; Arias and Drake, 1990; Fernández-Delgado *et al.*, 2000). Some individuals of each species were preserved and deposited in the ichthyological collection of the Department of Zoology of the University of Córdoba.

Results and Discussion

The fluvial systems of the Guadiamar River basin (marshland not included) host a typical Iberian low-diversity fish community composed by 17 species representing 8 families (Table 1). Cyprinidae represented the most diverse family followed by Centrarchidae and Mugilidae. Five of the native fishes (29.4%) caught are included in the IUCN red list with a threatened category (Table 1). Moreover, seven species (41.2%) were among the most important invasive fishes from the Iberian Peninsula (Ribeiro *et al.*, 2008; Almeida and Grossman, 2012; Ribeiro and Leunda, 2012).

Table 1. List of taxa collected from 78 sampling sites in the Guadiamar river basin. New records detected for the basin (*) and the Iberian status for each taxa (native vs. exotic) are shown.

Taxonomic list			**Iberian status**	**Conservation status (IUCN 2012)**
Anguillidae	*Anguilla anguilla* Linnaeus, 1758		native	CR
Atherinidae	*Atherina boyeri* Risso, 1810	(L)	native	VU
Cobitidae	*Cobitis paludica* (de Buen, 1930)	(L)	native	VU
Cyprinidae	*Alburnus alburnus* (Linnaeus, 1758)*		exotic	--
	Carassius gibelio (Bloch, 1782)*(1)		exotic	--
	Cyprinus carpio Linnaeus, 1758	(L)	exotic	--
	Pseudochondrostoma willkommii (Steindachner, 1866)	(L)	native	VU
	Iberochondrostoma lemmingii (Steindachner, 1866)		native	VU
	Luciobarbus sclateri (Günther, 1868)	(L)	native	LC
	Squalius alburnoides (Steindachner, 1866) complex	(L)	native	NE
	Squalius pyrenaicus (Günther, 1868)		native	NE
Poeciliidae	*Gambusia holbrooki* Girard, 1859*		exotic	--
Centrarchidae	*Lepomis gibbosus* (Linnaeus, 1758)	(L)	exotic	--
	Micropterus salmoides (Lacepède, 1802)	(L)	exotic	--
Ictaluridae	*Ameiurus melas* (Rafinesque, 1820)*		exotic	--
Mugilidae	*Liza ramada* (Risso, 1827)		native	LC
	Mugil cephalus Linnaeus, 1758		native	LC

(L) Larval stage detected in the main watercourse.
(1) Denomination of the species in accordance to the Kottelat and Freyhof (2007) information.

Four exotic species listed in this study (*A. alburnus, C. gibelio, G. holbrooki* and *A. melas*) were not present in the fish assemblage that was described by Doadrio (1996) before the accident. Nevertheless, the present taxonomic list (Table 1) was similar to current species composition detected in nearby watersheds from the south-western Iberian Peninsula (Clavero *et al* 2004, Blanco-Garrido, 2006; De Miguel *et al*, 2010; Fernández-Delgado *et al*, 2010). On the other hand, when Guadiamar River basin species richness was compared with that of the rest of northern Guadalquivir River tributaries (Fernández-Delgado *et al*, 2010), that of the Guadiamar reached the maximum value (17 species, Fig. 2; Table 2).

Figure 2. Guadalquivir Drainage location (grey) in the southern Spanish region of Andalusia. Northern tributaries are identified with roman numbers and the names of their watersheds are detailed in the Table 2. Guadiamar River basin is highlighted in dark.

Table 2. Northern Guadalquivir River tributaries, size of drainage basin (Km^2) and species composition.

	Área (Km^2)	Native species	Exoticspeciess	Richness
Guadalimar (I)	5241	7	5	12
Rumblar (II)	712	5	3	8
Jándula (III)	2570	5	4	9
Yeguas (IV)	2570	5	4	9
Guadalmellato (V)	1288	5	2	7
Guadiato (VI)	1491	6	3	9
Bembezar (VII)	1993	7	2	9
Retortillo (VIII)	358	6	3	9
Rivera de Hueznar (IX)	683	6	3	9
Viar (X)	1784	7	2	9
Rivera de Huelva (XI)	1979	10	7	17
Guadiamar (XII)	1325	10	7	17

These northern Guadalquivir tributaries shared similar drainage area and environmental conditions (climate, geology, geomorphology, hydrology and vegetation). However, both the Guadiamar River and Rivera de Huelva River mouths were located downstream from the first large dam in the Guadalquivir River main stem, which allowed the entrance of migratory species (*A. Anguilla, A. boyeri, M. cephalus and L. ramada*) from the estuary but interrupted it for the rest of upstream northern tributaries. Moreover, another reason for which there is an increase in species with respect to the other upstream northern tributaries was the accumulation of exotic individuals in the lower section of large Iberian drainages. This distribution is because of most exotic fish species introduced in the Iberian Peninsula are related to reservoirs (Elvira, 1995; Clavero, 2004; Clavero and Hermoso, 2011; Ribeiro and Leunda, 2012) and are asymmetrically spread, mostly downstream from reservoirs, where flow is continuous, rather than upstream, where introduced fish are not adapted to unstable stream conditions (Bernardo *et al*., 2003; Vinyoles *et al*., 2007; Ribeiro *et al*., 2008).

Generally, the fish assemblage in Mediterranean rivers show a dominance of just a few essential and more tolerant species widely distributed accompanied by others of reduced distribution, associated to particular habitats, and of limited expansion potential (Encina *et al*., 2006). According to the frequency of occurrence in catches throughout the samplings in the Guadiamar basin, *L. sclateri* (FO = 61.5%) and *S. alburnoides* (FO = 50.5%) were the dominant fish species (Table 3). The first is a generalist species (Encina and Granado-Lorencio, 1997) widely adapted to the unfavorable conditions (Herrera and Fernández-Delgado, 1992; Torralva *et al*. 1997; Doadrio *et al*., 2011). This makes the southern Iberian barbel the most ubiquitous and abundant species in southern Iberian river courses (Oliva-Paterna *et al*., 2003a; Fernández-Delgado *et al*., 2010). On the contrary, *S. alburnoides* avoids watercourses where anthropic pollution and sediment accumulation have a strong influence (Fernández-Delgado *et al*., 2010). Nevertheless, the successful reproductive strategy of this species (Fernández-Delgado and Herrera, 1994; Carmona *et al*., 1997) promotes its large populations and widespread distribution throughout watercourses where the degree of degradation is acceptable for the species (Fernández-Delgado *et al*., 2010).

However, focusing on the sampling sites within the affected area, frequency of occurrence changed considerably for most species. Among the dominant native species, *L. sclateri* maintained dominance, while *S. alburnoides* was almost absent. Regarding the exotics, *Lepomis gibossus* and *Cyprinus carpio* significantly increased their frequencies of occurrence to 71.4% and 64.3%, respectively, and the rest of exotic fish increased from 15.6 to 39.3% in this stretch. On the other hand, both native and exotic fish larvae caught in this affected area (Table 1) support local reproductive activity, and therefore, reflect not a temporary, but a consolidated fish assemblage (Pérez-Alejandre, 2009).

Sampling sites results suggest the predominant lack of exotic fish species in the upper section of the basin (except the Agrio reservoir) and in the upper reaches of the Guadiamar River tributaries, whereas downstream of the Agrio reservoir and the Aznalcóllar mines, the middle and lower section of the Guadiamar River connected with the Doñana marshland, may be considered as exotic fish species sources (Table 3).

Table 3. Current records of taxa from the Guadiamar River basin, with indications of tributaries extension range and frequency of occurrence (FO%) obtained in 78 sampling sites.

Species	Sector and tributaries	FO(%)
Natives		
Anguilla anguilla	Gup,Gmid,Glow,AR	11.5
Atherina boyeri	Gmid	<2.5
Cobitis paludica	Gup,Gmid,Glow,LF,CA,AL	37.2
Pseudochondrostoma willkommii	Gup,Gmid,LF,CA	39.7
Iberochondrostoma lemmingii	Gup,Gmid, LF	6.4
Luciobarbus sclateri	Gup,Gmid,Glow,LF,CA,AR,DC,AG	64.5
Squalius alburnoides complex	Gup,Gmid,LF,CA,AR	50.00
Squalius pyrenaicus	Gup,Gmid,LF,CA,AR	21.8
Liza ramada	Gmid,Glow	3.8
Mugil cephalus	Gmid,Glow	5.1
Exotics		
Alburnus alburnus	Gmid, Glow,AR,DC	13.5
Carassius gibelio	Gmid,Glow,AG	10.2
Cyprinus carpio	Gmid,Glow	23.1
Gambusia holbrooki	Gup,Gmid,Glow,LF,AR,MA	19.2
Lepomis gibbosus	Gup,Gmid,Glow, AR,AG	28.2
Micropterus salmoides	Gup,Gmid,LF	14.1
Ameiurus melas	Gup	<2.5

(Gup, Gmid and Glow: upper, middle and lower Guadiamar; AR: Ardachón; LF: Los Frailes; CA: Cañaveroso; AL: Alcarayón; AG: Agrio; MA: Majaberraque; DC: De La Cigüeña)

In summary, more than a decade after the accident, the overall Guadiamar River fish assemblage is far from that which could be considered as recovered (Doadrio, 1996). Compared with the pre-accident assemblage, there is a native species depletion and exotics increase in the affected reach. Quantitative information on abundance, population dynamics and fish species trends are needed in order to obtain appropriate evaluations of the current factors responsible for its current status. Such information may provide recommendations for further recovery measures and, in the long term, assist in the decision whether recovery actions without ichthyological perspective make sense or environmental quality control of polluted inputs will have to be dealt with as a management priority. The present study may serve as a comparative species richness index against which future fish assemblage drifts may be evaluated.

Acknowledgments

Data presented in this study was collected under projects: i)“Caracterización ecológica de las comunidades de peces en el río Guadiamar”, ii)“Fauna piscícola de la cuenca del río Guadiamar: estado de conservación, problemática y directrices de restauración”, iii) “Seguimiento del efecto del vertido tóxico de las minas de Aznalcóllar sobre la comunidad de peces del río Guadiamar” and iv)“Bases para la elaboración de un plan de conservación de los peces continentales autóctonos de Andalucía”, all of them funded by the Andalusian Regional Government. We thank Teresa Saldaña, Palmira Guarnizo, Diego García, Carmen García, Arnolf Fernández, Carmen Arribas, Javier Berná, Rocío Pérez, Antonio Barranco, David Redondo, Manuel Fernández, Enrique Pino, Alejandro Ramiro, Javier Peña, Francisco Aranda and Raquel Moreno for their help both in the field and with GIS.

PARTE II:

Análisis puntual del efecto del vertido a corto y medio plazo sobre la ictiofauna del Guadiamar.

II.1- Habitat quality affects the condition of Luciobarbus sclateri in the Guadiamar River (SW Iberian Peninsula): Effects of disturbances by the toxic spill of the Aznalcóllar mine.

(Trabajo publicado en: *Hydrobiologia* (2013) 700: 85-97.)

Abstract

This study analyzes the somatic condition of southern Iberian barbel *Luciobarbus sclateri* (Günther, 1868) in the Guadiamar River (SW Iberian Peninsula). This river was seriously affected by a toxic spill of about 4 million cubic meters of acidic water and 2 million cubic meters of mud rich in heavy metals. Once the spill removal works concluded, sites affected and unaffected by the accident were sampled to study its effects on the fish fauna. The ecological variables registered were related to water quality, physical state of reaches, ecological quality, resources exploited by fish, and potential intra-specific interactions. From an initial fifteen ecological variables, seasonal water flow and pH explained most of the variation in barbel condition. This study shows that the Guadiamar River, fifty-six months after the accident, is still undergoing a recovery process where, beyond ecological variables, proximity to the affected area is the most influential factor for fish condition.

Introduction

Since 1960, the International Commission on Large Dams has registered more than one major tailing dam failure every year (ICOLD, 2001). Tailing dam vulnerability, compared to other retention structures (e.g. water reservoirs), is related to several aspects: (i) dykes are often formed by accumulated fills from the mine; (ii) dams are subsequently raised with additional solid materials, and suffer a severe increase in effluent (increased by runoff from precipitation); (iii) lack of regulations on design criteria; (iv) dam stability requires monitoring, emplacement, construction and operation controls; (v) high cost of remediation after mine closure (Rico *et al.*, 2008). Several accidents have been caused by these weaknesses worldwide. For example, 268 people died in Trento, Italy, when a fluorite mine tailing pond released 200,000 cubic meters of waste along the Avisio river in 1985 (Van Niekerk and Viljoen, 2005); in 1996 all fish disappeared along a 500 km stretch of the Pilaya river, due to a mine spill from Porco, in western Bolivia (Macklin *et al.*, 2006); and after the 2000 Aural-Baia Mare gold mine spill, in north-eastern Romania, the dykes built to retain the cyanide and heavy metals from the spill broke and released these pollutants into the Lapus and Somes and Novat rivers, dramatically reducing the number of fish, plant and mollusc species (Cordos *et al.*, 2003).

On 25th April 1998, the tailing pond dike of the ‘‘Los Frailes’’ zinc mine, in Aznalcóllar (SW Spain) collapsed, releasing about 4 million cubic meters of acidic water and 2 million cubic meters of mud rich in toxic metals (Grimalt and Macpherson, 1999). As a consequence of this accident, 67 km of the Guadiamar River’s main channel were polluted with a toxic spill whose primary composition was S (35-40%), Fe (34-37%), Zn (0.8%), Pb (0.8%), As (0.5%), Cu (0.2%), Sb (0.05%), Co (0.006%), Tl (0.005%), Bi (0.005%), Cd (0.0025%), Ag (0.0025%), Hg (0.001%) and Se (0.001%) (Grimalt and Macpherson,1999). Mechanical removal of contaminants from the stream and flood plain caused the destruction of the natural protection against bank erosion (Gallart *et al.*, 1999). 37.4 tonnes of dead fish mixed with mud were removed from the marsh area, including carps (75-80%), mullets (10-16%), barbels (6-8%), eels (4%) and other species (5%) (Valls and Blasco, 2005). After the accident, several studies analyzed the effects of the toxic spill (Blasco *et al.*, 1999, Meharg *et al.*, 1999, Van Geen *et al.*, 1999, Alcorlo *et al.*, 2006, among others). Effects on the fish fauna were reported short after the spill for both the fluvial sector (Fernández-Delgado and Drake,

2008) and the marsh area (Drake *et al*., 1999). This paper addresses the mid-term effects of the spill by exploring the relationship between current habitat variables and fish condition.

The analysis of fish condition is standard practice in the management of fish populations as a measure of both individual and cohort fitness (Jakob *et al*., 1996). Condition measures are useful as indicators of tissue energy reserves and may reflect the environment in which fish live (e.g., habitat, prey availability, competition) (Vila-Gispert and Moreno-Amich, 2001; Oliva-Paterna *et al*., 2003a and 2003b; Verdiell-Cubedo *et al*., 2006a and 2006b). A poor body condition can negatively affect survival, maturity and reproductive effort in subsequent phases of fish life-history (Hoey and McCormick, 2004; Morgan, 2004). Therefore, fish condition indices are useful to assess population status, the impact of management actions, and anthropogenic influences on fish (Brown and Austin, 1996).

The southern Iberian barbel, *Luciobarbus sclateri* (Günther, 1868), is an endemic fish in the ecosystems of the central-southern Iberian Peninsula (Doadrio, 2001; Kottelat and Freyhof, 2007). *L. sclateri* is a useful indicator of fish community status because it has a widespread distribution, a long life-span (9-14 years for males and 12-19 years for females) (Lucena *et al*., 1979; Herrera *et al*., 1988), it is the most abundant fish in the fluvial section of the Guadiamar River basin (Fernández-Delgado and Drake, 2008), and its reproductive migration usually occurs within the same catchment (Herrera and Fernández-Delgado, 1992; Rodríguez-Ruiz and Granado-Lorencio, 1992; Torralva *et al*., 1997). Moreover, the relevant effects of habitat quality disturbances on body condition of this target species have previously been reported in studies from other semi-arid regions in the Iberian Peninsula (Oliva-Paterna *et al*., 2003a; 2003b and 2003c).

The objectives of this study were (1) to assess and compare body condition of *L. sclateri* from fluvial sectors inside and outside the area affected by the toxic spill and (2) to analyze the relationships between population condition at site level and environmental variables related to water quality, the physical state, ecological quality, possible resources exploited by fish and potential intra and inter-specific interactions. We hypothesized that the condition of barbels in the Guadiamar River basin is influenced by whether they are found inside the affected area or not.

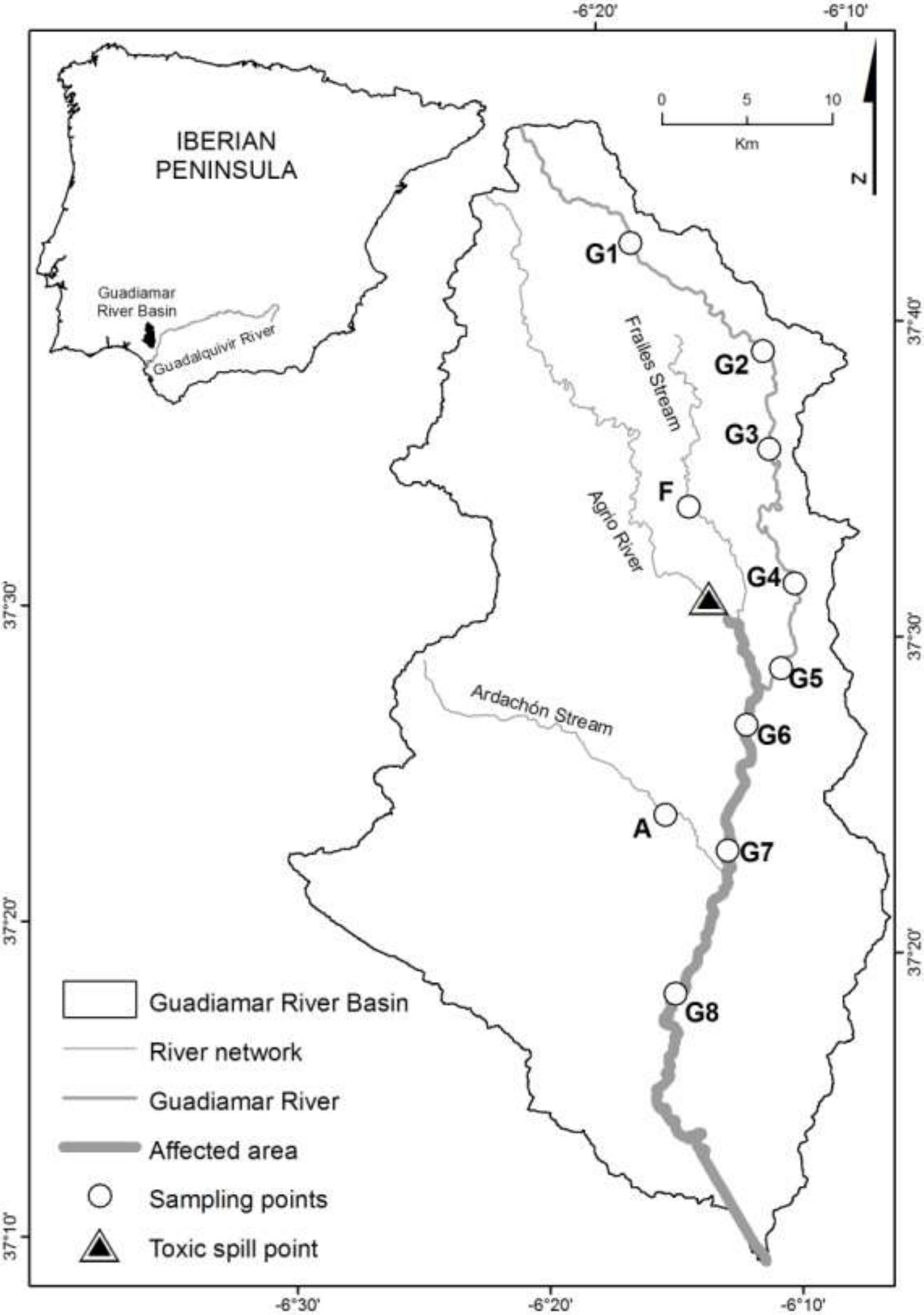

Figure 1. Sampling at sites at the Guadiamar River basin in the southern Iberian Peninsula. G1-G5: sampling sites located in the non-affected area of the Guadiamar River and G6-G8: sampling sites located in the affected area of the Guadiamar River; A and F: sampling sites located in the non-affected area of the Ardachón and Frailes tributaries, respectively.

Material and Methods

Study area

The Guadiamar River basin is located in the South-western Iberian Peninsula, and it is the last large tributary of the Guadalquivir River in its northern side. The basin covers an area of 1.880 km^2 (Borja *et al.*, 2001) (Fig. 1). The upper section flows through the western Sierra Morena, with typical xeric Mediterranean forests. Thereupon, the river crosses a mainly agricultural area on sedimentary hills and, finally, the southern end turns into a fine-material channelized marsh that flows into the Guadalquivir river mouth within the Doñana National Park (Borja *et al.*, 2001). From a hydrological point of view the Guadiamar is a typical Mediterranean river (Giudicelli *et al.*, 1985), with a severe summer drought, annual average temperature above 10 ºC and annual average rainfall of 600 mm (Aguilar *et al.*, 2003). Agrio, Frailes and Ardachón are the most important tributaries in the Guadiamar basin (Fig. 1).

Sampling

Ten sampling sites were selected (Fig. 1): seven in the area not affected by the toxic spill, including five (G1 to G5) in the main channel and two in the most important tributaries (A and F), and three sampling sites (G6 to G8) in the main channel affected by the toxic spill (Fig. 1). Fish were caught at each site in December 2002, fifty-six months after the toxic spill. Sampling during this period avoided the capture of pre-spawning and spawning fish, and ensured that variations in body condition were unaffected by gonad development (Herrera and Fernández-Delgado, 1994; Encina and Granado-Lorencio, 1997a and 1997b). Fish collected at each sampling station were considered as independent populations for several reasons: minimum distance along the river course between sampling sites was above 5 km; the Guadiamar River has several small dams that restrict fish migration (Arribas *et al.*, 2005); and the reported winter home-range for *L. sclateri* is below 1976 m^2 (Prenda and Granado-Lorencio, 1994).

Fish were sampled by electrofishing in wadeable sections of the river 100-300 m in length, depending on its width (wading upstream with one/two anodes using 240 V pulsed direct current). Two fishermen with electric dip-nets collected fish while walking from the lower towards the upper part of each sampling site. Fish were anaesthetized with benzocaine before furcal length (FL; ± 1 mm) and total mass (TM; ± 0.1 g) were recorded. Individuals smaller than 40 mm FL (<1+ age class) (Saldaña, 2006) were excluded from the analysis to avoid possible effects of differences in body shape between juveniles and adults (Murphy *et al.* 1990), and to minimize measurement errors associated with weighing small fish in the field (Vila-Gispert and Moreno-Amich, 2001).

Each sampling site was characterized by the following fifteen environmental variables: conductivity (μS cm^{-1}), oxygen (ppm), water temperature (ºC) and pH (fortnightly mean values for these four variables); seasonal water flow, dominant substrate, channel width (m) and land use index [based on the EEA's Corine land cover (2009)]; QBR [Riparian Ecosystems Quality Index *sensu* Munné *et al.* (1998)]; IBMWP [Iberian version of the Biological Monitoring Working Party *sensu* Hellawell (1978)] (Alba-Tercedor and Sánchez-Ortega, 1988) and IBG [Indice Biologique Global *sensu* Verneaux *et al.* (1982)]; fish diversity [(H') Shannon's diversity index], fish species richness (S), fish density (fish individuals m^{-2}) and *L. sclateri* density (*L. sclateri* individuals m^{-2}) (Table 1).

According to previous studies on the same species and other barbels (Vila-Gispert *et al.*, 2000; Oliva Paterna *et al.*, 2003a; 2003b and 2003c), we classified seasonal water flow as very unstable (0) when flow drastically decreased in summer and the stream was reduced to isolated pools, moderate (1) if the flow was continuous but with water level fluctuations in accordance with the wet-and-dry cycle, and very stable (2) if the flow remained relatively constant throughout the year. The dominant substrate was recorded according to the size of different particles: sand (100% sand, 2-5 mm), muddy-sandy-stony (equal percentages of mud, 1-2 mm, sand and stones, 25-100 mm), sandy-stony (over 50% sand, the remainder being stones), stony-sandy (over 50% stones, the remainder being sand) and stony (100% stones). Qualitative sampling of macroinvertebrates was carried out at each sampling site, using nets with 0.5 and 0.3 mm mesh. The content of each net was deposited periodically in trays to stop nets from collapsing. Each sampling was considered finished when sweeps provided no new taxa (Zamora-Muñoz *et al.*, 1995). The specimens were identified up to family level and a value was calculated according to two indices, IBMWP (very bad <15, bad 16-36, moderate 36-60, good 61-100, very good < 100) (Alba-Tercedor and Sánchez-Ortega, 1988) and IBG (0-20, where 0 indicates pollution and 20 no pollution) (Verneaux *et al.*, 1982). Finally, riparian forest quality was classified based on the QBR index range (>95: natural; 90-75: good quality; 70-55: acceptable quality; 30-50: poor quality; < 25: bad quality) (Munné *et al.*, 1998).

Table 1. Mean habitat variable values for each sampling site. S: Fish species richness. H': Fish diversity (Shannon's diversity index). QBR: Riparian Ecosystems Quality Index. IBMWP: Iberian version of the Biological Monitoring Working Party. IBG: Indice Biologique Global. *L. sclateri* density and Fish density were removed from the model selection protocol due to lack of data in G6 and G8 due to field sampling constraints.

Sampling sites	H'	S	Conductivity (µs cm^{-1})	Oxygen (ppm)	Tª (ºC)	pH	Seasonal water flow	Dominant substrate	Channel width (m)	QBR	Land use Index	IBMWP	IBG	Fish Density (ind m^{-2})	*L.sclateri* density (ind m^{-2})
G1	0.67	5	351	10.4	6.9	8.00	0	3.5	7	65	2.78	89	10	1.98	0.08
G2	0.73	5	329	10.7	9.8	8.18	0	4	6	70	3.36	87	10	3.22	1.30
G3	0.98	4	353	11,5	6.3	8.22	1	3.7	10	65	2.88	59	9	0.97	0.14
G4	0.98	5	419	10,9	8.6	8.39	1	3.3	7.5	50	3.05	53	9	1.42	0.11
G5	1.11	6	308	10,6	11.1	8.31	1	4	8.2	25	2.97	26	7	2,58	1.69
G6	0.85	4	1,107	9,8	9,0	6.78	2	5	12.5	20	3.54	14	5	--	--
G7	0.68	3	993	8,8	10.1	7.38	2	4	11	15	3.49	9	5	0.02	0.01
G8	0.70	3	1,350	10,5	11.5	6.50	2	3	12	25	3.42	--	--	--	--
A	0.64	3	1,256	10,1	11.3	7.57	1	1	3.5	30	3.06	37	7	0.76	0.16
F	0.83	4	209	7,7	10.3	7.91	1	3	6.5	70	3.12	70	9	0.48	0.07

Statistical analyses

The statistical analyses used to compare fish condition followed those used in two previous studies that dealt with the same species (Oliva-Paterna *et al*., 2003a and 2003b) and those proposed by García-Berthou and Moreno-Amich (1993). They include the application of univariate analysis of covariance (ANCOVA) using TM (total mass) as the dependent variable and FL (furcal length) as the covariate, and "sampling site" as factor. The relationship between TM and FL was clearly non-linear; therefore, the log-transformation of TM was used as dependent variable and log-transformation of FL as the covariate. We tested the homogeneity of the regression coefficients (parallelism as the assumption of equal slopes) of the dependent-covariate relationship with an ANCOVA design that analysed the pooled covariate-by-factor interaction. If the covariate-by-factor interaction (homogeneity of slopes) was not significant ($p > 0.05$), we developed a standard ANCOVA to test for significant differences in parameter *a* (the y-intercept) between populations as a condition index.

Additionally, the condition of *L. sclateri* was represented by residuals obtained from a least squares regression between TM and FL of all captured individuals (log-transformed data) (Sutton *et al*., 2000). This residual index (Kr) provides an alternative to other more traditional condition indices, e.g. relative condition factor and Fulton's condition factor, and removes body length effects. Some authors (García-Berthou 2001a, among others) have pointed out dangers in calculating residuals. However, later studies have demonstrated significant correlations between residuals and fat stores (Schulte-Hostedde *et al*. 2005). First, some of the key assumption underlying the use of residuals were verified: (1) the mass-length relationship was linear, (2) the residual index was independent of length (Regression test ANOVA $F_{(1,759)=}0.21$ p=0.889), and (3) the parallelism assumption. Secondly, the mean condition for *L. sclateri* at each site level was determined from the average Kr of individuals captured at each sampling site. The existence of significant differences between sampling sites was verified by ANOVA analysis and Tukey's HSD post hoc tests (Quinn and Keough, 2002).

Finally, we performed multiple regression analyses to determine the amount of variation in parameter *a* (the y-intercept) and Kr (residual index) associated with environmental variables. In order to reduce the number of predictor variables and detect the potential occurrence of collinearity, a bivariate analysis was carried out using Pearson's correlations between all quantitative variables, and Spearman's correlations for categorical variables (seasonal water flow, dominant substrate and land use index)

(Table 2). The final variables were selected according to the following criteria: first, groups of variables that were highly correlated (> 0.75) were identified and one variable was chosen according to its relevance for barbel condition or information from previous studies; second, those variables not highly correlated with others and pointed out as important by other studies were added to the list; and finally, if variables were of similar importance, the variable with the highest correlation with barbel condition was selected, trying always to build the most parsimonious model (Johnson and Omland, 2003). The final regression models were applied to a total of 10 cases (n=10) and a maximum of 5 predictor variables, since if we had a larger number of variables we would incur in a Type 2 error (Field, 2005). The residuals of preliminary models were checked for outliers and/or influential cases (Cook´s distance and Leverage, Cook, 1979), and no outliers were found. Once the final variables were chosen in each case, the best models supported by the data were selected using the Akaike Information Criterion (AIC), a model selection approach based on Information Theory (Burham and Anderson, 2002). The lack of both *L. sclateri* density and fish density values at two sampling sites (G6 and G8) due to problems during field sampling reduced the degrees of freedom and, therefore, the possibility of obtaining a significant model. For this reason we decided to remove *L. sclateri* density and fish density from the model selection procedure. Variance partitioning was used to differentiate the most influential variables when models selected more than one variable (Peres-Neto, *et al*., 2006). Statistical analyses were performed using R® software version 2.12 and packages: vegan, lattice, hier.part and mass (R Development Core Team, 2010).

Table 2. Correlation matrix of parameter *a* (y-intercept) of the mass-length relationship and mean Kr values with environmental variables (Pearson's correlation coefficient; Spearman's correlation coefficient in brackets). (*) Significance level $p < 0.05$. S: Fish species richness. H': Fish diversity (Shannon's diversity index). QBR: Riparian Ecosystems Quality Index. IBMWP: Iberian version of the Biological Monitoring Working Party. IBG: Indice Biologique Global. LUI: Land Uses Index.

Variables	H'	S	Conductivity	Oxygen	Tª	pH	Seasonal water flow	Dominant substrate	Channel width	QBR	Land uses index	IBMWP	IBG	Fish density	*L.sclateri* density
S	0.63*														
Conductivity	-0.52	-0.77*													
Oxygen	0.33	0.35	-0.07												
Water (Tª)	-0.18	-0.26	0.47	0.36											
pH	0.50	0.70*	-0.87*	0.26	-0.39										
Seasonal water flow	(0.05)	(-0.65)*	(0.64)*	(-0.38)	(0.34)	(-0.65)									
Dominant substrate	(0.41)	(0.37)	(-0.20)	(0.07)	(-0.38)	(0.08)	(0.12)								
Channel width	0.16	-0.24	0.35	0.03	-0.11	-0.59	(0.78)*	(0.47)							
QBR	0.05	0.37	-0.76*	0.10	-0.54	0.63*	(-0.81)*	(-0.33)	-0.48						
Land uses index	(-0.18)	(-0.46)	(0.34)	(-0.49)	(0.34)	(-0.65)*	(0.59)	(0.34)	(0.33)	(-0.42)					
IBMWP	-0.17	0.36	-0.67*	0.19	0.19	0.59	(-0.90)*	(-0.41)	-0.54	0.95*	(-0.51)				
IBG	0.03	0.47	-0.77*	0.33	0.47	0.77*	(-0.93)*	(-0.34)	-0.56	0.94*	(-0.54)	0.96*			
Fish density	0.24	0.83*	-0.50	0.55	-0.03	0.66	(-0.77)*	(0.39)	-0.26	0.30	(-0.38)	0.47	0.52		
L. sclateri density	0.43	0.67	-0.33	0.31	0.39	0.46	(-0.38)	(0.22)	-0.09	-0.10	(-0.24)	-0.02	0.04	0.81*	
a (y-intercept)	0.26	0.58	-0.78*	-0.17	-0.27	0.91*	(-0.87)*	(-0.27)	-0.82*	0.72*	(-0.54)	0.85*	(0.84)*	0.57	-0.27
Kr	0.03	0.39	-0.76*	-0.11	-0.44	0.74*	(-0.82)*	(-0.26)	-0.71*	0.84*	(-0.61)	0.87*	(0.84)*	0.12	-0.25

Results

Southern Iberian barbel was the most abundant fish species in the study area. Other species collected were *Anguilla anguilla* (L.), *Pseudochondrostoma willkommi* (Steindachner), *Iberochondrostoma lemmingii* (Steindachner), *Squalius pyrenaicus* (Günther), *Iberocypris alburnoides* (Steindachner), *Cobitis paludica* (De Buen), *Lepomis gibossus* (L.), *Micropterus salmoides* (Lacépède), *Cyprinus carpio* (L.) and/or *Gambusia holbrooki* (Agassiz), depending on the sampling site.

Parameters of the mass-length relationship in each site are presented in Table 3 and the results of the ANCOVA are shown in Table 4. There was a significant degree of homogeneity (P = 0.172) between sampling sites on slope (b) of the relationships between TM and FL (the preliminary design confirmed the parallelism assumption, Table 4), although the y-intercept (a) varied significantly (P < 0.0005) between sampling sites (Final design, Table 4). The first sector of Guadiamar River (G1) and Frailes stream (F) showed the highest fish condition, while areas affected by the toxic spill (G6, G7 and G8) showed the lowest values (y-intercept higher and lower respectively, Table 3 and Fig. 2a). As a result, sampling sites can be differentiated according to differences in parameter a of the mass-length relationship.

With respect to Kr values (Table 3 and Fig. 2b), we verified homogeneity of variances for the comparison among sampling sites (Levene test at site-level $F_{(9,759)}$ = 1.80; P = 0.065). ANOVA analysis showed significant differences in Kr values between sampling sites ($F_{(9,759)}$ = 105.02; P < 0.0005). G1 and Frailes stream (F) had the highest fish condition values and formed a significantly homogeneous group (Tukey’s HSD, Fig. 2b). G2, G3, G4, G5 and Ardachón stream were another significant group (Tukey’s HSD), with lower values than the first one; and finally G6, G7 and G8 constituted another significant group (Tukey’s HSD) with the lowest Kr values (Fig. 2b).

Bivariate relationships between the condition indices (parameter a of the mass-length relationship and Kr) and environmental variables, and among the latter, are presented in Table 2. Note that conductivity, pH, seasonal water flow, channel width, QBR, IBMWP and IBG presented significant correlations with parameters a and Kr.

Fish density, channel width, QBR, IBMWP and IBG were all highly correlated with seasonal water flow (Table 2), so the first five variables were not included in the models, whereas the last one was selected as a predictor. Seasonal water flow was selected based on its importance as a major structuring force of fluvial systems, and because its significant influence on fish condition has been shown by several other authors (Vila-Gispert *et al*., 2000; Oliva-Paterna *et al*., 2003a and 2003b). The final list included 4 variables (seasonal water flow, pH, dominant substrate and land use index).

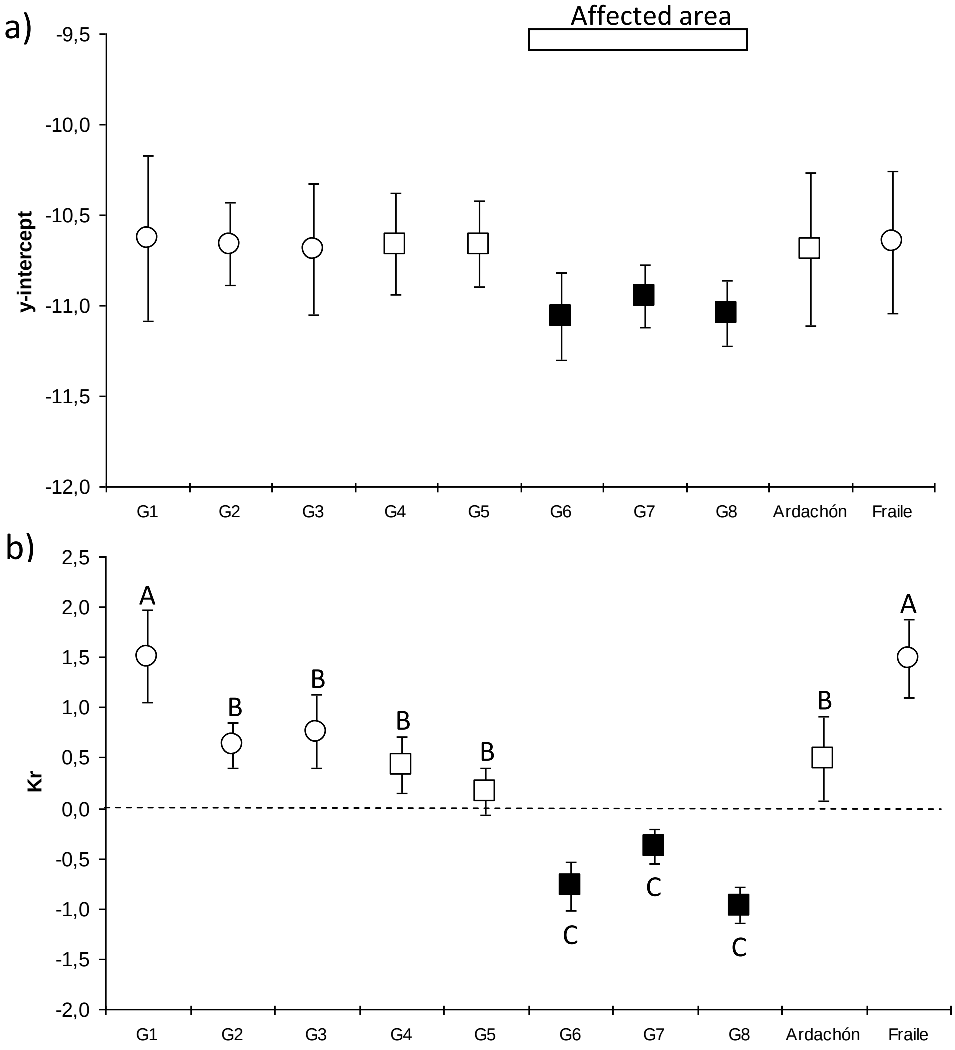

Figure 2. Mean fish condition estimated from the y-intercept of the mass-length relationships (a) and using residual values (Kr) (b) in each study site. Circles represent sites immersed in a forestry land use matrix, while squares are under agricultural land use (black squares are in the affected area). Fraile and Ardachón are two tributaries that meet the main course between G5 and G6, and just after G7, respectively (see Fig. 1). Different capital letters (A, B and C) represent significant differences in fish condition according to Tukey´s HSD post-hoc tests ($p < 0.05$).

Table 3. Regression (*a*, *b*), adjusted correlation coefficients (R^2adj) and residuals (Kr) of the log-transformed mass-length relationship in each sampling site.

Sampling site	**n**	***b*** **(slope)**	***a*** **(the y-intercept)**	**R^2adj**	**Kr (residuals)**	**Mean ± CL Furcal length (mm)**
G1	20	2.99±0.09	-10.63 ± 0.46	0.996	1.51± 0.20	81.3 ± 10.6
G2	174	2.98±0.03	-10.66 ± 0.23	0.996	0.63± 0.09	74.3 ± 4.8
G3	41	2.99±0.07	-10.69 ± 0.36	0.994	0.76± 0.18	85.4 ± 7.8
G4	33	2.98±0.07	-10.66 ± 0.28	0.976	0.43± 0.25	70.0 ± 13.6
G5	106	2.98±0.05	-10.66 ± 0.24	0.992	0.16± 0.10	64.9 ± 4.8
G6	66	3.04±0.05	-11.06 ± 0.24	0.996	-0.77± 0.20	109.6 ±22.6
G7	103	3.03±0.03	-10.95 ± 0.17	0.991	-0.38± 0.13	84.6 ± 7.7
G8	167	3.04±0.03	-11.04 ± 0.18	0.984	-0.97± 0.11	104.6 ± 7.4
A	24	2.99±0.08	-10.69 ± 0.42	0.995	0.49± 0.31	65.4 ± 14.5
F	26	2.99±0.07	-10.65 ± 0.39	0.996	1.49± 0.27	65.1 ± 10.0

Table 4. ANCOVA analyses of the mass-length relationship in *L. sclateri*: *F*-statistics, degrees of freedom (df) and *P* values. All variables (dependent and covariate) were log-transformed. Furcal length is the covariate.

Source of variation	***F***	**df**	***P***
Preliminary design (test for interaction)			
Length	77539.05	1, 759	<0.0005
Sampling site	2.677	9, 759	0.005
Length × Sampling site	1.429	9, 759	0.172
Final design (no interaction)			
Length	157476.30	1, 759	<0.0005
Sampling site	153.12	9, 759	<0.0005

This new model selected under Akaike's criterion accounted for 96% of the variance and pointed out pH, seasonal water flow and dominant substrate as the most influential variables, representing 53%, 35% and 11%, of the explained variance, respectively (Table 5). The relationship between parameter *a* and both seasonal water flow and dominant substrate was negative, whereas it was positive for pH. The multiple regression model with Kr as dependent variable accounted for 62% of the variance. This model highlighted seasonal water flow as the most influential variable for *L. sclateri* condition (negative relationship, Table 5).

Table 5. Multiple regression models used to determine the main environmental predictors of parameter *a* (y-intercept) and Kr of the mass-length relationships as fish condition indices for *L. sclateri* in the Guadiamar River. Significant variables in models and their relative weight are shown. ('***' $p < 0.001$ '**' $p < 0.01$ '*' $p < 0.05$). (% explained variance = variance explained by each variable according to variance partitioning using the hier.part package).

	Significant variables (% explained variance)	**Adjusted R^2**	**p value**	**Estimate**	**Std. Error**	**t_value**	**Pr(>|t|)**
Model *a*	pH (53%)	0.96	0.00003	0.17760	0.02529	7.022	0.000416***
	Seasonal Water Flow (35%)			-0.07524	0.02277	-3.305	0.016312 *
	Dominant substrate (11%)			-0.04401	0.01076	-4.088	0.006442 **
Model Kr	Seasonal Water Flow	0.62	0.00404	-0.9378	0.2354	-3.984	0.00404 **

Discussion

Our results showed that the condition of *Luciobarbus sclateri* was significantly different between sampling sites. All differences in parameter *a* of the mass-length relationship and in Kr values were related to differences in habitat conditions.

Both fish condition indices established a significant group with lowest condition values in the area affected by the toxic spill (G6, G7 and G8) and the best body condition in sites located in the upper parts of the basin (G1 and Frailes). This pattern coincides with that obtained for fish community indicators in an eight-year survey in the same study area (Fernández-Delgado and Drake, 2008) and with another study that focused on the macro-invertebrate community (Ferreras-Romero *et al.*, 2003). In contrast, other authors report no effects of toxic waste on the nektonic community (crustaceans and fish species) soon after the spill (Drake *et al.*, 1999). This may be due to the protection offered by several dykes that were constructed immediately after the accident to stop the advance of the flood and stop the spill from reaching the downstream Doñana National Park (López-Pamo *et al.*, 1999).

In our site-level analysis of habitat-fish condition relationships, the ecological variables that accounted for most of the variation in barbel condition in the Guadiamar River were seasonal water flow and pH. Nevertheless, due to the multivariate regression model requirements detailed above, several environmental variables highly correlated with those finally included in the analyses (fish density, IBMWP, IBG, QBR and channel width with seasonal water flow; conductivity and IBG with pH), must be taken into account, since they may also be influential factors.

According to previous studies with the same species (Oliva-Paterna *et al.*, 2003a) and with *Barbus meridionalis* (Vila-Gispert *et al.*, 2000; Vila-Gispert and Moreno-Amich, 2001), the stability of seasonal water flow is greatly responsible for the large variation in fish condition between populations, with better fish condition in streams with a continuous seasonal water flow, where fish are not confined in pools and find more shelter and food. In the present study, seasonal water flow also exerted a major influence on fish condition; however, in the opposite direction. The highest condition values were found in upstream stretches with the lowest seasonal water flow values, where summer drought restricts the flow to isolated pools. This negative effect probably occurs because reaches with the most stable flow are located in the affected area, and the presence of toxic remains (Gallart *et al*., 1999) affects fish condition and thus disrupts the natural gradient found by other authors (Vila-Gispert and Moreno-Amich, 2001; Oliva-Paterna *et al*., 2003a and 2003b).

The collinearity between seasonal water flow and fish density could offer another explanation for the reversion found with respect to natural gradients. Areas with the lowest seasonal water flow were those with greatest total fish density and *L. sclateri* density. High *L. sclateri* and total fish density may give rise to competitive interactions that could be an influential factor for fitness, growth, reproduction and survival (Wootton, 1998). The relationship between inter- or intra-specific abundance and fish condition has been mentioned in several studies with the same species and other Iberian barbels (Vila-Gispert *et al*., 2000; Oliva-Paterna *et al*., 2003a and 2003b). In particular, Saldaña (2006) found that an increase in intra-specific density of *L. sclateri* had a negative effect on somatic condition in a population located in the upper Guadiamar River. In contrast, our study presents the reverse situation, where a positive relationship between fish density and condition is observed. This apparently antagonistic result can be explained if we take into account that reaches with good habitat conditions in the Guadiamar River after the toxic spill can shelter both healthy and highly diverse fish populations (Fernández-Delgado and Drake 2008), while the affected reaches, poorer in resource availability, are not able to support abundant barbel populations, and individuals that can survive in these areas do it in a subsistence manner, as reflected by their low somatic condition. Specifically, reaches with the lowest condition and fish diversity coincide with the affected area, so it seems that a toxic effect still remains.

IBG, IBMWP and QBR were variables whose collinearity with those selected by the models suggests that their potential influence should be considered. These macroinvertebrate and riparian vegetation indices are well-known indicators of ecosystem health (e.g. Goede and Barton, 1990), and their positive relationship with fish condition has been reported before (Oliva-Paterna *et al*., 2003a and 2003b). Other authors (Prat *et al*. 1999; Ferreras-Romero *et al*. 2003) found few aquatic macroinvertebrate families in the affected area of the Guadiamar River, and those present were more opportunistic and linked to lentic environments than those that inhabited the unaffected area. In our study, the reaches with lowest IBG, IBMWP and QBR values coincide with the affected area, where the spill deteriorated the riparian vegetation (Murillo *et al*., 1999). Riparian vegetation provides suitable habitats for aquatic and terrestrial organisms that are important food items for *L. sclateri* (Encina and Granado-Lorencio, 1997). The QBR index was highly correlated with both seasonal water flow and the condition indices, suggesting that the quality and quantity of riparian vegetation has a positive effect on the condition of individuals in our population. Therefore, these indicators suggest that poor habitat conditions remain in certain parts of the study area.

The most influential variable in the model for parameter *a* (y-intercept) was pH. This variable had not been considered in other studies on Iberian barbels. Only one study that addressed the same species in reservoirs (Oliva-Paterna *et al*., 2003c) found a positive correlation between pH and condition. In our study area, the lowest pH values are found in the affected area, due to the input of dissolved sulphates from the pyritic mud that persists in the substrate (Van Geen *et al*., 1999). Furthermore, pH reduction favours the release of heavy metals retained by the substrate (Olías *et al.*, 2005), and causes bioaccumulation in benthonic macroinvertebrates such as *Procambarus clarkii* and fish, especially barbel (Alcorlo *et al*., 2006). These studies, carried out in the Guadiamar River after the mining accident, have shown an increase in the concentration of Pb and Cd in tissues of *P. clarkii* and *L. sclateri* when samples were taken close to the spill point (Moreno-Rojas *et al*., 2005; Alcorlo *et al*., 2006). This impact gradient is coincident with other results based on physical indicators such as the depth of the toxic mud layer (Gallart *et al*., 1999; López-Pamo *et al*., 1999), or even chemical indicators, since pH increases and heavy metal concentration in water decreases as we move away from the spill point (Olías *et al*., 2005). In addition, the high correlation and negative relationship between pH and conductivity coincides with results from previous studies (Oliva-Paterna *et al*., 2003a and 2003b).

Summarizing, the combination of variations in water level (seasonal water flow) and pH explain the variability in barbel condition at the Guadiamar River, with other related variables such as fish density (intra-specific density), landscape attributes (QBR), and water quality (IBG, IBMWP and conductivity) being of potential importance. The highest body condition values were found in stretches where individuals are concentrated in isolated pools, and this suggests that the remnants of the spill stop barbels form thriving in lower stretches with potentially better habitat conditions. pH values are also still significantly lower in the affected area, and this reinforces the conclusion that the variation in barbel condition at the Guadiamar River is determined, mainly, by whether they inhabit the affected area or not. Therefore, we conclude that fifty-six months after the accident, the environmental requirements needed to harbour a healthy barbel population in the Guadiamar River basin have not been reached yet.

Acknowledgements

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II.2- Habitat-fish community relationships in the Guadiamar River basin (SW Spain) 8 years after a toxic spill: is the spill still important?

Abstract

The Guadiamar River basin (SW Iberian Peninsula) suffered one of the most environmentally harmful toxic spills worldwide on April 25th 1998, when a tailing pond broke and released 4 m^3 of acid water and 2 m^3 of mud rich in heavy metals. Immediately, removal works started and a recovery program was designed for the affected area. Eight years later, every stream in the catchment was sampled to find the most influential variables for native and exotic fish, and to establish whether the spill is still exerting an influence on the fish community. GLMs were performed to analyze the relationship between fish species richness and diversity and environmental variables. The remaining effect of the toxic spill was assessed by comparing Guadiamar basin values with those of six similar basins that were not affected by the spill. Native fish of the Guadiamar basin prefer environments with low human influence, locations far away from reservoirs, a large drainage area and the presence of natural shelters in the river channel. For native fish, variables at both the catchment and site scales were equally relevant. Exotic fish were mainly favoured by site-scale factors downstream from the reservoir, where the alteration of the river channel and accumulated disturbances give them an advantage versus natives. In summary, eight years after the accident richness and diversity of the Guadiamar fish community are more influenced by anthropogenic impacts than by the long-term influence of the toxic spill. This work highlights that studies that aim to monitor the long-term effects of pollution events should take into account the previous and current impacts of other anthropogenic factors, such as upstream reservoirs or humanized land uses.

Introduction

On April 25th 1998, a 50-meter breach opened in the tailing pond dike of the Aznalcóllar mine (SW Spain). This breach caused the release of about 4 hm^3 of acidic water with dissolved metallic compounds and 2 hm^3 of mud mainly composed by floated pyrite (Aguilar *et al.*, 2003). To stop the spill from reaching Doñana National Park, downstream, several dams were constructed (López-Pamo *et al.*, 1999). Nevertheless, 67 km of the Guadiamar River's main channel were polluted with toxic spill whose primary composition was Fe (34-37%), S (35-40%), Zn (0.8%), Pb (0.8%), As (0.5%), Cu (0.2%), Sb (0.05%), Co (0.006%), Tl (0.005%), Bi (0.005%), Cd (0.0025%), Ag (0.0025%), Hg (0.001%) and Se (0.001%) (Grimalt and Macpherson, 1999). As a consequence, approximately 2000 ha of riparian vegetation forests were contaminated (Murillo *et al.*, 1999), causing the death of 170 kg of red swamp crayfishes and nearly a hundred different vertebrates (Aguilar *et al.*, 2003). Fish were the most affected vertebrates, and during the days following the accident 37.4 t were collected from different areas, including carps (75-80%), mullets (10-16%), barbels (6-8%), eels (4%) and other species (5%) (Valls and Blasco, 2005). Unfortunately, the coarse mechanical removal of contaminants from the stream and flood plain aggravated the effects of the toxic spill, with major implications for the geomorphological characteristics of the river (Gallart *et al.*, 1999). After these cleanup operations in the affected area, a Recovery Plan (PICOVER) was implemented, aimed at repairing the damaged ecosystems and transforming the affected area into a green corridor between two well conserved ecosystems: Sierra Morena in the north and Doñana National Park in the south (Cárdenas and Hidalgo, 2007; Márquez-Ferrando, 2009). The dimensions and effects of heavy metal contamination resulting from the spill were immediately studied in different environments such as soil (Cabrera *et al.*, 1999), groundwater (Manzano *et al.*, 1999), and sediments and water (Van Geen *et al.*, 1999). The consequences for biota within or around the affected area were also readily investigated, focusing on plankton, periphyton and macroinvertebrates (Prat *et al.*, 1999); molluscs, crustaceans and fish (Blasco *et al.*, 1999; Drake *et al.*, 1999); plants (Murillo *et al.*, 1999) and birds (Hernández *et al.*, 1999; Benito *et al.*, 1999). Over the years, several studies have monitored the recovery of previous abiotic and biotic states (e.g., soil, Fernández *et al.*, 2007; water, Olias *et al.*, 2005; vegetation, Madejón *et al.*, 2010;

coleopteran communities, Cárdenas and Hidalgo, 2006; ant communities, Luque *et al.*, 2007; crayfish, Alcorlo *et al.*, 2006; reptile community, Marquez-Ferrando *et al.*, 2009; mice, Bonilla-Valverde *et al.*, 2004; otters, Delibes *et al.*, 2009; etc.), providing different results about the remaining spill influence. Unfortunately, few studies have focused on monitoring the recovery of the fish community (but see Fernández-Delgado and Drake, 2008). To the best of our knowledge, this is one of the first studies that address the recovery of the fish community in the Guadiamar River and its relationships with habitat conditions several years after the toxic spill.

Fish community structure is determined by a wide range of biotic and abiotic processes, and community composition changes over time and space (Magalhaes *et al.*, 2002a, 2002b; Blanchet *et al.*, 2009). Community relationships with these processes should be considered at different scales, since fish have both local and catchment mobility (Tolonen *et al.*, 2003; Pinto *et al.*, 2006). The recovery of aquatic systems from anthropogenic impacts depends not only on revealing the causes of impairment, but also on anticipating their responses to stressors operating at multiple scales (Ferreira *et al.*, 2007). Recent attempts to meet these needs include studies identifying ecological indicator metrics (e.g., Karr, 1991; Wright, 1995; Barbour *et al.*, 1999; Norris and Hawkins, 2000; Hermoso *et al.*, 2010) and efforts comparing the effects of anthropogenic land use at various scales, from the stream reach to the entire basin (e.g., Roth *et al.*, 1996; Lammert and Allan, 1999; Sponseller *et al.*, 2001; Wang *et al.*, 2001; Wasson *et al.*, 2010). Consequently, it is important to consider the scale at which habitat variables are recorded (Allan *et al.*, 1997a, b; Allan, 2004; Pinto *et al.*, 2006; Hughes *et al.*, 2009; Hermoso *et al.*, 2010), and thus identify the spatial scale at which the most important variables for the community are acting.

In this study, the main aims were 1) to identify the main environmental variables that currently determine both native and exotic fish species richness and diversity in the Guadiamar River basin at different scales, and 2) to assess whether the toxic spill can still be considered influential for fish richness and diversity eight years after the accident.

Materials and Methods

Study area

The Guadiamar River basin is located in the South-western Iberian Peninsula at latitude 37º 10' to 37º 45' N and longitude 6º 10' to 6º 25' W, near the Guadalquivir River mouth in the Atlantic Ocean (Fig. 1). The basin area is 1.325 km^2, and altitude ranges from 4 to 544 m.a.s.l. Climate is sub-humid Mediterranean with oceanic influences, with average temperatures between 9ºC in winter and 29ºC in summer. Mean annual rainfall is 624 mm, oscillating between 754 mm in the source and 543 mm in the mouth. Rain falls abundantly in autumn, winter and spring and is completely absent in summer. This severe drought causes the drying of most small streams or the creation of isolated pools of different sizes (Gasith and Resh, 1999). The basin shows a geological transition linked to river section type. The upper section (near the source) runs between narrow and steep valleys formed by Palaeozoic slates and quartzite crests. The middle section crosses quaternary alluvial deposits, loams, sands and edge limestone rolling hills. Finally, the lower section flows through clay and lime deposits forming a marshland. Pine forests (*Pinus* spp.) and dehesas (cleared, savannah-like *Quercus* spp. Mediterranean forest) are the predominant vegetation in the upper section; olive groves, cereal fields and vineyards form a mosaic in the middle section; and the marsh starts in the lower section, close to Doñana Natural Park, dominated by *Salicornia* spp. shrubs, and pastures with *Sylibus marianum*, *Hordeum murinum* and *Plantago coronopus*. The hydrological network is disrupted by three dams (Fig. 1). Two small ones that collect less than 4 hm^3 in the source area and one large reservoir (20 hm^3) in the Agrio River, slightly upstream from the spill point (Borja *et al*., 2001).

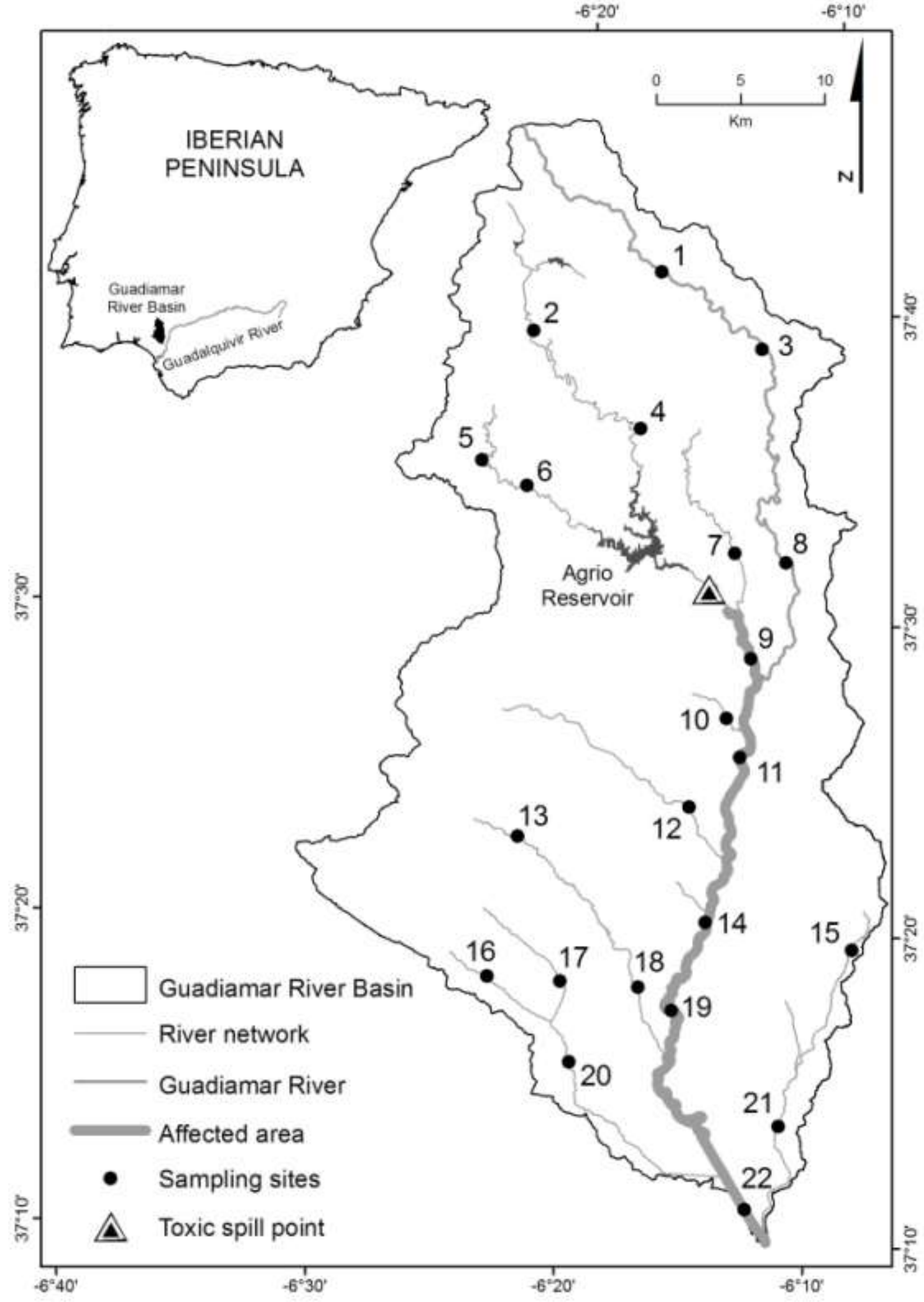


Figure 1. Location of the Guadiamar River Basin and the 22 sampling sites.

Sampling protocol

The sampling period was divided into three campaigns: winter (2006) for the low flow streams and spring and summer (2007) for the high flow courses that were not wadeable during the rainy period (winter). In order to cover the whole perennial stream network (except the marshland), 22 sampling sites were selected.

Using a geographic information system and high-resolution maps provided by the Andalusian Government, the perennial stream network (291.94 km) was split into hydrological fragments (part of a stream with similar hydrological features, located between two tributaries or large disruptions). These hydrological fragments were divided into approximately 10 km segments, and finally, every segment was divided into high, mid-slope or lowland areas, and one sampling site was allocated to each of these areas. The exact sampling site location was finally chosen in the field, whenever a wadeable reach was found within the selected area. Fish abundance at each sampling site was estimated by electrofishing (220 V, 2-5 A, C.C.). Backpacking (1300 W) was the selected procedure for 1 m depth and 5 m width reaches. A more powerful engine fixed to the riverbank (2400 W) was needed in order to maintain the same sampling effort on larger reaches (Lobón-Cerviá, 1991). Caught fish were identified to species and released into the stream again. Sampling effort was constant, collecting the maximum number of individuals in one sweep.

Habitat data were collected at two different scales: catchment (considered as the land surface covered by all permanent streams that drain onto a sampling site) and site (the stream section where fishing takes place and field data are recorded). Throughout the paper, both catchment and site will refer to scale, whereas basin, watershed, reach and stretch are used to indicate specific areas. A total of 71 environmental variables thought to be relevant for fish species richness and diversity were recorded at each sampling site, by means of different methods (see Fernández-Delgado *et al.*, 2010 for further details) and at different sampling scales (Appendix 1). In several cases, variables were summarized by means of PCAs (see Statistical analyses section and Appendix 1).

All catchment scale data and several site scale data were extracted from digital maps provided by the Andalusian Government, using ArcGis® software (see Fernández-Delgado *et al.*, 2010).

Statistical analyses

In order to identify the main factors that determine species richness (S) and diversity (H’, Shannon’s diversity index; Shannon and Weaver, 1949) in the Guadiamar River basin General Linear Models (GLM) were used. Predictor variables were considered at two different scales: catchment and site. First, a general model was created using all variables at both scales, including their interactions. This model would reveal the scale at which the most important factors for fish diversity are acting. Second, two further models were derived, one for each scale, in order to find out which were the most relevant drivers at that particular scale.

The following dependent variables were considered: native species richness (S-na); native diversity (H-na); exotic species richness (S-ex). Three models were computed for each dependent variable: 1) general model (with predictor variables recorded at both scales); 2) catchment model (with predictor variables recorded at the catchment scale); 3) site model (with predictor variables recorded at the site scale). The diversity of exotic species was not considered for analyses because they were absent in many of the sampling sites, resulting in a large proportion of zeroes and, as a result, a weak model. Therefore, a total of 9 models were computed (3 for each dependent variable).

A priori variable selection was carried out due to the large number of predictor variables (see Appendix 1), their collinearity and ecological redundancy, and parsimony considerations. First, some groups of related variables that implied ecological redundancy were summarized by means of PCAs. In all cases, the Kaiser (1960) criterion (eigenvalue >1) was used to define the principal components to be chosen as the final variables.

PCA 1 – Habitat characteristics at the site scale. This included variables recorded in the field, at each sampling site, which describe the local habitat conditions, as well as the physicochemical characteristics of water (Appendix 1). The analysis yielded two axes with eigenvalue >1 that accounted for 31.5% (axis 1) and 23.7% (axis 2) of the total variance. The first axis, which represents a gradient from pools and fine material to riffles and coarser substrates, was used for analyses. This axis reflects the common gradient in running water systems, from areas with slow-running water with pools where fine material accumulates (negative end of the axis) to areas with boulders and other coarse substrates and fast-running water (positive end).

PCA 2 – Factors affecting bank stability at the site scale. This analysis included variables that inform about the erosion risk at a given site (Appendix 1). There was one principal component that accounted for 26.8% of total variance. This axis represented a gradient from highest to lowest risk of erosion (greatest risk at the positive end and lowest at the negative end).

PCA 3 – Land uses at the site scale. This analysis included data about land uses in the area right next to the sampled site (Appendix 1). It yielded a main principal component that accounted for 37.5% of total variance. This axis represented a gradient from greater human impact (urban and agricultural areas at the positive end) to less humanized uses (native forests at the negative end).

PCA 4 – Land uses at the catchment scale. This PCA represents the main land uses in the whole catchment area of each sampling site (% of each type of land use, Appendix 1). It yielded one principal component that accounted for 40.3% of total variance. This axis represented a gradient from greater human impact (urban and agricultural areas at the negative end) to less humanized uses (native forests at the positive end).

The second step to reduce the number of variables was to test for collinearity between the remaining ones (Appendix 1) using Pearson´s correlations. Whenever the correlation coefficient between two variables was greater than 0.75, one of the variables was chosen for the regression models.

Once the final predictor variable list was defined (Table 1), preliminary regression models were computed and their residuals were analyzed for outliers and/or influential cases (Cook, 1979). No outliers were detected in the dataset, so the final regression models were applied to a total of 22 cases (n=22) and a maximum of 6 predictor variables, since a larger number of variables would lead to a Type 2 error (Field, 2005). The final maximum of 6 variables for each model included those that showed a high correlation with the relevant dependent variable and low collinearity with the other selected variables. A list of all the variables included in the 9 models is presented in Table 1.

The final models were fitted with a normal error distribution and an identity link function. The best models supported by the data were selected using the Akaike Information Criterion (AIC), a model selection approach based on Information Theory (Burham and Anderson, 2002). This allowed us to rank a set of competitive models and decide which explained the most variance whilst being most parsimonious (Johnson and

Omland, 2004). Variance partitioning of the significant variables selected for the general model was performed to identify the most important scale in each case. The potential influence of spatial autocorrelation was tested using a matrix of the reticular distance between all sampling points with each other, and calculating Moran's I of the residuals of preliminary regressions. Moran's I value obtained was always <0.05 (p >0.05), therefore spatial autocorrelation was unlikely to influence the model results. This analysis was carried out using SAM (Spatial Analyses in Macroecology, Rangel *et al.*, 2006). All other analyses were performed using R version 2.12 and packages: vegan, hier.part, gtools and asbio (R Development Core Team, 2010).

In order to assess whether the spill had a relevant influence on species richness and diversity in the Guadiamar River basin eight years after the accident, a binomial variable "Spill" was initially included in models, indicating whether the sampling site was located within the originally affected area (1) or not (0). However, since the spill point is located 5 km downstream from the main reservoir in the Agrio River (Agrio reservoir hereafter), there is an almost complete overlap between the affected area and that influenced by the reservoir, and these two factors are expected to have a negative influence on richness and diversity. In addition, both variables (Spill and Number of reservoirs upstream) are collinear (almost 90%), and only two sampling sites under reservoir influence are upstream from the spill point (Spill = 0). Therefore, the spill influence was assessed using a different approach. Species richness and diversity values downstream from the Agrio reservoir were compared with richness and diversity values downstream of reservoirs from similar watersheds within the Guadalquivir river basin that were not affected by the spill. It was expected that if the spill still exerts a significant influence for the fish community, values from the Guadiamar River basin would be lower than those from other watersheds. For this purpose, data from an ongoing survey carried out for the whole Guadalquivir River basin that uses the same fishing methods (Fernández-Delgado *et al.*, 2010) were used. Six basins with similar reservoirs and environmental characteristics were chosen (Cala, Pintado, Huesna, Montoro, Rumblar and Fernandina) (Fig. 2). Fish richness and diversity data from points located in reaches downstream from the main reservoir in each of these basins were compared with values downstream of the Agrio reservoir using ANOVA. This analysis was also carried out using R 2.12 (R Development Core Team, 2010).

Table 1. Selected variables and regression model results for native (-na) and exotic (-ex) fish species richness (S) and diversity (H) at the catchment (_C) and site (_S) scales. The first column shows the variables selected after testing for collinearity. S.E. = standard error, p = probability, Adjusted R^2 = coefficient of determination, Pr(>|t|) = significance.

	Selected variables for the model	Significant variables selected by models and percentage of variance explained after variance partitioning	Adjusted R^2	p	Estimate (coefficient)	S.E.	Student t_value	Pr(>|t|)
S-na	PCA4 -Land uses at catchment scale, No. reservoirs upstream, Drainage area, PCA1-Habitat, Distance from mouth and Riparian vegetation width	PCA4-Land uses at catchment scale (33%) No. reservoirs upstream. (28%) Drainage area (20%) PCA1-Habitat (19%)	0.70	0.0000361	0.673360 -1.986970 0.006492 0.383857	0.161439 0.345598 0.001301 0.151632	4.171 -5.749 4.991 2.532	0.000641 *** 0.000023 *** 0.000112 *** 0.021512 *
S-ex	PCA4-Land uses at catchment scale, Drainage area, PCA1-Habitat, Riparian vegetation width, Mean valley width and River length covered by reservoirs upstream	Mean valley width	0.42	0.0007046	0.0005621	0.0001406	3.999	0.000705 ***
H-na	PCA4-Land uses at catchment scale, No. reservoirs upstream, Drainage area, PCA1-Habitat, Distance from mouth and Riparian vegetation width	PCA4-Land uses at catchment scale (46%) No. reservoirs upstream (31%) Drainage area (23%)	0.52	0.0009089	0.2182641 -0.5225907 0.0016197	0.0474672 0.1198613 0.0004264	4.598 -4.360 3.798	0.000223*** 0.000377*** 0.001316**
S-na_C	PCA4-Land uses at catchment scale and Drainage area	PCA4-Land uses at catchment scale	0.23	0.01297	0.4792	0.1757	2.728	0.0130 *
H-na_C	PCA4-Land uses at catchment scale and Drainage area	PCA4-Land uses at catchment scale	0.11	0.06885	0.10043	0.05223	1.923	0.0689 .
S-na_S	No. reservoirs upstream, PCA1-Habitat, Distance from mouth, Riparian vegetation width, PCA3-Land uses at site scale, Distance to source	PCA1-Habitat (45%) Distance to source (23%) No. reservoirs upstream (21%) PCA3-Land uses at site scale (11%)	0.53	0.001565	0.6634 0.00008422 -1.463 -0.4129	0.1481 0.00002134 0.3837 0.1879	4.480 3.946 -3.812 -2.198	0.000329 *** 0.001042 ** 0.001394 ** 0.042109 *
S-ex_S	PCA1-Habitat, Riparian vegetation width, Mean valley width and River length covered by reservoirs upstream (RLCRU)	Mean valley width (55%) RLCRU (45%)	0.53	0.00002841	0.0004093 0.00004679	1.406e-04 0.00001920	2.911 2.437	0.00896 ** 0.02483 *
H-na_S	No. reservoirs upstream, PCA1-Habitat, Distance from mouth, Riparian vegetation width, PCA3-Land uses at site scale, Distance to source	Distance to source (54%) No. reservoirs upstream (46%)	0.35	0.04623	0.00002708 -0.4311	0.0000748 0.1263	3.620 -3.412	0.00252 ** 0.00386 **

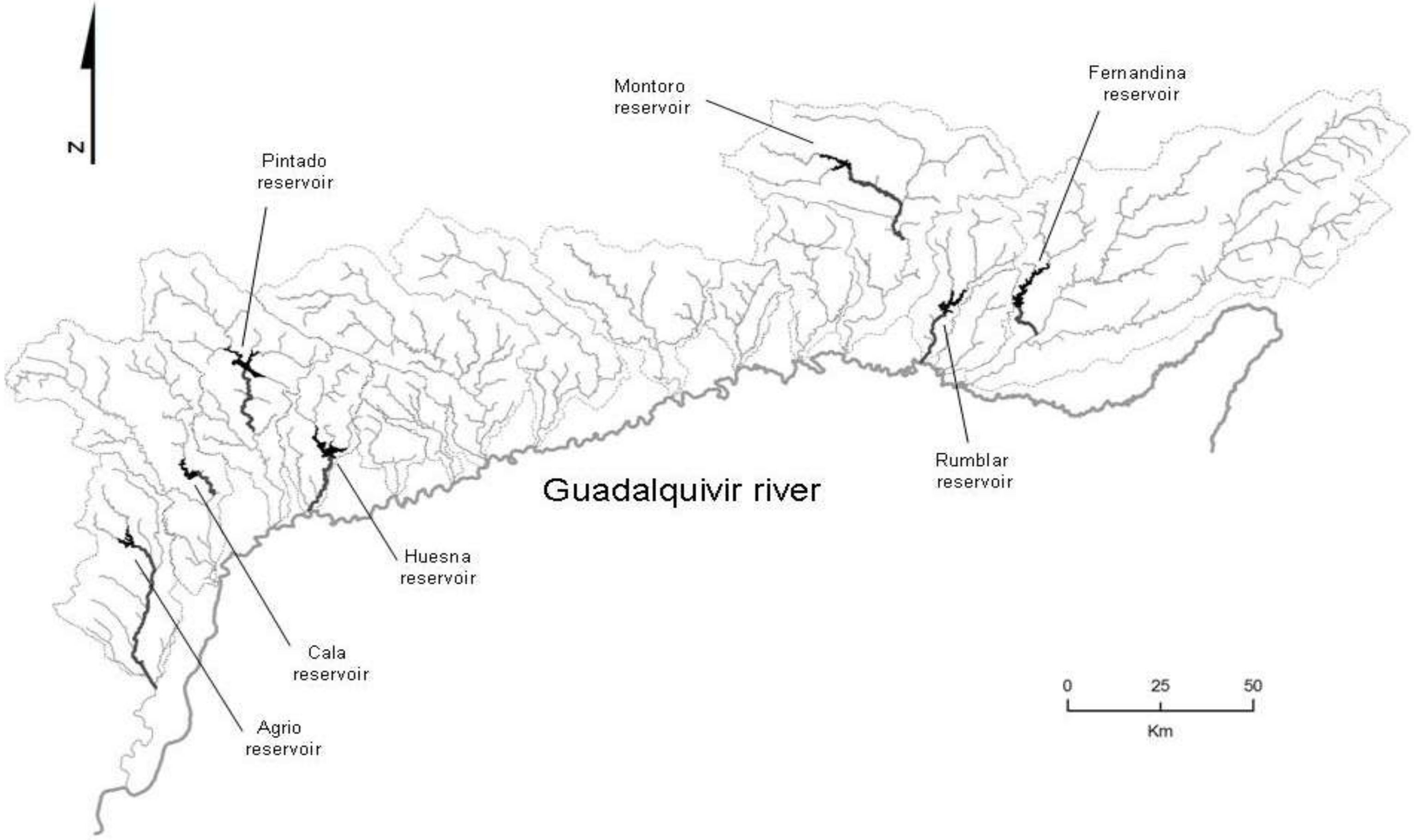


Figure 2. Tributary network of the Guadalquivir river (northern side). Stretches in bold show the six reservoirs and their downstream reaches selected to compare with the values for the Agrio reservoir and its downstream reach (first from the left).

Results

Fish community

The following 9 native species were found in the study area: Southern Iberian barbel, *Luciobarbus sclateri* (Günther 1868); Southern straight-mouth nase, *Pseudochondrostoma willcommii* (Steindachner 1866); Calandino, *Squalius alburnoides* (Steindachner 1866); Southern Iberian chub, *Squalius pyrenaicus* (Günther 1868); Iberian arched-mouth nase, *Iberochondrostoma lemmingii* (Steindachner 1866); Southern Iberian spined-loach, *Cobitis paludica* (de Buen 1930); European eel, *Anguilla anguilla* (Linnaeus 1758); Thinlip grey mullet *Liza ramada* (Risso 1826) and Flathead grey mullet, *Mugil cephalus* (Linnaeus 1758).

In addition, 5 exotic species were found: Carp *Cyprinus carpio* (Linnaeus 1758); Prussian carp, *Carassius gibelio* (Linnaeus 1758); Pumpkinseed, *Lepomis gibbosus* (Linnaeus 1758); Eastern mosquitofish, *Gambusia holbrooki* (Agassiz 1859) and Largemouth bass, *Micropterus salmoides* (Lacèpéde 1802).

Native species

a) General models

Significant models were obtained for native species richness (S-na) and diversity (H-na), which accounted for 70% (R^2 = 0.70) and 52% (R^2 = 0.52) of the variance, respectively. The main factors included in the best models were similar for S-na and H-na (Table 1). These models identified PCA4 ("Land use at the catchment scale") and "Number of reservoirs upstream" as the most influential factors. Variance partitioning using *hierpart* showed that they accounted for 33% and 28% of the explained variance, respectively, in the case of S-na; and 46% and 31%, respectively, in the case of H-na. A positive relationship was found between PCA4 and both dependent variables, and this means that higher native fish richness and diversity are found in natural forest areas with respect to those with agricultural or urban land uses. In contrast, a negative relationship with "Number of reservoirs upstream" was found, which indicates that the more reservoirs upstream from a site, the lower the native fish richness and diversity. "Drainage area" and PCA1 ("Habitat characteristics at the site scale") were a second group. These variables accounted for 20% and 19% of the explained variance, respectively, for S-na. For H-na, "Drainage area" explained 23% of the variance. A positive relationship with S-na and H-na was observed for "Drainage area", which simply reflects the species-area relationship that occurs as you go downstream: drainage area increases and so does the number of species found. Axis 1 of PCA1 showed also a positive relationship with S-na, indicating that S-na is higher in those reaches with coarser substrate and clearer water.

Variance partitioning performed with the significant variables at both site and catchment scale accounted for a similar overall proportion of the variance (0.51 and 0.55, respectively) for S-na, and also for H-na (0.45 and 0.55, respectively). This means that both scales are equally important for native fish richness and diversity, so further models were developed including only variables measured at each scale.

b) *Catchment models*

As expected given the general results, when only variables at the catchment scale were considered, significant models were obtained for native species richness (S-na_C) and diversity (H-na_C), which accounted for 23% ($R^2 = 0.23$) and 11% ($R^2 = 0.11$) of the variance, respectively (Table 1). Again, the main axis of PCA4 (“Land use at the catchment scale”) was selected as the main driver for S-na_C and H-na_C. This positive relationship suggests that, at a wide scale, native fish richness and diversity are higher in areas where land uses are more natural.

c) Site models

Similarly, when only variables at the site scale were included in the models, they were significant for native species richness (S-na_S) and diversity (H-na_S), accounting for 53% ($R^2 = 0.53$) and 35% ($R^2 = 0.35$) of the variance, respectively (Tables 1). The main axis of PCA1 (“Habitat characteristics at the site scale”) was identified as the most influential factor for S-na_S and variance partitioning showed that it accounted for 45% of the explained variance, whereas it was not selected in the case of H-na_S. This positive relationship between the main axis of PCA1 and S-na_S reinforces the same trend described for the General model (native richness is higher in those reaches with coarser substrate and clearer water). “Distance to source” however, was the most important factor for H-na_S and the second most important for S-na_S. According to variance partitioning, this factor accounted for 54% and 23% of the explained variance, respectively. This positive relationship between “Distance to source” and H-na_S and Sna_S, reflects a similar explanation to that suggested for “Drainage area” in the General model, showing the species-area relationship that occurs as you go downstream: distance to source increases and so does the number of species found.

“Number of reservoirs upstream” was selected as the second most important factor for H-na_S and the third for S-na_S, accounting for 46% and 21% of the explained variance, respectively. This negative relationship concurs with that observed in the General model and reinforces the idea that the more reservoirs upstream from a site, the lower the native fish richness and diversity. The last variable selected by the model at the site scale was the main axis of PCA3 (“Land uses at site scale”), which accounted for 11% of the explained variance for S-na_S. In this axis human impact is located at the positive end, so this negative relationship shows how, at the site scale, native fish richness is lower in areas where land uses are more humanized, the same trend as that observed in the General and catchment-scale models.

Exotic species

a) General model

A significant model was found for S-ex (Table 1). “Mean channel width” was the most influential variable, accounting for 42% of the variance ($R^2 = 0.42$). This positive relationship suggests that exotic fish richness in the Guadiamar River is greater in the wider valleys of the lower sections of the river, away from the narrow valleys near the source. The final model included only this variable, measured at the site scale, which suggests that this is the most important scale for exotic species richness. Therefore, only a more detailed site model was computed for exotic species richness.

b)Site model

The model for exotic species richness that included only variables at the site scale (Sex_S) was significant and explained 53% of the variance ($R^2 = 0.53$) (Table 1). As in the General model, “Mean valley width” was identified as the most influential variable, followed by “River length covered by reservoirs upstream” in this case, accounting for 55% and 45% of the explained variance, respectively. The positive relationship between “Mean valley width” and exotic species richness confirms the results of the General model, an increase in the number of exotic species as the channel becomes wider further away from the source. The other positive relationship (“River length covered by reservoirs upstream”) shows how exotic species are linked to reservoirs upstream.

Spill Effect

The ANOVA and post-hoc t-tests between reaches downstream from the Agrio reservoir and each one of the six selected reservoirs, showed significant differences in native species richness between the selected Guadiamar reach and reaches downstream from three reservoirs (Huesna, Rumblar and Fernandina), whereas there were no differences with three others (Cala, Pintado and Montoro) (Table 2). In addition, no significant differences were found between native species diversity downstream from the Agrio reservoir and any of the other six selected reaches. Similarly, no significant differences were found for exotic species.

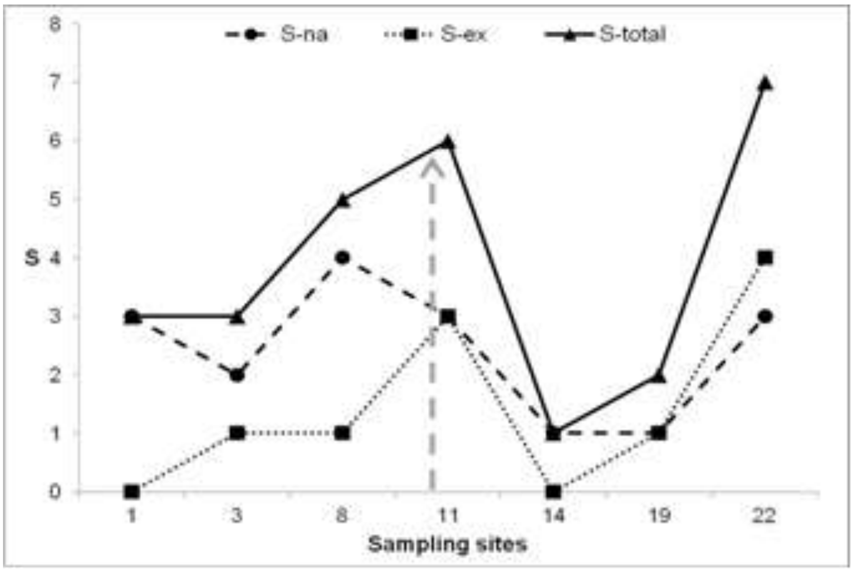
Table 2. p-Values for t-test of native (-na) and exotic (-ex) richness (S) and diversity (H) values between the reach downstream from the Agrio reservoir and reaches downstream from reservoirs in 6 other basins with similar environmental characteristics to the Guadiamar. (* $p < 0.05$).

	Cala p	**Pintado p**	**Huesna p**	**Montoro p**	**Rumblar p**	**Fernandina p**
S-na	0.07	0.02*	0.17	0.65	0.04*	0.02*
H-na	0.95	0.08	0.48	0.82	0.26	0.26
S-ex	0.23	0.06	0.31	0.47	0.46	0.49
H-ex	0.11	0.11	0.55	0.66	0.34	0.35

Fish assemblage longitudinal variation

Figure 3 shows how both native fish richness and diversity increased with distance from the source and as the size of main river channel increases. However, when the river reaches the stretch affected by the Agrio reservoir, which is also the area affected by the spill, this native species trend is interrupted. The highest total richness and diversity values were found in this area due to the incorporation of exotic species from these disturbed stretches. Downstream, beyond the reservoir and the area affected by the spill, the lowest total richness and diversity values were detected. Progressively, total richness and diversity values increased again as the river reaches its mouth.

a)



b)

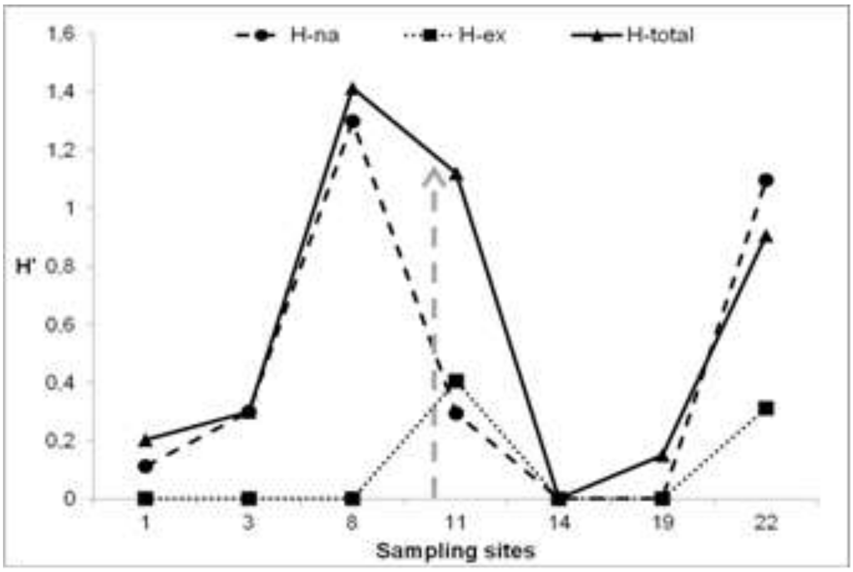


Figure 3. Richness (a) and diversity (b) at the seven sampling sites located in the Guadiamar River main channel. The segmented grey arrow indicates the confluence with the Agrio river (where both reservoir and spill impact start to exert their effects on the Guadiamar main channel). In both figures, the segmented line represents native species (S-na or H-na); the dotted line represents exotic species (S-ex or H-ex); and the sum of native plus exotic species (S-total or H-total) is given by the solid line.

Discussion

Results revealed the main environmental variables that currently influence the Guadiamar River fish community. Richness and diversity followed similar trends, whereas differences were found at the site and catchment scales between native and exotic species. It was difficult to determine whether there is still an influence from the spill on these parameters, since there were no significant differences between Guadiamar data and data from other watersheds, and the Agrio reservoir exerted a confounding effect.

Indeed, there was a strong influence of a catchment-scale factor such as land use on the native species community of this basin. In accordance with other authors (e.g., Corbacho and Sánchez, 2001; He *et al.*, 2010), more natural areas present higher native species richness and diversity than those with some human impact (agricultural or urban land uses). This is probably because the life cycle requirements of the fish species considered are not fulfilled in areas with increasing denaturalisation of environmental conditions (Blanck *et al.*, 2007; Hughes *et al.*, 2010). Deforestation at both the catchment scale and of local riparian vegetation due to agricultural practices decreases shelter availability in riverbanks and increases erosion and water turbidity (Aguiar and Ferreira, 2005). Furthermore, urban land uses raise the organic load through sewage discharges, thus reducing the concentration of oxygen in the water (Ferreira *et al.*, 2005). The extent of these effects will determine the presence or absence of certain species and therefore, affect the overall diversity of the fish community.

At the site scale, the presence of reservoirs upstream acts as the other main influence for native richness and diversity, representing a pivotal point for fish distribution in the basin under study. According to variance partitioning, upstream reservoirs are even more important than the well-known species-area relationship trend of higher richness with greater drainage area (McArthur and Wilson, 1967; Sheldon, 1988), which is evident near the Agrio reservoir (Fig. 3). At this point, however, the trend is reversed and downstream from the dam, native species richness decreases dramatically. This decline is due to the artificial conditions of the reach immediately downstream from the reservoir, such as the absence of necessary habitat elements and constant predation exerted by the exotic species after dam release periods (Moyle and Leidy, 1992; Clavero and Hermoso, 2011).

In addition, and in agreement with Ferreira *et al*. (2007), the analysis shows how at the site scale, native species prefer coarser substrates and fast-flowing water. This preference reflects the typical conditions of natural areas where, first, a coarser substrate indicates absence of fine material overload from agricultural erosion, reservoir deposits upstream or urban pollution (Doadrio, 2001); and secondly, calm waters are found in higher proportion in the middle and lower sections of the river, where the increase of both habitat degradation and exotic species has been cumulative, creating an unsuitable environment for native species (Ferreira *et al*., 2007).

Regarding exotic species in the Guadiamar River basin, results confirm that the number of exotic species increases from the source to the mouth of the river (Moyle and Light, 1996a, 1996b; Gido and Brown, 1999; Kopp *et al*., 2009). This is probably due to the accumulation of pernicious effects as the river reaches its lower section (Sheldon, 1988; Corbacho and Sánchez, 2001), accumulating exotic individuals from upstream reservoirs and those going upstream from the mouth (Ruiz, 1998; Clavero *et al*., 2004). Moreover, in the Guadiamar River basin, initial habitat degradation after the spill favoured the rapid colonization of exotic species (Olias *et al*., 2005; Fernández *et al*., 2007). Toxic mud removal works inevitably caused the elimination of important natural elements for native species such as riparian vegetation or rocky shelters, leaving an altered area where exotic species, generalists and better adapted to degraded zones (Corbacho and Sánchez, 2001), have established more successfully than natives.

Exotic species establishment in the Guadiamar River basin is a consequence of fishermen and government introductions for sport fishing (Fernández-Delgado, 2003). The reservoir therefore becomes a source of exotic species, but their dispersal is not homogeneous along the river course. Downstream colonization is more effective than upstream, since individuals barely go upriver towards the source streams. This asymmetrical movement may have a twofold explanation. First, the exotic species in the Guadiamar River basin posses either a flattened body adapted to lentic ecosystems, such as centrarchids and cyprinids, or a small size, such as the eastern mosquitofish. Both body shapes have not evolved to be efficient in dealing with upstream colonisation of the turbulent streams that fill the reservoir (Bernardo *et al*., 2003), while the fusiform native species find no problems to overcome these currents and even use upstream areas as spawning sites (Hubbs, 1940; Nikolsky, 1963; Herrera and Fernández-Delgado, 1992). The second cause may also be related to the adaptation process of exotic species to the stable conditions of the water bodies where they originally inhabit (Elvira and

Almodóvar, 2001). These stable conditions can be found in reservoirs and their regulated downstream tailwaters, but reaches immediately upstream suffer large fluctuations with strong flows during rainy periods and drought during summer, so they are inappropriate environments for these exotic species (Moyle, 1986; Maghalaes *et al*., 2002 and 2007).

Unfortunately, the attempt to discern between spill and reservoir effects did not yield a clear result, but suggests a combination of events. The observed native species richness and diversity depletion caused by reservoirs in other river basins, similar to Guadiamar, provides a range of values, and those observed in the Guadiamar River fit within that range. Therefore, the current potential effects of spill remnants are not strong enough to cause abnormal fish species richness and diversity values. The analysis performed with exotic species yields the same result, which suggests that the set of factors that promote exotic species richness in the Guadiamar River basin are equal to those found in other similar river basins, not affected by the spill. This conclusion suggests that eight years after the accident, the habitat recovery works have minimized the spill effect and the reach originally affected is now exposed to the same impacts that it suffered before this event.

Finally, with respect to the main aspects of the Guadiamar River basin fish community, results agree with several studies carried out for other Mediterranean watersheds (Godinho and Ferreira, 1998 and 2000, Moran-López *et al.*, 2006) that report a pivotal point with the highest species richness and diversity in the area just upstream from a reservoir. In this study, there is a steady increase from the source in parallel to river order and suddenly, downstream from the reservoir, the trend is interrupted. Thus, the highest values are observed at the last sampling site upstream from the reservoir. This could be because the reservoir may also acts as a refuge from drought for several native species during the dry season (Schlosser, 1987; Rodríguez-Ruiz and Granado-Lorencio, 1992; Poff *et al*., 2007) and although the exotic species found in the reservoir have a limited ability to swim large distances against the main current, they may be attracted to a nearby area upstream, rich in nutritional resources (mainly native species juveniles). In consequence, both native and exotic fish coincide in a complicated equilibrium that results in maximum in richness and diversity at a specific place.

In summary, the native species of the Guadiamar River basin are favoured by environments with low human influence, locations far away from reservoirs, a large drainage area and the presence of natural shelters in the river channel; therefore, both catchment and site scale approaches must be taken into account when relevant factors for native species are addressed. On the contrary, exotic species thrive mainly due to site-scale factors downstream from the reservoir, where the alteration of the river channel and accumulated disturbances as the river flows towards the mouth give them an advantage versus natives. The disruption that the toxic spill causes after eight years cannot be considered more important than other human disturbances acting on this watershed and on the other biogeographically similar watersheds considered. Currently, the Agrio reservoir seems to be the main disruptor of the natural fish community dynamics in the Guadiamar River basin. This work highlights that studies that aim to assess or monitor similar accidents should take into account the previous and current impacts of other anthropogenic factors, such as upstream reservoirs or humanized land uses.

Acknowledgements

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PARTE III:

Evolución de la comunidad de peces del Guadiamar 13 años después del vertido tóxico.

III.1- Recolonization process and fish assemblage dynamics in the Guadiamar River (SW Spain) after the Aznalcóllar mine toxic spill.

Abstract

The fish assemblage recovery process in the Guadiamar River (SW Iberian Peninsula) was monitored over 13 years. This river received a major toxic spill (6 hm^3) from a tailing pond in 1998 that defaunated 67 km of the main stem. Following early mud removal works, the fish assemblage was annually monitored at four affected sampling sites and one located in the upstream non-affected reach of the Guadiamar River as reference. Comparison with pre-disturbance using Jaccard's similarity index, fish richness, diversity, species abundance and assemblage structure were analyzed and a principal response curve (PRC) was applied to assess the recovery trends and to identify the dominant species. A non-metric multidimensional scaling (NMDS) ordination and PERMANOVA were applied to evaluate changes in fish assemblage structure between sites and years.

Overall, the affected reaches harboured fish within two years of the spill. Colonists arrived mainly from the upstream and downstream non-affected Guadiamar River reaches, and to a lesser extent from three lateral tributaries. It is likely that the proximity, connectivity and environmental conditions of non-affected fish sources greatly influenced the recolonization process in each site.

The structure of the fish community in the affected sites was initially similar to that in the unaffected reference stretch, but changed dramatically with time and each site followed its own trajectory. Currently, long-term threats such as mining leachates, urban sewage, agricultural pollution and exotic fish species expansion, have probably exceeded the initial spill effect. This highlights the large effect of anthropogenic factors

on freshwater ecosystem resilience, and the need to significantly reduce both pollution and exotic species if the affected reach of the Guadiamar River is to recover fully.

Introduction

An ecological perturbation consists of an initial natural or anthropogenic disturbance and a subsequent response from the ecosystem (Bender *et al.*, 1984; Glasby and Underwood, 1996). Disturbances occur when potentially damaging forces kill or displace the organisms present in an ecosystem, deplete consumable resources or degrade habitat structure, leaving this space to be used by individuals of the same or different species (Townsend and Hildrew, 1994; Lake, 2000). Such disturbances may be categorized according to temporal patterns. "Press" disturbances are long-term disturbances that are maintained over time, while discrete perturbations that produce an immediate important response are defined as "pulse" disturbances (Bender *et al.*, 1984; Lake, 2000). Ecosystem opposition to change (resistance) and the ability of biota to recover pre-disturbance status (resilience) (Lake and Barmuta 1986; Schlosser, 1990) are fundamental concerns in the face of any disturbance. In aquatic ecosystems, infrequent natural events such as a sudden flood or long drought could be considered disturbances (e.g. Matthews, 1986; Adams and Warren, 2005; Roghair and Dolloff, 2005), especially in short upstream courses where unstable flow implies low resilience (Junk *et al.*, 1989; Grigg, 1996; Lake, 2000). Aquatic assemblages have evolved to withstand this environmental variability (Resh *et al.*, 1988; Schlosser, 1990), yet anthropogenic impacts may decrease resilience increasing ecosystem vulnerability (Poff and Ward, 1989 and 1990) to large pulse perturbations such as tailing pond failures, distinguished from common industrial or sewage spills due to the huge volumes usually involved (Macklin *et al.*, 2003).

Fish assemblages have often been used in biological monitoring to reflect the stress applied to an aquatic ecosystem (e.g. Larimore and Smith, 1963; Albanese at al., 2009, Kubach *et al.*, 2011; Ryon *et al.*, 2011). Whenever a disturbance causes partial or total defaunation, subsequent fish responses include initial habitat recolonization and subsequent assemblage recovery (Sheldon and Meffe, 1995; Coker *et al.*, 2001). Fish recolonization processes mainly depend on both habitat fragmentation and species traits. Physical or chemical barriers between colonists and the defaunated area may reduce their potential recolonization rates after a disturbance (Niemi *et al.*, 1990; Pringle, 2003; Kubach *et al.*, 2011). This rate is positively related to species abundance, mobility and, to lesser extent, spawning. Thereby, abundant species supply more colonist individuals and may be more likely to settle within reaches because they are better matched to local habitat conditions than species that were previously scarce (Sheldon and Meffe, 1995). Species with greater mobility will also recolonize more rapidly than those with low movement rates (Albanese *et al*., 2009); and multi-spawner species often recover more rapidly than simple-spawner species (Ensign *et al*., 1997). After large-scale disturbances, fish often start the recolonization process triggered by floods from non-affected reaches and tributaries within the basin (e.g. Matthews, 1986; Roghair and Dolloff, 2005; Lake, 2000; Kubach *et al*., 2011).

One of the most harmful anthropogenic aquatic disturbances ever registered in Europe took place in the Guadiamar River, South-western Spain. On 25 April 1998, a tailing pond located in Aznalcóllar (Seville) ruptured, discharging 4 hm^3 of acidic water and 2 m^3 of metallic mud (Aguilar *et al*., 2003). The spill, composed mainly of iron, sulphur and heavy metals, flowed into the Agrio River and reached the Guadiamar River, where over 60 km of the fluvial course were defaunated (Grimalt and Macpherson, 1999). Several dams were built in the channelized Guadiamar River mouth to stop the toxic sludge from reaching Doñana National Park (López-Pamo *et al*., 1999). Among the dead organisms, fish were the most affected vertebrates, and during the days following the spill, 37.4 t were collected from the downstream marshland (Del Valls and Blasco, 2005). Several days after the spill, the tailing dam was sealed, mud and contaminated soils were mechanically removed, sediments were chemically stabilized (mainly with carbonates), a barrier of wells was installed in the perimeter in order to collect any leachates and remediation works were implemented in the area (Ayora *et al*., 2001; Arenas *et al*., 2008). Unfortunately, all these urgent measures aggravated the effects of the toxic spill, with major implications for the geomorphological, hydrological and geochemical characteristics of the river (Gallart *et al*. 1999). As a last long term measure, a Recovery Plan (PICOVER) was implemented not only to repair the damaged ecosystems, but aiming to transform the affected area into a green corridor between two well conserved ecosystems: Sierra Morena in the north and Doñana National Park in the south (Cardenas and Hidalgo, 2007; Márquez-Ferrando, 2009). Once the restoration tasks were over, several studies have monitored the recovery of the affected ecosystem with different targets (e.g., soil, Fernández *et al*., 2007; water, Olias *et al*., 2005; vegetation, Madejón *et al*., 2010; coleopteran communities, Cárdenas and Hidalgo, 2006; ant communities, Luque *et al*., 2007; crayfish, Alcorlo *et al*., 2006; reptile community, Marquez-Ferrando *et al*., 2009; mice, Bonilla-Valverde *et al*., 2004;

otters, Delibes *et al*., 2009). These studies have reported diverse results about the remaining spill influence. Nonetheless, the few studies that addressed the recovery of fish assemblages (Fernández-Delgado and Drake, 2008; Pérez-Alejandre; 2009) provided ambiguous early conclusions that considered an ongoing recolonization process that tends to the pre-disturbance conditions.

The purpose of this study was to evaluate the long-term effects of the Aznalcollar toxic spill on the Guadiamar River fish assemblage. The specific objectives were to: (1) study the recolonization process, pinpointing the main colonist sources, obstacles and dominant species dynamics; and (2) assess whether the fish assemblage in the affected reach can be considered recovered 13 years after the toxic spill.

Material and Methods

Study area and sampling design

The Guadiamar River basin is located in the South-western Iberian Peninsula covering an area of 1.880 km^2 (Borja *et al*., 2001) (Fig. 1). The upper section flows through the western Sierra Morena, with typical xeric Mediterranean forests. Thereupon, the river crosses a predominantly agricultural area on sedimentary hills and, finally, the southern end turns into a channelized marsh stretch that flows into the Guadalquivir river mouth within the Doñana National Park (Borja *et al*., 2001). From a hydrological point of view the Guadiamar is a typical Mediterranean river (Giudicelli *et al*., 1985), with a severe summer drought, annual average temperature above 10 ºC and annual average rainfall of 600 mm (Aguilar *et al*., 2003). The main river network in the basin consist of the Guadiamar River main stem and its most important tributaries, such as the Agrio River, the Ardachón stream, the Alcarayón stream, the De la Cigüeña stream and the Majaberraque stream (Fig. 1). This Agrio River, located in the boundary between the upper and middle section of the basin, was the first watercourse to receive the spill and hence, it flowed to the Guadiamar River mouth into the Doñana National Park (Fernández-Delgado and Drake, 2008; Fig. 1).

Fish assemblage was monitored at five sampling sites located in the Guadiamar River main stem (longitudinal sampling design). Due to the need for quick information after the spill, each site was selected according to accessibility and trying to maximise coverage of the affected fluvial reach. Unfortunately, the hazardous nature of the toxic spill and rapid decomposition of fish impeded collection or identification of dead fishes. Therefore, these fish kill data could not be used to quantify pre-disturbance fish assemblage structure. On the other hand, available information on the Guadiamar River fish assemblage before the spill was scarce. The closest pre-disturbance survey was carried out in 1996 and it provided species presence/absence data (species richness) from the same locations within the affected reach that were subsequently used as sampling points (pre-disturbance data) (Doadrio, 1996 and 2001). Given this scarce previous information, an additional sampling site was established 6 km upstream from the affected reach to represent non-affected assemblage conditions in the context of the mining spill, hereafter referred to as reference site (E1 in Fig. 1). Downstream, within the affected reach, the four original sampling sites were named E2, E3, E4 and E5 (Fig. 1). The first site affected by the spill (E2) was located at the confluence with the Agrio and Guadiamar rivers, whereas E3, E4 and E5 were situated 9 km, 19 km and 26 km, downstream of this confluence, respectively (monitoring stretch-: 32 Km from E1 to E5, Fig. 1). For our objective of evaluating fish assemblage recovery processes, we assumed that all the affected sampling sites (E2, E3, E4 and E5) began the recovery from the same state of disturbance.

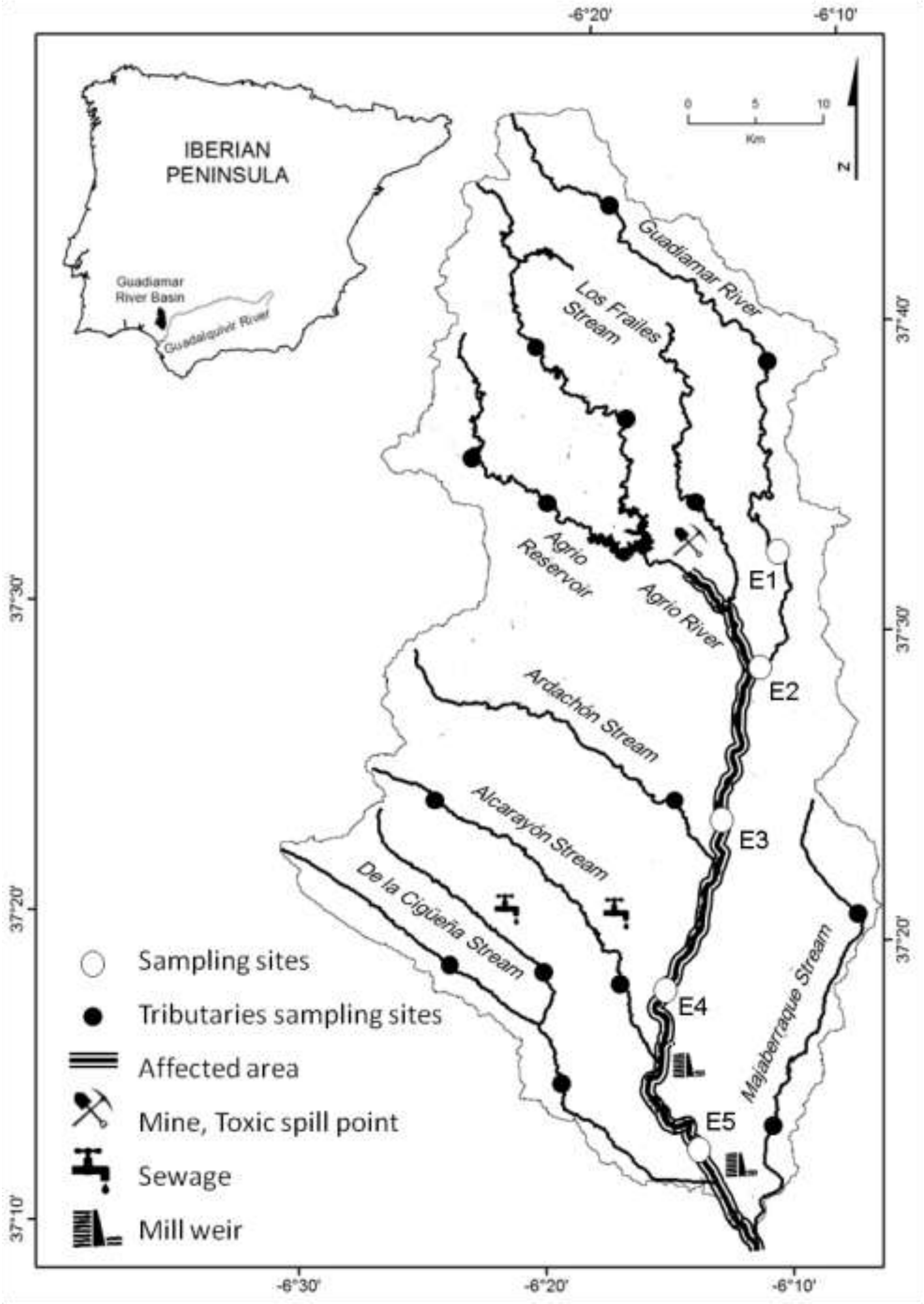

Figure 1. Location of the Guadiamar River basin where main river network, longitudinal sampling sites, affected reach, non-affected sampling sites in the main tributaries and potential major chemical and physical barriers are shown.

The Guadiamar River network is disrupted by several physical and chemical barriers. Some of these disturbances represent an important interruption to fish movement and therefore, an obstacle for recolonization processes. The Agrio reservoir in the Agrio River is the largest transversal obstacle in the watershed. Nevertheless, two other major barriers located in the Guadiamar River main stem were likely a direct obstacle to fish recolonization from downstream sources. Los Molinos mill is placed 2 km downstream of the lowest sampling site (E5) in the longitudinal design. This ancient mill dam (height = 2 m) has a drain that allows some flow to pass below the structure. Upstream, La Patera mill is the second obstacle that potential downstream colonists find. This former weir (height = 1.5 m) is located between E5 and E4, at 3 km and 4 km from these points, respectively. Moreover, three major chemical barriers may also hamper the recolonization process. Specifically, leachates from Aznalcóllar mines to the Agrio River in the upper section (Cabrera *et al.*, 1984, 1987; Arambarri *et al.*, 1996); sewage from Pilas towards the lower section of the Alcarayón stream in the middle section (Fernández-Delgado *et al.* 2010); and sewage from Hinojos and Villamanrique de la Condesa to the channelized De la Cigüeña stream in the lower section (Fernández-Delgado *et al*. 2010).

Fish were sampled once a year at each sampling site at the time of low annual flow (July-August) for nine years. Because of safety restrictions and cleaning works after the spill, the first sampling was carried out in 1999, and monitoring was uninterrupted until 2006. Additional funds allowed a last sampling effort in 2011. Altogether, 45 surveys were conducted in this longitudinal sampling design. Monitoring at the five sampling sites took place in streches with low-flow conditions (runs or pools); water width and depth of sampling stretches averaged 15 m and 2 m, respectively; clay and sand were the predominant substrate, with some gravel and a few boulders. At site level, fish were caught using two passive sampling methods: (i) setting ten minnow-traps (Harrison *et al.*, 1986; 0.5 m length, 0.03 m diameter entrance), distributed only in the bank of pools, for roughly 18 hours; and (ii) one multi-mesh gillnet (30 m long and 1.5 m deep) placed transversely running from the bank of pools, with mesh sizes ranging from 10 mm to 200 mm, soaking time approximately 18 hours.

In addition, the most important tributaries that flow into the Guadiamar River main stem (Fig. 1) and a Guadiamar stretch, just downstream of the river-marsh transition (Doñana marshland), were sampled and considered as non-affected fish sources after the spill. In these non-affected sources, fish were sampled twice, in 2003 and 2006, and only information about species richness was obtained. Electrofishing following the CEN standard protocol (CEN, 2003) was the sampling gear used in the tributaries and the same passive methods used in the sites of the longitudinal sampling design was the method used in the river-marsh site.

Data analyses

Following the spill, the riparian habitat of the affected reach was physically altered because of both toxic sludge dragging and the subsequent cleaning works. So, although we use the term fish assemblage recovery, we expected significant assemblage structure changes, in comparison to the pre-disturbance data (Doadrio, 1996). Jaccard's similarity index was applied to identify patterns in an initial comparison between the pre-disturbance species composition in the affected reach (1996) and the annual sampling data from the four affected sampling sites (E2, E3, E4 and E5). This index is used as a measure of similarity based on presence/absence data, and quantifies fish assemblage similarity between all possible site pairs within each time period (Gillette *et al.*, 2012).

In surveys carried out from 1999 onwards, species richness (S) and diversity (H', Shannon's diversity index; Shannon and Weaver, 1949) were calculated. Fish abundance was estimated using catch per unit effort (CPUE), standardizing total species catch with both passive sampling methods to 24 hours.

Sampling site E1 (reference site) was considered representative of non-affected fish assemblage conditions, so a principal response curve (PRC) was used to test differences between the affected sites and the reference site through time. The PRC approach constitutes a multivariate method, based on redundancy analyses, which describes changes in assemblage response over time in relation to a control (Van den Brink *et al.*, 2003; Silva *et al.*, 2010). The principal component is plotted against time, giving a PRC of the fish assemblage for each sampling site. A quantitative interpretation of the effects at species level is possible by scoring the species weight, according to each species accounting for the deviances (Van den Brink *et al.*, 2003). PRC were performed considering fish abundance at the species level. Monte Carlo permutations tests commonly carried out to test the significance of the axis (Van den Brink *et al.*, 2003; Silva *et al.*, 2010) could not be performed because of lack of sampling replicates in the same year.

Non-metric multidimensional scaling (NMDS) ordination was used, after CPUE log(x+10) transformation, to extract spatio-temporal patterns in fish assemblage structure (Kruskal, 1964a,b; Mather, 1976; Kruskal and Wish, 1978). NMDS is a general ordination procedure recommended for non-normal or questionably distributed data and calculates ranked ecological distances (Clarke, 1993; McCune and Grace, 2002), providing a relative measure of proportional similarity in fish assemblage structure (Kubach *et al*., 2011). NMDS estimates distances between samples out of a derived “sample by sample” matrix. This matrix is obtained by transforming the original matrix using a dissimilarity measure. NMDS is not restricted to Euclidean distance measure but any dissimilarity measure can be used, which can also relax the requirement of normality of data (Van den Brink *et al*., 2003). We used the Bray–Curtis dissimilarity distance to compute the resemblance matrix among sites. In this study, distances between reference site data and those from the affected reach were used to detect fish community recovery trends.

The statistical significance of differences in fish assemblages between years was tested using a semi-parametric permutational multivariate analysis of variance using the Bray-Curtis distance matrices (henceforth PERMANOVA). One PERMANOVA was performed per site, species abundances acted as the dependent variables, and both axes (time and site) were factors, so axes weight in each case was also assessed. Abundance values from E4 in 2005 were not included because during this year the sampling site was confined to an isolated pool where fish abundance (mainly *Luciobarbus sclateri*) was overestimated.

All statistical analyses were performed using R version 2.12.1 (R Development Core Team, 2012) and its package 'vegan' (Oksanen *et al*., 2011).

Results

Fish assemblage composition

A total of 6243 fish representing 13 species (7 native and 6 exotics) were caught during the whole monitoring period of the longitudinal sampling sites (Table 1). The dominant family was Cyprinidae, which accounted for 46.1% of the total species richness within the monitored stretch, followed by Centrarchidae and Mugilidae. Five species were detected in the five longitudinal sampling sites during any monitoring period: *L. sclateri, S. alburnoides* complex, *A. alburnus, C. carpio* and *L. gibossus*.

There were some differences in the fish species found in the affected reach respect to the pre-disturbance assemblage data from 1996 (Table 1). Three native species (*A. anguilla, I. lemmingii* and *S. pyrenaicus*) previously caught were not captured during surveys after the spill; however, five new exotics were detected.

Table 1. List of the fish species caught and locations within the Guadiamar River basin during the pre-disturbed sampling in 1996, affected reach monitoring (1999-2011) and the non-affected main river network (2003 and 2007).

	Pre-disturbance	**Longitudinal sampling sites**				**Non-affected**
Species	**Doadrio (1996)**	**1999**	**2000**	**2001-2006**	**2011**	**fish sources**
Natives						
Anguilla anguilla	(+)					M
Atherina boyeri	(+)				E2	M
Cobitis paludica	(+)	E1	E1	E1,E2,E3	E1,E3	Gup, AG, AR, AL
Pseudochondrostoma willkommii	(+)	E1	E1,E2	E1,E2,E4	E1,E2	Gup, AG
Iberochondrostoma lemmingii	(+)					Gup, AG
Luciobarbus sclateri	(+)	E1	E1,E2,E3,E4	E1,E2,E3,E4,E5	E1,E2,E3,E4,E5	Gup, M, AG, AR, DC
Squalius alburnoides complex	(+)	E1	E1,E2,E4	E1,E2,E3,E4,E5	E1	Gup, AG, AR
Squalius pyrenaicus	(+)					Gup, AG, AR
Liza ramada	(+)			E4,E5	E4	M
Mugil cephalus	(+)			E5		M
Exotics						
Alburnus alburnus	( )				E1,E2,E3,E4,E5	M
Carassius gibelio	( )	E1	E1	E3,E4,E5		M, AG
Cyprinus carpio	(+)	E1	E1,E3,E4	E1,E2,E3,E4,E5	E3,E5	M
Gambusia holbrooki	( )		E3,E4,E5	E2,E4,E5	E2,E3	M, AG, AR, MA
Lepomis gibbosus	( )	E1	E1,E2,E3,E4	E1,E2,E3,E4,E5	E1,E2,E3,E4,E5	Gup, M, AG, AR
Micropterus salmoides	( )	E1	E1,E2	E1,E3,E4,E5		Gup, AG

Longitudinal sampling sites: non-affected (E1), affected (E2, E3, E4 and E5); pre-disturbance data only in the affected reach (E2-E5), (+) present and ( ) absent; non-affected fish sources (Gup: upper Guadiamar, M: Doñana marshland sampling site, AG: Agrio River, AR: Ardachón stream, AL: Alcarayón stream, MA: Majaberraque stream and DC: De La Cigüeña stream).

Previous vs. post spill fish composition

There were no clear patterns in dissimilarity between the pre-disturbance species composition (1996) and the annual post-disturbance data in the four affected sites (1999-2006, 2011). Despite an initial large difference (Jaccard's distance = 1 in E2, E3 and E5, and 0.67 in E4) between the pre-disturbance fish composition and that of the first sampling period after the spill (1999), from 2000 onwards Jaccard's distance tended to either decrease or increase without any clear pattern (Fig. 2).

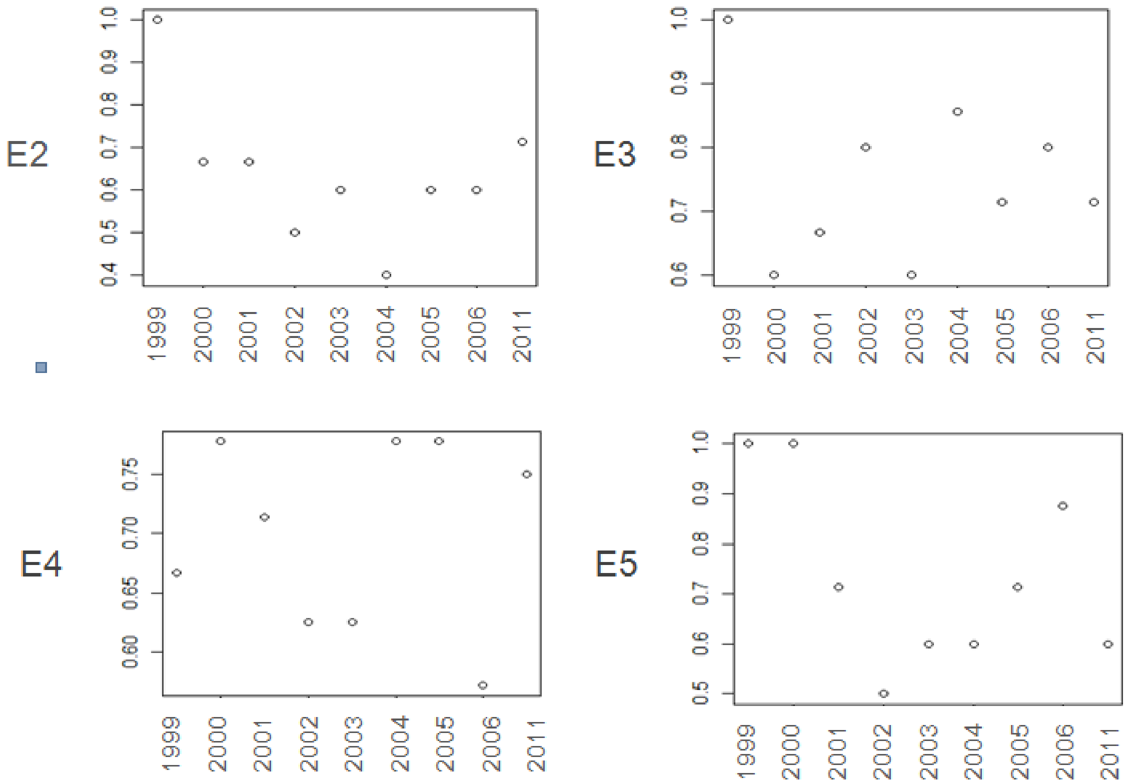

Figure 2. Comparison of fish assemblage composition at each site of the affected reach in the Guadiamar river (E2-E5) with its pre-disturbance assemblage in 1996 (Jaccard´s distance, y-axis), through time (x-axis).

Abundance, species richness and diversity

During the monitoring period, two species were present in all sites every year: one native, *L. sclateri*, accounting for 50% on average (range 30%-73%) of all CPUEs collected, and one exotic, *L. gibbosus*, accounting for 16% on average (range 3%-31%). *L. sclateri* was the dominant species, except in the reference site (E1), where it was often codominant with *P. willkommii* (36% of total captures). This last species was considerably less abundant in E2, and absent in the rest of the monitoring stretch. Although *S. alburnoides* complex was present in every sampling site, it was the least abundant native species, accounting for just over 3% of all individuals collected. It occurred in the reference site but was almost absent in the affected reach. Among the exotic species, the second most dominant was *A. alburnus*, accounting for 12% (range 4%-26%) of all individuals collected on average, but absent in the reference site. *G. holbrooki* and *M. salmoides* accounted for 9% on average (range 6%-13%) and 10% (range 4%-17%), respectively. *M. salmoides* was present in all sampling sites, whereas *G. holbrooki* was caught only in the affected reach. No other species accounted for more than 3% of all individuals collected at any sampling site, nevertheless, all species have also been taken into account for assemblage structure analyses.

Native species richness in the reference site maintained a stable fluctuation between 2 and 4 species during the monitoring period (Table 1; Fig. 3), whereas in exotics it decreased from 4 to 1-2 (Fig. 3). In contrast, both native and exotic species richness at the affected sites increased from 0 or 1 species in 1999 to a constant fluctuation mainly between 1 and 3 (sporadically 4) species in the following years. Finally, at the end of the monitoring period (13 years after the spill), the reference site showed its maximum number of native species, whereas exotics dropped to an average value.

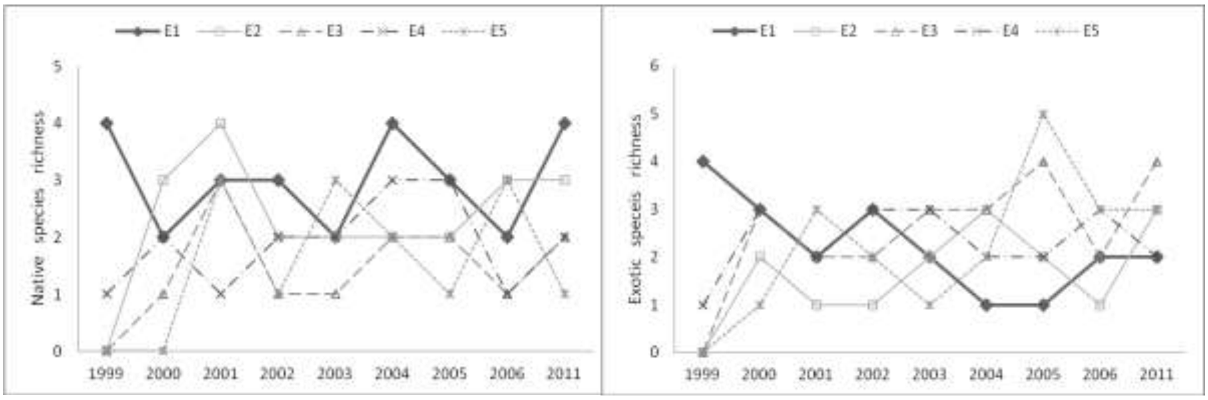

Figure 3. Species richness dynamics for natives (left) and exotics (right) at the reference (E1) and affected (E2-E5) sites following the April 1998 spill.

Fish diversity increased in each sampling site during the two years following the spill, especially in the affected reach (except in E5; Fig. 4). After this initial recovery, a second stable phase followed (2001-2004), with constant diversity values. However, from 2005 onwards, started a third fluctuating phase, with maximum values in E2 in 2011.

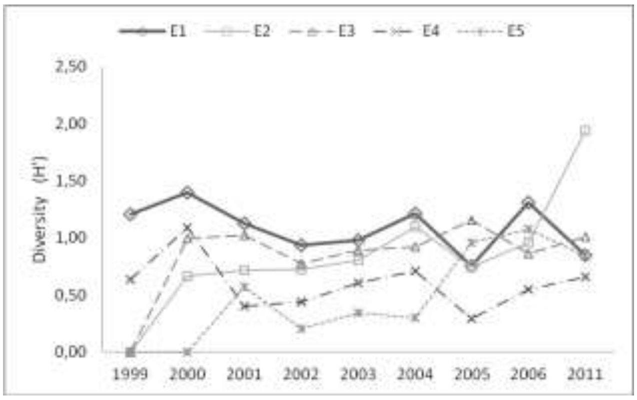

Figure 4. Species diversity (H´) dynamics at the reference (E1) and affected (E2-E5) sites following the April 1998 spill.

During this study, at least three different phases could be distinguished for fish abundance trends in the affected sites. First, early spill removal works resulted in an increase from the lowest initial values (1999) to a maximum in the second year after the spill (2000), reaching similar abundance values between the reference and the affected sites (Fig. 5). However, in 2001 native species abundance, and especially that of exotics, decreased to a minimum. Between 2002 and 2004, there was a stable phase for both native and exotic species in most sampling sites, with a slight increase for natives. The third phase could be considered the fluctuating trend that sampling sites underwent from 2005 to 2006, when each sampling site showed different trends in native and exotic species abundance. Finally, the last sampling in 2011 showed how exotic species abundance mightily increased in the affected reach and decreased to a minimum in the reference site, resulting in higher values for exotic species in the affected reach than in the reference site at the end of the study period. On the contrary, native species abundance in the reference site remained above that in the affected reach.

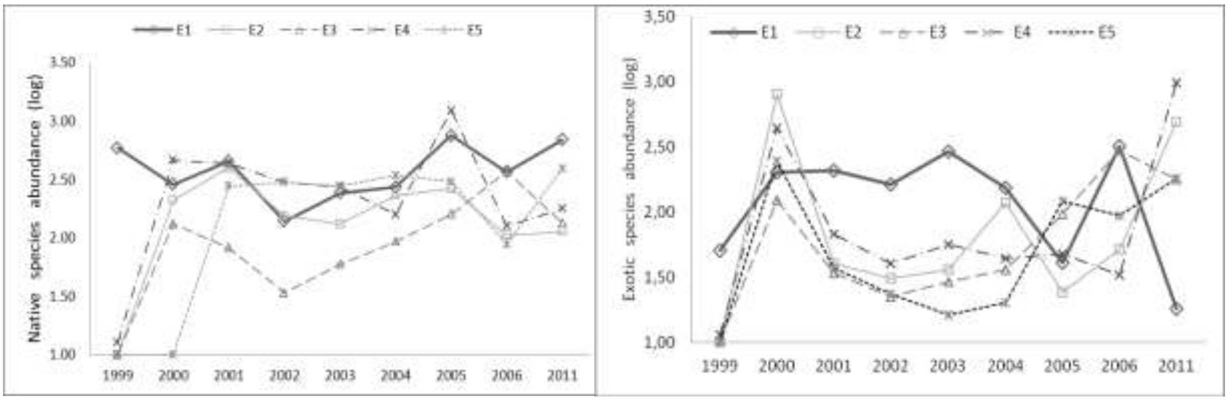

Figure 5. Fish abundance dynamics for natives (left) and exotics (right) at the reference (E1) and affected (E2-E5) sites following the April 1998 spill.

Assemblage structure dynamics

River channel conditions after the spill triggered large differences between the affected sites (E2, E3, E4 and E5) and the non-affected upstream reference site (E1). This divergence started to decrease after two years (Fig 6). Then, between 2002 and 2004, assemblages from the affected sites maintained a similar structure to that of the reference site. However, from 2005 all assemblages started to diverge, becoming very different by the end of the study period. These assemblage trends were more influenced by some species than others. PRC identified *A. alburnus, L. gibbosus, P. willkommii* and *L. sclateri* as the species with greatest weight on assemblage structure (Fig. 6). As previously mentioned, lack of sampling replicates made the quantification of the species' influence by PRC impossible. PERMANOVAs were used to test this influence.

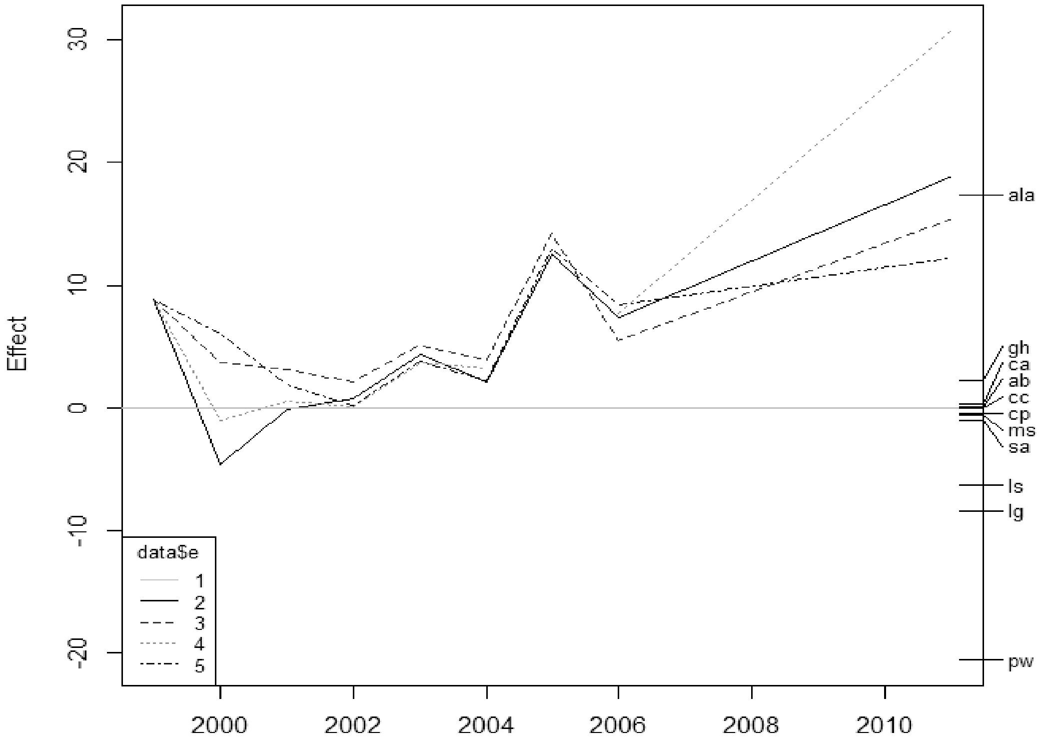

Figure 6. Principal response curves (PRC) representing the fish assemblage of the affected sites (2-5 are E2-E5) in relation to the reference site (1 is E1) following the April 1998 spill. The left y-axis represents the effect of deviances from the control (E1). The right side of the figure represents species weight, accounting for the deviances of the PRC (ala: *A. alburnus,* gh: *G. holbrooki*, ca: *C. gibelio,* ab: *A. boyeri,* cc: *C. carpio,* cp: *C. paludica,* ms: *M. salmoides,* sa: *S. alburnoides,* ls: *L. sclateri,* lg: *L. gibbosus* and pw: *P. willkommii*).

NMDS ordination (Fig. 7) revealed a similar spatio-temporal recovery pattern of fish assemblage structure to that displayed by PRC. Along Axis 1, the position of the reference site showed relatively little variability across time. All samples from the reference site occupied a localized area towards the negative end of this axis, indicating relative stability in assemblage structure. In 1999, affected sites were in the opposite end of Axis 1 and in the positive part of Axis 2. From 2000 to 2004, the affected sites increased in similarity with respect to the reference assemblage on Axis 1. E2 reached the reference site area in 2001 and then maintained a close resemblance for 3 more years. However, from 2005, affected sites tended to diverge from the reference assemblage again. This trend did not derive towards the initial dissimilar starting point at the positive ends of both axes, but it is directed towards the negative end of Axis 2, where no sites appeared before (Fig. 7).

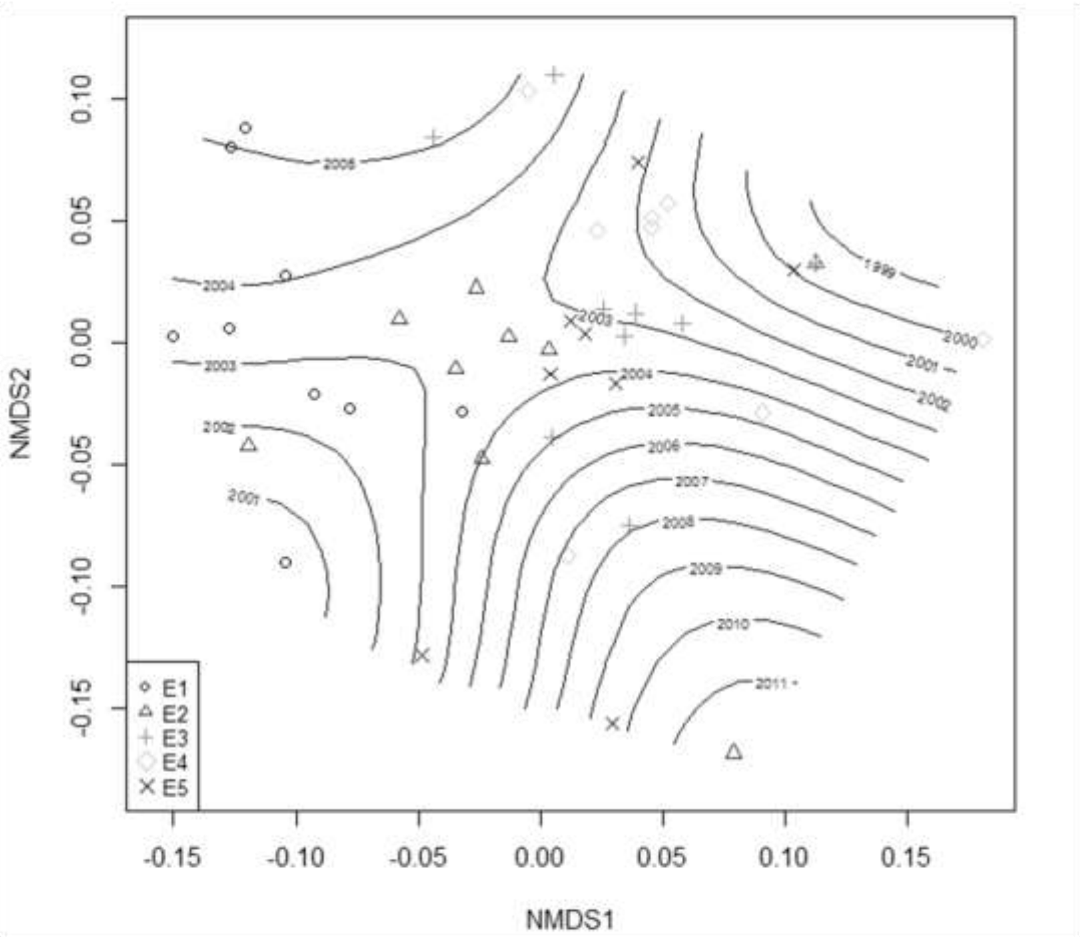

Figure 7. Fish assemblage dynamics in sampling sites along the Guadiamar River following the April 1998 spill illustrated in a non-metric multidimensional scaling ordination plot. Each sampling site (E1-E5) is represented by a symbol and the different positions are defined by the fish assemblage structure in each sampling. Annual curves indicate the overall fish assemblage trend throughout the sampling period. Axis NMDS1 and NMDS2 represents the majority of variation among samples.

PERMANOVA revealed no significant differences between years in E1 ($F_{(1,8)}$= 1.187; p = 0.345). However, these differences were significant for E2 ($F_{(1,8)}$= 4.4854; p = 0.008), E4 ($F_{(1,8)}$= 3.2358; p = 0.015) and marginally significant ($p < 0.1$) for E3 ($F_{(1,8)}$=2.0664; p = 0.091) and E5 ($F_{(1,8)}$= 3.2667; p = 0.056). In the PERMANOVA with site, sample and site-year interaction, site accounted for 31% (p = 0.001) of the variance explained by the model; year accounted for 10% (p = 0.001); and site-year interaction accounted for 10% (p = 0.032). Thus, the model explained 52 % of the variance.

The four species with highest weight in the assemblage dynamics deserve special attention. *L. sclateri* maintained a high abundance in all sites since 2001, resulting slightly higher in E5 at the end of the study period (Fig. 8a). *P. willkommii* was mainly restricted to the upstream non-affected reach of the Guadiamar River, even though it was common for some individuals to reach the adjacent affected E2. Since 2005, seven years after the spill, *P. willkommii* abundance has increased in the reference site (E1), but decreased in the affected reach (Fig. 8b). Among the exotic species within the affected sites, *L. gibbosus* greatly increased in 2000, but abundance decreased from the following year, and values were stable hereinafter (Fig. 8c). *A. alburnus* was absent until the last sampling year. However, in that year this species was the second most abundant in the affected reach. This last abrupt expansion of a particular species confirms a deep change in fish assemblage structure.

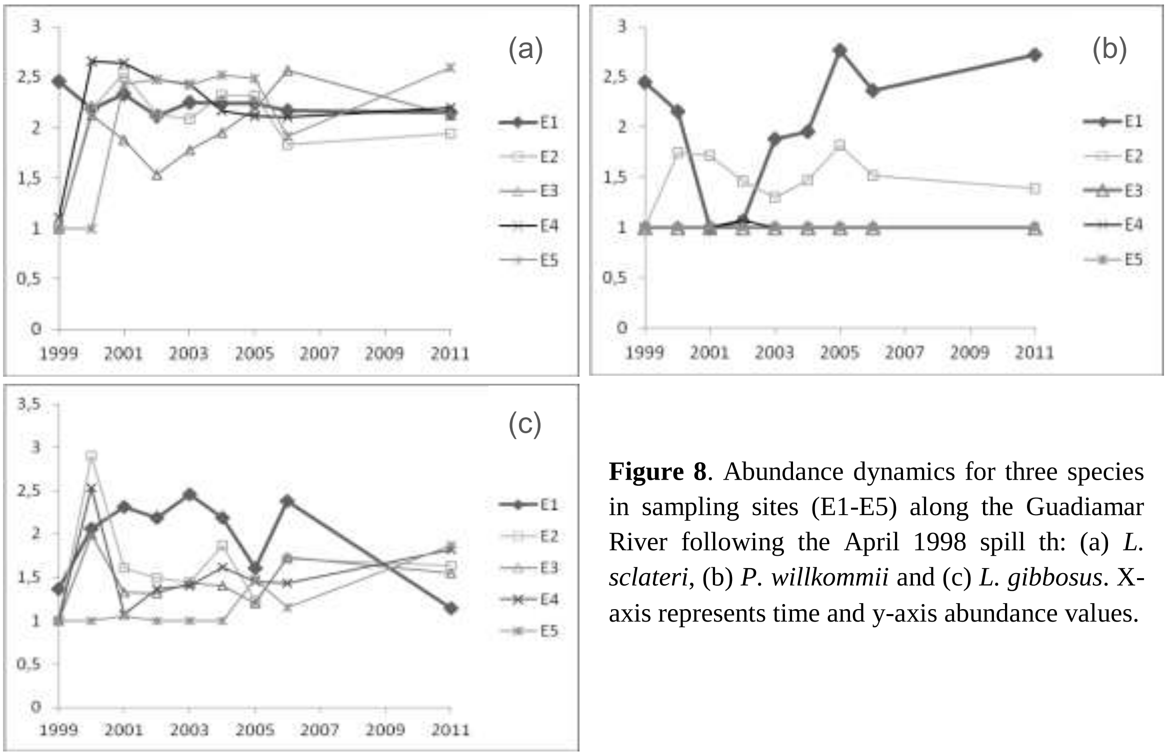

Figure 8. Abundance dynamics for three species in sampling sites (E1-E5) along the Guadiamar River following the April 1998 spill th: (a) *L. sclateri*, (b) *P. willkommii* and (c) *L. gibbosus*. X-axis represents time and y-axis abundance values.

Fish recolonization sources

Sampling of non-affected tributaries and Doñana marshland area identified fish assemblages that were a likely source of colonizing individuals after the spill removal works (Table 1; Fig. 9). The largest native species assemblage was found in the upstream Guadiamar River main stem (Table 1). This source supplied six native species, *L. sclateri, P. willkommii, S. alburnoides, S. pyrenaicus, C. paludica* and *I. lemmingii,* together with two exotics, *L. gibbosus* and *M. salmoides*. On the other hand, the largest exotic species assemblage was detected downstream in the Doñana marshland sampling site (Table 1; Fig. 9). Regarding the tributaries, Agrio River and Ardachón stream were potentially the largest lateral contributors, providing native species such as *L. sclateri, S. alburnoides and S. pyrenaicus*, together with the exotic *G. holbrooki* and *L. gibbosus* (Table 1). Downstream, *C. paludica* was the only species caught in the Alcarayón stream, and Majaberraque stream was the last tributary holding likely colonists, in this case *G. holbooki* (Fig. 9).

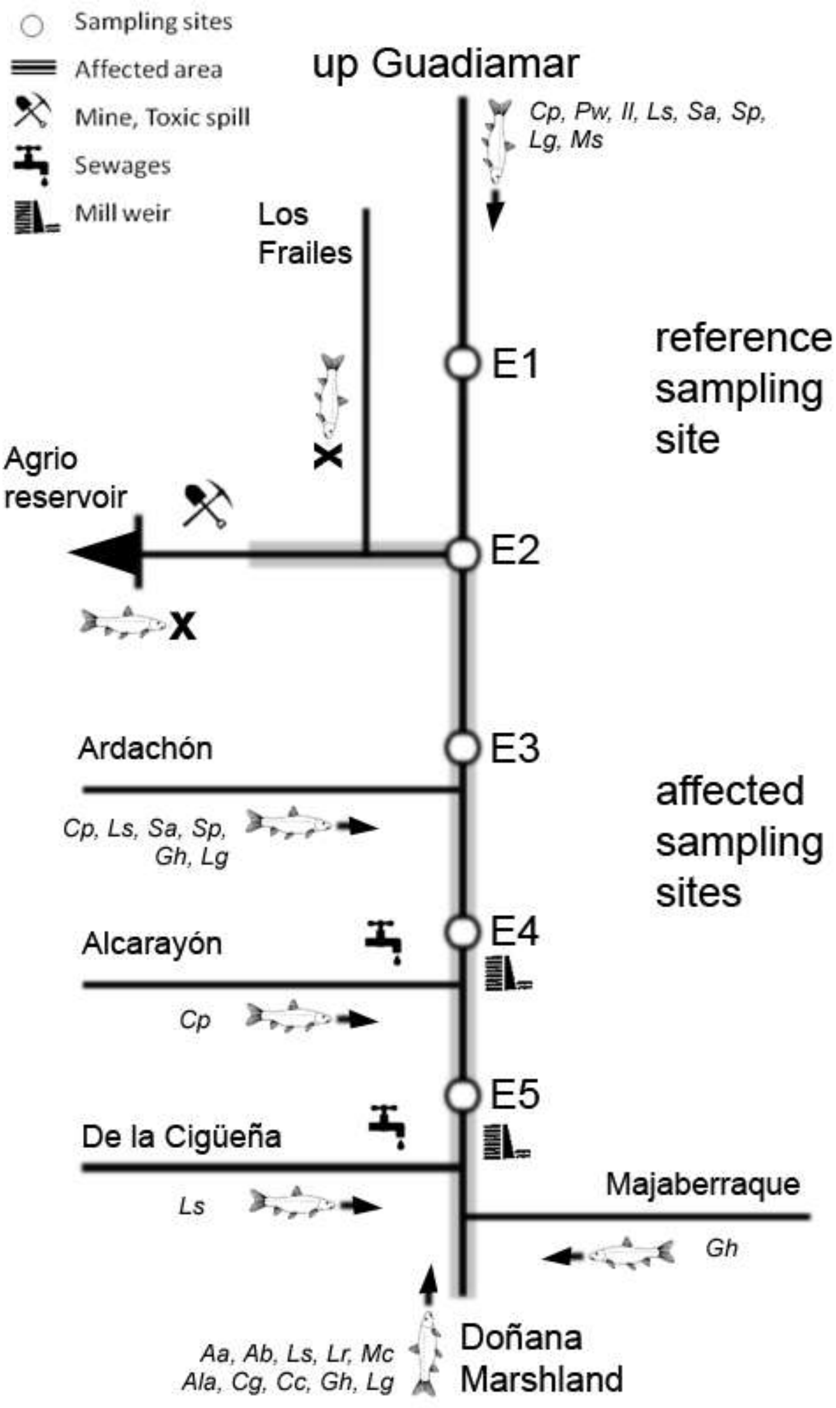

Figure 9. Diagram of the main potential sources of fish colonists for the affected reach of the Guadiamar River after the April 1998 spill. Physical and chemical barriers (crosses denote insuperable conditions and arrows those permeable for fish) and fish species caught in each source are shown (Ala: *A. alburnus*, Gh: *G. holbrooki*, Cg: *C. gibelio*, Ab: *A. boyeri*, Cc: *C. carpio*, Cp: *C. paludica*, Ms: *M. salmoides*, Sa: *S. alburnoides*, Ls: *L. sclateri*, Lg: *L. gibbosus*, Pw: *P. willkommii*, Aa: *A. anguilla*, Mc: *M. cephalus*, Lr: *L. ramada*, Il: *I. lemmingii*, Sp: *S. pyrenaicus*).

Discussion

Guadiamar River fish assemblages at the different sampling sites evolved in different ways throughout the 13 years following the spill. Several barriers hampered recolonization from tributaries; however, this process was carried out and is still underway.

The fish assemblage after the spill event almost disappeared, and was therefore, highly dissimilar to that found in the previously undisturbed Guadiamar River main stem (Doadrio, 1996 and 2001). Different trajectories at each sampling site reduced or increased this similarity through time, but no clear patterns were found. Available pre-spill information was limited, so only species presence could be used as reference data. Species richness commonly ranged from 1 to 3 every year for both native and exotic species. Therefore, species presence was a weak changing variable for pre- and post-spill similarity comparisons. Although fish diversity allowed us to draw a general outline of assemblage dynamics, this approach did not help us identify the dynamics of fish assemblage structure through time. The PRC and NMDS analyses, based on abundance, offered both overall and specific approaches to explain the observed patterns. First, the early spill effect and subsequent cleaning works, especially the withdrawal of vast amounts of soil in the summer of 1999 that cut and dried several main stem reaches (Arenas *et al*., 2008), impeded fish establishment in the affected reach (E2-E5) until 2000 - Fernández and Drake (2008) caught some individuals before but were scarce and probably reduced during these soil movements (personal observation) -. From that year, fish assemblage structure in affected sites tended towards that of the reference site (E1), where native species were dominant and exotics were scarce (Table 1, Fig. 6 and 7). The increase in assemblage similarity was especially relevant in E2, which was the nearest sampling site to the reference. Thus, between 2002 and 2004 (four-six years after the spill), fish assemblage structure in affected sites stabilized, with slight increases or decreases in similarity, depending on the sampling year, to that of the reference site (Fig. 5). These first signs of recovery were considerably belated compared with those of other studies where a defaunated river stretch, experimentally or by accident, was considered. In Illinois (USA), a fish assemblage took between 3 and 10 days to reach background levels in a river without barriers and drained by many tributaries (Peterson and Bayley, 1993). In another case, in South Carolina (USA), several downstream pools recovered in 1 month, while upstream sites took longer to recover (Sheldon and Meffe, 1995). Several studies both in Europe and in the USA (e.g. Ensign and Leftwich, 1997; Meade, 2004; Kennedy *et al*., 2012) also mention a time lapse of 1 year to overall assemblage recovery; 2-3 years or longer were needed for certain species or specific age structures to reach previous conditions. Nevertheless, two recent studies showed a recolonization process similar to our case. First, colonization experiments in Virginia (USA) concluded that most fish populations recovered 2 years after defaunation and only species with low movement rates took longer (Albanese *et al*., 2009). Second, a study investigating the effects of an oil spill in South Carolina (USA) placed the time of recovery in fish assemblage structure at 4.3 years after the spill (Kubach *et al*., 2011).

Interestingly, from 2005 onwards, fish assemblage structure in the affected reach diverged from that of the reference site again (Fig. 6 and 7). This year was the driest in the sampling period (SAIH, 2012) and native species, better adapted than exotics to drought (Ribeiro and Collares-Pereira, 2010), were favored in those upstream reaches where flow was mightily reduced (E1 and E2, Fig. 5). However, exotic species thrived in the affected reach because flood shortage enhanced the lentic nature and stable flow of this area (Clavero and Hermoso, 2011). In subsequent years, native species decreased in the upstream sites (E1 and E2) because of both downstream displacement by floods and recovery of interactions with exotics (Ribeiro and Leunda, 2012). Nevertheless, at the end of the sampling period, native species abundance returned to average values for each sampling site. On the other hand, exotic species abundance recovered in upstream sites (E1 and E2) and both floods that displaced individual downstream and upstream migration from Doñana marshland, increased the abundance of exotics in the affected reach at the end of the sampling period (Fig. 5).

Regarding recolonization sources, the unaffected upstream and downstream Guadiamar River main stem seemed to be the most relevant fish source (Fig. 9). Areas upstream from the spill provided mainly native species from a low disturbance area where natural conditions still remain. Introduced centrarchids present upstream, were occasionally displaced with floods. Potential colonists from downstream sources may be mainly migratory native and exotic species present in the highly human-modified marshland. Lateral sources from tributaries contributed to recolonization to a lesser extent because of accumulation of urban sewage, water collection and diffuse agricultural pollution, that largely reduced water quality (Fernández-Delgado and Drake, 2008) and caused fish assemblage to become poorer or absent as the tributaries go downstream. Nevertheless, floods enhance fish drift (Harvey, 1987) and dilute pollution (Cánovas *et al.*, 2010), so upstream fish may be able to reach the tributary mouth and swim into the Guadiamar River main stem. After such pulse events, Ardachón stream could be considered as the third main fish source due to the highest richness species value among the tributaries (Table 1, Fig. 9). Alcarayón and Majaberraque streams may have only a slight contribution to recolonization, but in a monospecific and antagonistic way. The first could be the source of a native species (C. *paludica*) while the second of an exotic one (*G. holbrooki*) (Fig. 9).

When considering the relevance of barriers, mining leachates in the Agrio River were likely the most harmful for recolonization. Although the Agrio reservoir may be restraining downstream fish displacement from the upstream tributaries to the affected reach, fish from Los Frailes stream, that connects onto the Agrio River downstream from the dam, were also absent near the confluence with the Guadiamar River (E2) (Fig. 9). This fish absence may be because the Agrio River crosses the mining area in this stretch, and becomes contaminated by acid mine drainage (Olías *et al.*, 2006). This mining pollution is previous to the April 1998 spill (Cabrera *et al.*, 1984, 1987; Arambarri *et al.*, 1996) and it has not been adequately addressed yet. A second considerable chemical barrier was urban sewage that fills the De la Cigüeña stream, which may have stopped upstream fish from reaching the affected reach (Fernández-Delgado and Drake, 2008). On the contrary, the two mills in the main stem lower section did not represent a significant enough obstacle to prevent upstream fish recolonization because catadromous species (*L. ramada* and *M. cephalus),* whose only source could be the downstream marshland, were present upstream from the mills (E4, Table 1) during the study period (Fig. 9).

Most species underwent an initial rise in abundance because a continuous flow was restored after the cessation of the main cleaning works. However, most of these species maintained a low abundance in the affected reach during the sampling period. Only *L. sclateri, P. willkommmii* and *L. gibbosus* maintained stable populations through the entire sampling period. These three species together with *A. alburnus* were identified by the PRC as the species with greater weight on assemblage structure (Fig. 6). Consequently, the overall fish assemblage recovery process in the affected reach must be addressed taking into account the dynamics of these four species that stood out in the fish assemblage patterns.

The southern Iberian barbel, *L. sclateri,* was the dominant species in both the affected and non-affected reaches of the Guadiamar River. This native species maintained an abundant population in the study area since 2000, even though the sampling site close to the Doñana marshland (E5) took one year longer in harboring it (Fig 8a). This delay suggests that this species recolonized the affected reach mainly from both the non-affected upstream areas of the main stem and the Ardachón stream (Fig. 9). *L. sclateri* is a potadromous species well adapted to the unfavorable Mediterranean summer conditions (Rodríguez-Ruiz and Granado Lorencio, 1992; Encina *et al*., 2006), with high temperature in isolated pools in the upper section, and organic matter accumulation in the lower section (Herrera y Fernández-Delgado, 1992; Torralva *et al*. 1997; Doadrio *et al*., 2011). This intrinsic resistance to adverse conditions makes *L. sclateri* the most ubiquitous and abundant species in southern Iberian courses, withstanding the increasing pollution that other native species are not able to face (Oliva-Paterna *et al.*, 2003a; Encina *et al*., 2006; Fernández-Delgado *et al*., 2007). Moreover, its fast growth rate and large size provide this species a wide mobility and high capacity for dispersal (Saldaña, 2006), including *L. sclateri* in the fast recolonist group (Scholosser, 1990; Detenbeck *et al*., 1992). Consequently, and in agreement with others studies on recolonization processes (e.g. Ensign and Leftwich, 1997; Lonzarich *et al*., 1998; Kubach, 2011), high abundance (Sheldon and Meffe, 1995) and large mobility (Albanese, 2009) identified *L. sclateri* as the best colonist of the affected reach. To a lesser extent, scarce rainfall in 2002 together with the last soil movements (Arenas *et al*., 2008) hampered the southern Iberian barbel population, especially in E3, but in the following years, the species reached stable levels (Fig. 8a).

Southern straight-mouth nase, *P. willkommii,* was the co-dominant species in the upstream non-affected reach of the Guadiamar River, together with *L. sclateri*. However, it was almost absent in the affected area (Table 1, Fig 8b). This native fish is a potadromous species highly sensitive to pollution and habitat fragmentation (Rodríguez-Ruiz and Granado-Lorencio, 1992; Doadrio *et al*., 2011). Furthermore, the species' feeding habits consist on scraping algae or macroinvertebrates fixed to the stony riverbed (Bellido *et al*., 1989). Since the affected reach lacks many of those macroinvertebrates (Solà *et al.*, 2004; Ferreras-Romero *et al*., 2003) and both anthropic pollution and sediment accumulation are still increasing (Carrascal *et al*., 2008), *P. willkommii* will rarely recolonise the affected reach as long as this trend is not changed. Only the stretch nearest to the upstream non-affected reach of the Guadiamar River can currently harbor the species (Table 1). This is because upstream areas are hardly polluted, low in sediments and have clean waters that are a source of macroinvertebrates and algae for *P. willkommii* individuals that successfully establish in this reach. This species' dynamics in the reach where it is confined seemed to be influenced by both rainfall and habitat fragmentation. Thus, rainy years enhanced *P. willkommii* drift towards the nearest affected reach, whereas the driest year (2005; SAIH, 2012) promoted an increase in this species' larvae (personal observation) due to two events. First, floods shortage during the period when individuals are most vulnerable to be killed by dragging (Pérez-Alejandre, 2009); and second, regarding exotic species interactions, the *L. gibbosus* population that predate on *P. willkommii* larvae or compete for the same habitat (García-Berthou and Moreno-Amich, 2000), decline due to their poor adaption to drought (Ribeiro and Collares-Pereira, 2010). On the other hand, early soil movements were probably hampering the free displacement along the river needed for the *P. willkommi* life-cycle (Herrera and Fernández-Delgado, 1994; Encina and Granado-Lorencio, 1997), probably reducing upstream *P. willkommii* abundance year after year. Since the cessation of the cleaning works (2002; Arenas *et al*., 2008), this species increased its upstream population until a maximum was reached in the last sampling year. However, *P. willkommii* was confined to the upstream non-affected reach of the Guadiamar River (E1) and its adjacent area (E2, Fig. 8b).

Regarding exotic species, *L. gibbosus* was one of the most abundant in the upstream non-affected reach of the Guadiamar River and its dynamics were considered as the third representative recovery pattern in the study area. This large population favored rapid recolonization by this species from the upstream non-affected reach of the Guadiamar River and, to a lesser extent, from the Ardachón stream (Fig. 9). After the removal of the polluted sediments, when several weirs were built (Solà, 2004), the first flood that connected the Guadiamar River main stem occurred in October 12, 1999 (SAIH, 2012). This flood discharged 90 mm in only a few hours, the second maximum discharge in the study period (SAIH, 2012). Consequently, the largest fish displacement was triggered. Among them, *L. gibbosus* < 10 mm (Total Length) should have been one of the most displaced downstream towards the affected reach (Harvey, 1987). The absence of centrarchid juveniles or adults in the defaunated stretch probably prevented intraspecific predation of larvae (Harvey, 1991), so most larvae of this species reached the next age-group (juveniles). These young-of-the-year, < 41 mm in males and < 62 mm in females (Gutierrez-Estrada *et al*., 2000; Ribeiro and Collares-Pereira, 2010), were probably able to grow successfully through the winter since at this early stage juveniles mainly feed on littoral microcrustaceans (García-Berthou and Moreno-Amich,

2000) displaced downstream by the flood. *L. gibbosus* has high life-history plasticity (Fox, 1994; Robinson and Wilson, 1996; Fox and Crivelli, 2001) that allows it to survive stochastic events such as seasonal flash floods (Ribeiro and Collares-Pereira, 2010) and a large ability to exploit prey in different types of habitats (Robinson *et al*., 1993; Godinho and Ferreira, 1996; Vila-Gispert *et al*. 2005, 2007). Therefore, this species underwent the rapid maturation observed in new receiving courses (Ribeiro and Collares-Pereira, 2010) and may have provided, through multiple spawning behaviors (Vila-Gispert *et al*. 2005), a large second generation reaching the first year of age (Gutierrez-Estrada *et al*., 2000; Ribeiro and Collares-Pereira, 2010). Moreover, the abundance of this new cohort probably increased further because recently introduced *L. gibbosus* populations exhibit higher reproductive effort than long existing populations (Copp and Fox, 2007; Ribeiro and Collares-Pereira, 2010).

This ability to adapt to the environmental conditions of novel environments (Vila-Gispert *et al*., 2007) probably gave them advantage over other recolonizing fish species that were not stable enough to compete for resources yet. However, this *L. gibbosus* demographic explosion decreased to a low but stable level throughout the following years (Fig. 8c). This decrease may be due to the sum of several changes in food availability, as well as inter- and intraspecific competition. When *L. gibbosus* juveniles mature, feeding habits turn to the riverbed (Kieffer and Colgan 1993), predominantly of the macrobenthos (e.g. Magalhaes 1993a, 1993b, Collares-Pereira *et al*. 1995), where they find mollusks, insect larvae, crustaceans, etc. (García-Berthou and Moreno-Amich, 2000; Domínguez *et al*., 2002); and where Diptera larvae were probably more vulnerable than others that are swimming (Godinho *et al*., 1997a, 1997b). In the affected area, the disturbed post-cleaning riverbed was not able to shelter most macroinvertebrates present in the upstream non-affected reach of the Guadiamar River, such as *Atyaephyra* spp., lothic Odonata nymphs, Ephemeroptera families with a low tolerance to pollution, Coleoptera or Mollusks (Solà, 2004; Ferreras-Romero *et al*., 2003); resulting in an important shortage of food. Moreover, the presence of juveniles and adults in the affected reach may have triggered intraspecific predation on larvae (Harvey, 1991) and eggs (García de Jalón *et al*. 1993, García-Berthou and Moreno-Amich 2000). In addition, different piscivorous species such as *M. salmoides* that directly feed on *L. gibbosus* juveniles (Ribeiro and Collares-Pereira, 2010) and food competition with cyprinids, even when dietary overlap is limited (Olson *et al*. 1995), may have diminished this initial large *L. gibbosus* population (Fig. 8c). Nevertheless, individuals were displaced from sources (Fig. 9) downstream towards the affected area during rainy years (2004 and 2009-2011, SAIH, 2012), slightly increasing the population in both mouth reaches (E2 and E4, Fig. 8c). On the contrary, during the driest year (2005) *L. gibbosus* abundance decreased in those upstream reaches (E1 and E2, Fig. 8c) where the flow was not stable and reduced to isolated pools. Under these circumstances, native species have an advantaged (Ribeiro and Collares-Pereira, 2010).

A. alburnus however, was not present in the Guadiamar River until the last sampling period (2011, Table 1), but during this year it shared exotic co-dominance with *L. gibbosus* in the affected reach. This exotic species was deliberately introduced in Iberian reservoirs by anglers to be used either as prey for piscivorous fish species or as live bait (Vinyoles *et al*., 2007). From 1992, *A. alburnus* spread throughout the rest of the Iberian Peninsula (Almodóvar *et al*., 2012), and the first occurrence in the Guadalquivir basin was reported in 2006 (Fernández-Delgado *et al*., 2007; Vinyoles *et al*., 2007). Introduced individuals are largely linked to reservoirs where they were released, with a greater potential for expansion downstream than upstream (Vinyoles *et al*., 2007). Consequently, the most likely source of bleak in the Guadiamar River Basin is the Agrio reservoir. This species depends on reservoirs and upstream tributaries (Hladík and Kubecka, 2003), where it finds shallow riffles adequate for multiple spawning (Kottelat and Feyhof, 2007; Doadrio *et al*., 2011). This need for upstream migration is not shared by other exotic species introduced in the Guadiamar River (Table 1), and it suggests a second hypothesis based on *A. alburnus* colonization from downstream water bodies. According to Fernández-Delgado *et al*. (2010), until 2009 *A. alburnus* was present in the Guadalquivir River main stem and in several tributaries with reservoirs, but also in others without dams. In the latter, *A. alburnus* was caught not far from the mouth of the Guadalquivir River. Between 2009 and 2011, maximum rainfall values were recorder (SAIH, 2012), and this increase in flow should have favoured upstream migration of *A. alburnus* from the Guadalquivir River through the many channels built in the mouth area, promoting the first successful colonization of the Guadiamar and other Guadalquivir tributaries (personal observation). Furthermore, *A. alburnus* was neither caught in the Agrio reservoir nor in the Agrio River backwater when these tributaries were sampled as part of this study (2003, 2006) or during other studies carried out in 2007 (CMA, 2007; Fernández *et al*., 2010).

In summary, most fish species recolonized the affected reach within two years of the spill, after the main cleaning works ceased and the first large flood took place. This recolonization process came mainly from the upstream and downstream non-affected Guadiamar River reaches, and to a lesser extent from three lateral tributaries. Our results suggest that differences in the proximity and connectivity of non-affected fish sources greatly influenced the recolonization process in each site mainly in the early recovery phase. The structure of the fish assemblage at the affected reach was initially similar to that in the unaffected reference stretch. However, in the last sampling dates, the fish assemblage in the affected reach became more dissimilar from the upstream non-affected reach of the Guadiamar River. At the end of the study period, the upstream non-affected reach of the Guadiamar River held a fish assemblage abundant in native species, while exotics were most abundant in the affected reach. This result is consistent with other fish assemblage changes after severe fish kill events (Winston *et al.*, 1991; Cambray, 2003; Dextrase and Mandrak, 2005; Badino *et al.*, 2007). However, poor previous information cannot prove whether these differences began as a result of the spill or if it was an on-going process. According to our results, currently long-term threats such as mining leachates, urban sewage, agricultural pollution and exotic fish species expansion, have exceeded the initial spill effect, and this highlights the great effect of anthropogenic factors on freshwater ecosystem resilience. Therefore, in spite of the large effort invested in the recovery of the affected area, from the ichthyological point of view, the affected reach of the Guadiamar River will not recover unless both pollution and exotic species are seriously reduced.

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RESUMEN DE RESULTADOS y DISCUSIÓN

Tras la liberación de 6 hm^3 de lodos tóxicos y aguas ácidas al río Guadiamar en abril de 1998, la comunidad de peces desapareció a lo largo de un tramo aproximado de 67 km de cauce fluvial debido principalmente a la acidez del agua y a la asfixia que causó la fina granulometría de dichos lodos (Arenas *et al*., 2008).

Además de este episodio de contaminación aguda, la cuenca del río Guadiamar, especialmente en su tramo medio y bajo, lleva décadas sufriendo una serie de impactos antrópicos de carácter crónico. Entre ellos podemos destacar el aporte constante de aguas residuales urbanas e industriales insuficientemente depuradas, los lixiviados ineficientemente tratados procedentes de las minas de Aznalcóllar-Los Frailes, la contaminación difusa proveniente de la matriz agrícola, la notable fragmentación del sistema fluvial mediante un gran número de obstáculos transversales (azudes, represas, vados, pequeños puentes, etc.) y, por último, la incesante expansión de las especies exóticas (Cabrera *et al*., 1984, 1987; Arambarri *et al*., 1996; Fernández-Delgado and Drake, 2008; Pérez-Alejandre, 2009).

El objetivo principal de la presente Tesis Doctoral ha sido analizar la evolución de la comunidad piscícola desde el colapso sufrido tras el accidente hasta la actualidad.. Mediante este estudio hemos pretendido evaluar el proceso de recolonización y analizar el impacto de las alteraciones del vertido sobre las comunidades de peces del Guadiamar a diferentes escalas temporales y espaciales, aunque centradas en el tramo afectado del cauce principal. Para ello, también se han analizado aspectos relacionados con otras perturbaciones ambientales que han condicionado la dinámica de la estructura de estas comunidades piscícolas durante dicho proceso de restauración.

Inicialmente, entre la primavera y otoño de 1998 se llevó a cabo la urgente retirada de los lodos. Para ello fueron necesarias una serie de actuaciones transversales sobre el sistema y drenajes en propio cauce imprescindibles para la limpieza del tramo fluvial afectado (Arenas *et al*., 2008). Éstas labores provocaron, además de la destrucción de los distintos mesohábitats previos al vertido (Pérez-Alejandre, 2009), el aislamiento del tramo afectado impidiéndose así la recolonización de los peces desde las fuentes potenciales hasta la conclusión de esta primera fase crítica de actuaciones. A lo largo de 1999 fueron retirándose los diques de contención (Arenas *et al*., 2008), pero la llegada del estío no favoreció la continuidad de la corriente óptima para iniciar una rápida colonización. De este modo, en nuestro estudio sobre la evolución temporal de la comunidad en el tramo afectado hemos observado cierto retraso en el tiempo de recolonización en comparación con trabajos similares en contextos catastróficos parecidos (Peterson and Bayley, 1993; Sheldon and Meffe, 1995; Ensign and Leftwich, 1997; Meade, 2004; Kennedy *et al*., 2012). Así, el tiempo de recolonización en procesos de extinción de comunidades de peces que podrían asemejarse al ocurrido en el río Guadiamar, siempre se ha relacionado con el tiempo de eliminación o remediación de la afección provocada. En nuestro caso, el tiempo de remediación podemos considerarlo prolongado y, por ejemplo, los lodos permanecieron ejerciendo un importante efecto negativo hasta su retirada mecánica. De este modo, el primer muestreo en julio de 1999 de nuestro seguimiento temporal reflejó la ausencia de peces en las estaciones de muestreo localizadas en el tramo afectado del río Guadiamar (Cap. 4). Sin embargo, las intensas lluvias de octubre de ese mismo año provocaron fuertes riadas que aumentaron notablemente la continuidad longitudinal en el cauce principal del Guadiamar (SAIH, 2012). Es muy probable que la primera recolonización del tramo afectado se produjera con éstas mediante el arrastre de numerosos peces desde los tramos no afectados localizados aguas arriba y desde algún tributario. A partir del segundo muestreo, en julio de 2000, del estudio de seguimiento realizado en el tramo afectado, se capturaron abundantes individuos de especies como *Luciobarbus sclateri* y *Lepomis gibbosus*. Además, en estas primeras fases temporales la evolución de la estructura y composición de la comunidad de peces del tramo afectado mostró mayor grado de similitud con la observada en el tramo fluvial de referencia no afectado por el vertido minero (Cap. 4).

En diciembre de 2002, cuatro años después del accidente, se llevó a cabo un primer análisis del efecto del vertido, aún considerado a corto plazo, mediante un estudio comparado de parámetros descriptores de las poblaciones de *L. sclateri* en el eje principal del río Guadiamar (Cap. 2). En esta primera aproximación fue seleccionado el barbo común por ser la especie dominante en la cuenca en su conjunto (Cap. 2) y también la más abundante en la fase de recolonización objeto. Además seleccionamos la condición somática de los individuos como índice comparativo entre los tramos afectado y no afectado del eje principal. Las poblaciones de la especie objetivo mostraron diferencias espaciales significativas que fueron principalmente explicadas por variables descriptoras de la estabilidad estacional del caudal y el pH del agua. Los valores más bajos de condición correspondieron con los tramos fluviales afectados por el vertido (en su comparación con tramos no afectados). En éstos el pH del agua también era significativamente inferior debido a la presencia de restos de lodos que provocaban la acidificación (Olías *et al.*, 2005) y, además, la estabilidad del cauce a lo largo del año se mostró también mayor (Cap. 2). La interpretación de conjunto de los resultados obtenidos, nos podría sugerir que, cuatro años después de producirse el accidente, las alteraciones del sistema provocadas por el vertido seguían ejerciendo un efecto negativo sobre la especie más abundante del tramo, con mayor importancia que cualquier otra variable ambiental.

A pesar de la influencia del vertido, la calidad del agua fue mejorando significativamente a partir de 2002 (Olías, 2006) y la estructura de la comunidad de peces siguió mostrando cierta similitud a la observada en la estación de referencia no afectada (Cap. 4). A partir de 2005, el año más seco de todo el período de estudio (SAIH, 2012), la estructura de la comunidad de peces detectada en el tramo afectado empezó a mostrar claras diferencias con la comunidad de referencia. Este proceso es probable que haya estado relacionado, por un lado, con la mejor adaptación de las especies autóctonas a las fuertes restricciones del caudal provocadas por la sequía (Ribeiro and Collares-Pereira, 2010), que en nuestro caso se refleja de forma más consistente en los tramos altos del río Guadiamar. Por otro lado, también se produjo un aumento significativo de las exóticas en el tramo afectado, que podría estar favorecido por el carácter léntico predominante en el cauce durante las fases de sequía (Clavero and Hermoso, 2011). De hecho, en el año siguiente (2006), observamos un aumento de las autóctonas en el tramo afectado y de exóticas en el de referencia probablemente relacionado con el aumento en la precipitación media (SAIH, 2012) (Cap. 4).

En el período 2006-2007, ocho años después del accidente, realizamos un estudio comparativo para el conjunto de la cuenca del río Guadiamar con la intención de evaluar el efecto que, considerado a medio plazo, pudiera seguir ejerciendo el vertido sobre la comunidad de peces (Cap. 3). Para ello, se llevó a cabo un muestreo representativo de la red fluvial de la cuenca identificando las variables ambientales, a escalas espaciales diferentes, que determinaban la riqueza y diversidad de especies de peces exóticos y autóctonos (Cap. 3). En esta aproximación a nivel de la comunidad se observó que las especies nativas preferían, como en otros estudios similares en la Península Ibérica (e.g. Godinho and Ferreira, 1998, 2000; Corbacho and Sánchez, 2001; Ferreira *et al.*, 2005, 2007; Hermoso *et al.*, 2010), aquellos tramos inmersos en una matriz poco antropizada, con una mayor área de drenaje, lejos del efecto de los embalses y con abundantes refugios naturales; mientras que las exóticas, como también reflejan otros autores (e.g. Corbacho and Sánchez, 2001; Clavero *et al*., 2004; Vinyoles *et al*., 2007; Clavero and Hermoso, 2011; Ribeiro and Leunda, 2012), se veían favorecidas prioritariamente por factores a pequeña escala como establecerse en tramos bajo la influencia de un embalse (Cap. 3). Sin embargo, la localización del origen del tramo afectado por el vertido a tan sólo 5 km aguas abajo del embalse del Agrio, provoca un grado de solapamiento elevado en la influencia de efectos derivados justo en el tramo afectado del río Guadiamar. Para intentar discernir entre efectos, se comparó este tramo afectado con otros tramos aguas abajo de embalses en cuencas similares de otros tributarios de la margen norte del río Guadalquivir (Cap. 3). Al encontrarse los valores de riqueza y diversidad del tramo perteneciente al Guadiamar dentro del rango obtenido para las otras cuencas seleccionadas (Cap. 3), se concluyó que el efecto, ocho años después del vertido, de las alteraciones y perturbaciones provocadas por la catástrofe minera sobre la riqueza y diversidad de los peces del río Guadiamar, no es mayor que el que pueda estar producido de forma crónica por otros factores de impacto antrópico comunes en la propia cuenca.

Finalmente, en el último año de los muestreos correspondientes a este estudio (2011), trece años después del accidente, la comunidad de peces del tramo afectado tiende definitivamente a distanciarse de aquella presente en el tramo de referencia. Es probable que la llegada de una nueva especie exótica, *Alburnus alburnus*, al tramo afectado del río Guadiamar tenga un importante peso en este patrón (Cap. 4). Esta especie se encontraba con anterioridad en el cauce principal del río Guadalquivir (Fernández-Delgado *et al*., 2010) y, probablemente, los máximos de precipitación registrados entre 2009 y 2011 hayan favorecido la conexión con la sección fluvial de la cuenca el río Guadiamar, mostrando esta especie una amplia distribución actual en el eje principal (Cap. 4).

En resumen, la recuperación de la comunidad de peces del tramo afectado comienza año y medio después del accidente, cuando las principales labores de limpieza llegan a su fin y las fuertes lluvias del otoño de 1999 unifican de nuevo el cauce. Esta comunidad tiende a ser similar a la observada en tramos no afectados localizados aguas arriba, aunque, a corto plazo, se siga observando que el vertido ejerce efectos perniciosos para las poblaciones de la especie dominante en la cuenca, el barbo. Sin embargo, a medio plazo, puede percibirse cómo las especies exóticas se ven favorecidas por el efecto que ejercen el cúmulo de perturbaciones antrópicas sobre el tramo afectado, sin destacarse significativamente el vertido, mientras que las autóctonas se ven potenciadas en tramos fluviales más naturales con un nivel inferior de dichas perturbaciones. Al final, trece años después del vertido, la mayoría de los individuos que habitan en el tramo afectado corresponden a especies exóticas, mientras que las especies nativas dominan aquellos tramos bien conservados de la cuenca. De este modo, mientras no se disminuyan las perturbaciones que actualmente siguen degradando el tramo afectado del río Guadiamar, no se recuperará una comunidad de peces óptima en la zona afectada por el vertido minero.

CONCLUSIONES

1. La riqueza actual de peces presentes en la cuenca del río Guadiamar consta de 10 especies autóctonas y 7 exóticas, encontrándose entre las subcuencas con mayor riqueza observada para la totalidad de la cuenca del río Guadalquivir. La familia Cyprinidae es la que presenta un mayor número de especies, siendo *Luciobarbus sclateri* (Günther, 1868) la especie dominante.

2. Cuatro especies exóticas detectadas en el tramo afectado, *Gambusia holbrooki* Girard, 1859, *Carassius gibelio* (Bloch, 1782), *Alburnus alburnus* (L. 1758) y *Ameiurus melas* (Rafinesque, 1820), no estaban presentes en inventarios de la comunidad previos al vertido minero.

3. Trece años después del vertido minero, la comunidad de peces en el tramo afectado refleja un descenso de especies autóctonas y un aumento de exóticas en comparación con datos previos a dicho accidente.

4. La condición somática de *L. sclateri* mostró diferencias espaciales significativas que fueron mayormente explicadas por variables descriptoras de la estabilidad estacional del caudal junto con los valores de pH del agua. Así, los valores más elevados fueron detectados en tramos no afectados por el vertido, pero donde los individuos están concentrados en pozas aisladas y sufren un estrés ambiental muy elevado durante el estío.

5. En 2002, cuatro años después del accidente, los efectos derivados del vertido minero muestran aún su impacto negativo sobre el estado de salud de las poblaciones de *L. sclateri*.

6. La riqueza y diversidad de especies autóctonas presentes en la cuenca del río Guadiamar se ven afectadas tanto por variables a escala de tramo como de drenaje al punto. Estas especies se ven favorecidas en tramos fluviales de las zonas menos antropizadas con mayor área de drenaje, poco afectadas por la regulación de embalses y con mayor naturalidad en sus riberas.

7. Por el contrario, la riqueza y diversidad de peces exóticos se ven influenciadas principalmente por un factor ambiental a escala de tramo fluvial como es la presencia de un embalse aguas arriba. Interpretamos que las alteraciones y perturbaciones sobre el sistema fluvial que provoca este factor ambiental favorecen la proliferación de especies exóticas frente a las nativas.

8. En base a las comparaciones con otras cuencas, ocho años después del vertido minero, la influencia de las alteraciones derivadas de éste sobre la comunidad de peces del tramo afectado del río Guadiamar no puede considerarse más significativa que la ejercida por otros impactos antrópicos que, de forma crónica, actúan sobre su diversidad y la del conjunto de la cuenca hidrográfica.

9. La primera fase de recolonización en el Guadiamar comenzó con *Pseudochondrostoma willkommii, Luciobarbus sclateri, Cobitis paludica, Squalius alburnoides* entre las autóctonas y con *Cyprinus carpio, Gambusia holbrooki, Lepomis gibbosus* y *Micropterus salmoides* entre las exóticas. En esta primera fase, la estructura de la comunidad tendió a asemejarse a la del tramo de referencia no afectado, llegando a estabilizarse entre 2002 y 2004. Sin embargo, a partir de 2005, la disminución de especies autóctonas y el aumento de exóticas ha hecho que tienda a distanciarse. Esta diferencia con la estructura de la comunidad de referencia se ve aumentada 13 años más tarde con la aparición de dos especies exóticas: *Alburnus alburnus* y *Ameirus melas*.

10. Las principales fuentes de recolonización del tramo afectado fueron sectores fluviales del propio río Guadiamar. Por un lado, el tramo alto habría aportado mayormente especies autóctonas, aunque también los Centrárquidos exóticos proviniesen de dicho sector, y por otro, el tramo bajo, que habría proporcionado especies nativas de carácter diádromo, junto con la mayoría de exóticas abundantes en la marisma. Entre los tributarios, únicamente los arroyos Ardachón, Alcarayón y Majaberraque podrían haber aportado individuos de especies tanto nativas como exóticas. El resto difícilmente podrían ser considerados como fuentes, ya que las barreras existentes, básicamente de carecer químico, deben haber resultado infranqueables para individuos de poblaciones presentes en las cabeceras no contaminadas. A nuestro entender, el proceso de colonización sigue aún en marcha.

11. Las especies con mayor influencia en la dinámica de la comunidad a lo largo del proceso de recolonización fueron *Luciobarbus sclateri, Pseudochondrostoma willkommmii, Lepomis gibbosus* y *Alburnus alburnus*. El resto de especies colonizadoras fueron poco abundantes o no presentaron cambios significativos que repercutieran considerablemente en la tendencia de la comunidad con respecto a la de referencia.

12. Podemos sugerir que, actualmente, el constante aporte de aguas residuales urbanas e industriales insuficientemente depuradas, los lixiviados ineficientemente tratados procedentes de las minas de Aznalcóllar-Los Frailes, la contaminación difusa proveniente de la matriz agrícola, la secuencia de obstáculos transversales que dificultan el movimiento longitudinal de los peces y la expansión de las especies exóticas, son la principales causas que impiden la recuperación de la integridad biótica en la comunidad de peces del tramo afectado.

BIBLIOGRAFÍA

Adams, S.B. and M.L. Warren. (2005). Recolonization by warmwater fishes and crayfishes after severe drought in upper Coastal Plain hill streams. *Transactions of the American Fisheries Society* 134: 1173–1192.

Aguiar, F. and M.T. Ferreira. (2005). Human-disturbed landscapes: effects on composition and integrity of riparian woody vegetation in the Tagus River basin, Portugal. *Environmental Conservation* 32: 1–12.

Aguilar, J., R. Bellver, C. Dorronsoro, J. Fernández, I. Fernández, I. García, A. Iriarte, F. Martín, I Ortiz y M. Simón. (2003). *Contaminación de los suelos tras el vertido tóxico de Aznalcóllar*. Editorial Universidad de Granada y Consejería de Medio Ambiente Junta de Andalucía, Sevilla.

Albanese, B., P.L. Angermeier and J.T. Peterson. (2009). Does mobility explain variation in colonisation and population recovery among stream fishes?. *Freshwater Biology* 54: 1444–1460.

Alba-Tercedor, J. and A. Sanchez-Ortega, 1988. Un método rápido y simple para evaluar la calidad biológica de las aguas corrientes basado en el de Hellawell (1978). *Limnetica* 4: 51–56.

Alcaraz, C. and E. García-Berthou. (2007). Life history variation of invasive mosquitofish (*Gambusia holbrooki*) along a salinity gradient. *Biological Conservation* 139: 83–92.

Alcorlo, P., M. Otero, M. Crehuet, A. Baltanás and C. Montes. (2006). The use of the red swamp crayfish (*Procambarus clarkii*, Girard) as indicator of the bioavailability of heavy metals in environmental monitoring in the River Guadiamar (SW, Spain). *The Science of the Total Environment* 366(1): 380-390.

Allan, D., D. Erickson and J. Fay. (1997a). The influence of catchment land use on stream integrity across multiple spatial scales. *Freshwater Biology* 37: 149–161

Allan, J.D. and L.B. Johnson. (1997b). Catchment-scale analysis of aquatic ecosystems. *Freshwater Biology* 37: 107–111.

Allan, J.D. (2004). Landscapes and riverscapes: the influence of land use on stream ecosystems. *Annual Review of Ecology, Evolution and Systematics* 35: 257–284.

Almeida, P.R. (2003). Feeding ecology of *Liza ramada* (Risso, 1810) (Pisces, Mugilidae) in a south-western estuary of Portugal. *Estuarine, Coastal and Shelf Science* 57: 313-323.

Almeida, D. and G.D. Grossman. (2012). Utility of direct observational methods for assessing competitive interactions between non-native and native freshwater fishes. *Fisheries Management and Ecology* 19(2): 157-166.

Almodóvar, A., G.G. Nicola, S. Leal, M. Torralva and B. Elvira. (2012). Natural hybridization with invasive bleak *Alburnus alburnus* threatens the survival of Iberian endemic calandino *Squalius alburnoides* complex and Southern Iberian chub *Squalius pyrenaicus*. *Biological Invasions* 14: 2237–2242.

Alves, M.J., M.M. Coelho and M.J. Collares-Pereira. (1998). Diversity in the reproductive modes of females of Rutilus alburnoides complex (Teleostei, Cyprinidae): a way to avoid the genetic constaints of uniparentalism. *Molecular Biology and Evolution* 5(10): 1233-1242.

Andreu-Soler, A., F.J. Oliva-Paterna, C. Fernández Delgado and M. Torralva. (2003). Age and growth of the sand smelt, *Atherina boyeri*, Risso 1810, in the Mar Menor coastal lagoon (SE Iberian Peninsula). *Journal of Applied Ichthyology* 19: 202-208.

Andreu-Soler, A., F.J. Oliva-Paterna y M. Torralva. (2003). Estrategia de crecimiento de *Atherina boyeri* Risso, 1810 (Pisces: Atherinidae) en la laguna costera del Mar Menor (SE Península Ibérica). *Munibe* 54: 95-112.

Arambarri, P., F. Cabrera and R. González-Quesada. (1996). Quality evaluation of the surface waters entering the Doñana National Park (SW Spain). *The Science of the Total Environment* 191: 185–196.

Arenas, J.M. (2003). La dimensión del vertido minero de Aznalcóllar y la gestión de la catástrofe. *II Jornadas sobre la Restauración del Río Guadiamar después del vertido minero de Aznalcóllar. Resumen de ponencias y conclusiones*. Sevilla.

Arenas, J.M., F. Carrascal, A. Gil and C. Montes. (2008). Breve historia de la construcción del Corredor Verde del Guadiamar. In: *La restauración ecológica del río Guadiamar y el proyecto del Corredor Verde*. Consejería de Medio Ambiente, Junta de Andalucía, Sevilla, pp. 263-281.

Arenas J.M. y C. Montes (Coord.). (2008). *La restauración ecológica del río Guadiamar y el proyecto del corredor verde.* Consejería de Medio Ambiente, Junta de Andalucía, Sevilla. 486pp.

Arribas C, P. Guarnizo, T. Saldaña and C. Fernández-Delgado. (2005). Human impacts and riparian conservation status in the last 67 Km, of the Guadiamar River. In: *Integrated assessment and management of the ecosystems affected by the Aznalcóllar mining spill (SW, Spain)* (Edited by TA del Valls and J. Blasco). UNESCO/Unitwin, Cádiz, Spain. pp 115-124.

Ayora, C., A. Guijarro, C. Doménech, I. Fernández, P. Gómez, M. Manzano, A. Mora, L. Moreno, P. Navarrete, M. Sánchez, J. Serrano. (2001). Actuaciones para la corrección y el seguimiento de la contaminación hídrica. *Boletín Geológico y Minero* 112: 123–136.

Badino, G., F. Bona, A. Candiotto and S. Fenoglio. (2007). Changes in fish assemblages of a previously highly polluted river: the role of environmental recovery and alien fish invasion in the Bormida River (Italy). *Journal of Freshwater Ecology* 22(2): 255–260.

Barbour, M.T., B.D. Gerritsen, B.D. Snyder and J.B. Stribling. (1999). *Rapid Bioassessment Protocols for use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish*. Second Edition. EPA 841-8-99-002. US Environmental Protection Agency, Office of Water, Washington D.C.

Bartulovic, V., B. Glamuzina, A. Conides, J. Dulcic , D. Lucic , J. Njire and V. Kozul. (2004). Age, growth, mortality and sex ratio of sand smelt, Atherina boyeri Risso, 1810 (Pisces: Atherinidae) in the estuary of the Mala Neretva River (middle-eastern Adriatic, Croatia). *Journal of Applied Ichthyology* 20: 427–430.

Bellido, M., J.A. Hernando, C. Fernández-Delgado and M. Herrera. (1989). Alimentación de la Boga del Guadiana (*Chondrostoma polylepis willwo*mmii, Stein. 1866) en la interfase rio-embalse de Sierra Boyera (Córdoba. España). *Doñana Acta Vertebrata* 16(2): 189-20.

Bender, E.A., T.J. Case and M.E. Gilpin. (1984). Perturbation experiments in community ecology; theory and practice. *Ecology* 65: 1–13.

Benito, V., V. Devesa, O. Muñoz, M.A. Suñer, R. Montoro, R. Baos, F. Hiraldo, M. Ferrer, M. Fernández and M.J. González. (1999). Trace elements in blood collected from birds feeding in the area around Doñana National Park affected by the toxic spill from the Aznalcóllar mine. *The Science of the Total Environment* Special Issue 242: 309-323.

Bernardo, J.M., M. Ilhéu, P. Matono and A.M. Costa. (2003). Interannual variation of fish assemblage structure in a Mediterranean river: implications of streamflow on the dominance of native or exotic species. *River Research and Applications* 19: 521–532.

Bianco, P.G. (1989). Some hypotheses on the origin of primary freshwater fishes in southern Europe. *Biologia Gallo-Hellenica* 15: 59–64.

Bianco, P.G. (1990). Potential role of the paleohistory of the Mediterranean and Paratethys basins on the early dispersalof Euro-Mediterranean freshwater fishes. *Ichthyological Exploration of Freshwaters* 1: 167–184.

Blanchet, S., F. Leprieur, O. Beauchard, J. Staes, T. Oberdorff and S. Brosse. (2009). Broad-scale determinants of nonnative fish species richness are context-dependent. *Proceedings of the Royal Society of London B: Biological Sciences* 276: 2385–2394.

Blanck, A., P.A. Tedesco and N. Lamoroux. (2007). Relationships between life-history strategies of European freshwater fish species and their habitat preferences. *Freshwater Biology* 52: 843–859.

Blanco-Garrido, F. (2006). *Ecología, Distribución y Conservación de Peces Continentales en el Cuadrante Suroccidental Ibérico.* Tesis Doctoral. Universidad de Huelva.

Blasco, J., A.M. Arias and V. Saenz. (1999). Heavy metals in organisms of the River Guadalquivir estuary: possible incidence of the Aznalcollar disaster. *The Science of the Total Environment* Special Issue 242: 249-259.

Bonilla-Valverde, D., J. Ruiz-Laguna and A. Muñoz. (2004) Evolution of biological effects of Aznalcollar mining spill in the Algerian mouse (*Mus spretus*) using biochemical biomarkers. *Toxicology* 197: 123-138.

Borja, F., J.A. López-Geta, M. Martín-Machuca, R. Mantecón, C. Mediavilla, P. Del Olmo, M. Palancar and R. Vives. (2001). Marco geográfico, geológico e hidrológico regional de la Cuenca del Guadiamar. *Boletín Geológico y Minero* 112: 13–34.

Brown, M.L. and D.J. Austin. (1996). Data management and statistical techniques. In Murphy, B.R. and Willis, D.W., eds. *Fisheries Techniques*. Bethesda, MD: American Fisheries Society, pp. 17-61.

Burham, K.P. and D.R. Anderson. (2002). *Model selection and multimodel inference*. Springer, New York.

Busack, C.A. and G.A.E. Gall. (1983). An initial description of the quantitative genetics of growth and reproduction in the mosquitofish *Gambusia affinis*. *Aquaculture*, 32: 123-140.

Cabrera, F., L. Clemente, E. Díaz-Barrientos, R. López and J.M. Murillo. (1999). Heavy metal pollution of soils affected by the Guadiamar toxic flood. *The Science of the Total Environment* Special Issue 242: 117–129.

Cabrera, F., J.M. Murillo, P. Madejón, M.T. Domínguez, P. Burgos, E. Madejón, T. Marañón, A. Pérezd de Mora, R. Cordón, F. Moreno Lucas y I.F. Firón Moreno. (2008). La historia de un paisaje emergente. In: *La restauración ecológica del río Guadiamar y el proyecto del Corredor Verde*. Consejería de Medio Ambiente, Junta de Andalucía, Sevilla, pp. 145-164.

Cabrera, F., M. Soldevilla, R. Cordón and P. Arambarri. (1987). Heavy metal pollution in the Guadiamar River and the Guadalquivir estuary (South West Spain). *Chemosphere* 16: 463–468.

Cabrera, F., C.G. Toca, E. Diaz and P. Arambarri. (1984). Acid mine water and agricultural pollution in a river skirting the Doñana National Park (Guadiamar River, South West Spain). *Water Research* 18(12): 1469–1482.

Cambray, J.A. (2003). Impact on indigenous species biodiversity caused by the globalisation of alien recreational freshwater fisheries. *Hydrobiologia* 500: 217–230.

Cánovas, C.R., M. Olías, J.M. Nieto, L. Galván. (2010). Wash-out processes of evaporitic sulfate salts in the Tinto river: Hydrogeochemical evolution and environmental impact. *Applied Geochemistry* 25: 288–301.

Cárdenas, A.M. and J.M. Hidalgo. (2006). Ecological impact assessment of the Aznalcollar mine toxic spill on edaphic coleopteran communities in the Guadiamar River basin Southern Iberian Peninsula. *Biodiversity and Conservation* 15: 361-383.

Cárdenas, A.M. and J.M. Hidalgo. (2007) Application of the mean individual biomass (MIB) of ground beetles (Coleoptera, Carabidae) to assess the recovery process of the Guadiamar Green Corridor (southern Iberian Peninsula). *Biodiversity and Conservation* 16: 4131–4146.

Cardona L. (2000). Effects of Salinity on the habitat selection and growth performance of mediterranean flathead Grey Mullet *Mugil cephalus* (Osteichthyes, Mugilidae). *Estuarine, Coastal and Shelf Science*, 50: 727–737.

Cardona, L., B. Hereu and X. Torras. (2007). Juvenile bottlenecks and salinity shape grey mullet assemblages in Mediterranean estuaries. *Estuarine, Coastal and Shelf Science* 77(4): 623-632.

Carmona, J.A., O.I. Sanjur, I. Doadrio, A. Machordom and R.C. Vrijenhoek. (1997). Hybridogenetic Reproduction and Maternal Ancestry of Polyploid Iberian Fish the *Tropidophoxinellus alburnoides* Complex. *Genetics* 146: 983–993.

Carrascal, F., J.M. Arenas, M. Ramos and C. Montes. (2008). Evolución de los principales indicadores de calidad ambiental en el Corredor Verde del Guadiamar. In: *La restauración ecológica del río Guadiamar y el proyecto del corredor verde*. Consejería de Medio Ambiente, Junta de Andalucía, Sevilla. pp. 451-486.

Casterlin, M.E. and W.W. Reynolds. (1977) Aspects of habitat selection in the mosquitofish *Gambusia affinis*. *Hydrobiologia* 55(2): 125-127.

CEN. (2003). *Water quality. Sampling of fish with electricity*. EN 14011. European Committee for Standardization, Brussels.

Clarke, K.R. (1993). Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology* 18: 117–143.

Clavero, M., F. Blanco-Garrido and J. Prenda. (2004). Fish fauna in Iberia Mediterranean river basins biodiversity, introduced species and damming impacts. *Aquatic Conservation: Marine and Freshwater Ecosystems* 14: 575‑585.

Clavero, M., F. Blanco-Garrido, L. Zamora and J. Prenda. (2005) Size-related and diet variations in microhabitat use of three endangered small fishes in a Mediterranean coastal stream. *Journal of Fish Biology* 67(B): 72–85.

Clavero, M. and V. Hermoso. (2011). Reservoirs promote the taxonomic homogenization of fish communities within river basins. *Biodiversity and Conservation* 20: 41-57.

Coelho, M.M., M.J. Martins, M.J. Collares-Pereira and A.M. Pires. (1997). Diet and feeding relationship of two Iberian cyprinids. *Fisheries Managament and Ecology* 4: 83-91.

Coker, G.A., C.B. Portt and C.K. Minns. (2001). Morphological and ecological characteristics of Canadian freshwater fishes. *Canadian Manuscript Reports in Fisheries and Aquatic Sciences* 2554.

Collares-Pereira, M.J., M.F. Magalhaes, A.M. Geraldes and M.M. Coelho. (1995). Riparian ecotones and spatial variation of fish assemblages in Portuguese lowland streams. *Hydrobiologia* 303: 93–101.

Consejería de Medio Ambiente, Junta de Andalucía (ed.). (2001). *Libro Rojo de los Vertebrados Amenazados de Andalucía*. 336 pp.

Cook, R.D. (1979). Influential observations in Linear Regression. *Journal of the American Statistical Association* 74: 169-174.

Copp, G.H. and M.G. Fox. (2007). Growth and life history traits of introduced pumpkinseed (*Lepomis gibbosus*) in Europe, and the relevance to its potential invasiveness. In: *Biological Invaders in Inland Waters: Profiles, Distribution, and Threats* (Gherardi, F., ed.), Dordrecht: Springer-Verlag. pp. 289–306.

Corbacho, C. and J.M. Sanchez. (2001). Patterns of species richness and introduced species in native freshwater fish faunas of a Mediterranean-type basin: the Guadiana river (southwest Iberian Peninsula). *Regulated Rivers: Research and Management* 17: 699–707.

Cordos, E., R. Rautiu, C. Roman, M. Ponta, T. Frentiu, A. Sarkany, L. Fodorpataki, K. Macalik, C. McCormick, D. Weiss. (2003). Characterization of the rivers system in the mining and industrial area of Baia Mare, Romania. *European Journal of Mineral Processing and Environmental Protection* 3(3): 324-335.

Cowx, I.G. and M.J. Collares-Pereira. (2002). Freshwater fish conservation: options for the future. In: *Conservation of Freshwater Fish: Options for the Future*, Collares-Pereira MJ, Cowx IG, Coelho MM (eds). Fishing News Books/Blackwell Science: Oxford. Pp. 443–452.

Cowx, I.G. and R.L. Welcomme (eds). (1998). *Rehabilitation of Rivers for Fish*. Fishing News Books/ Blackwell Science, Oxford.

Crivelli, A.J. (1981). The biology of the common carp, *Cyprinus carpio* L. in the Camargue, southern France. *Journal of Fish Biology* 18(3): 271-290.

De Buen, F. (1929). La invasión de nuestras aguas dulces por las Gambusias(*Gambusia holbrooki* Grd.). *Revisión de Biología Forestal y Limnología* 1(1): 49-53.

De Miguel, R.J., E. Pino, A. Ramiro, F. Aranda, J. P. Peña, I. Doadrio and C. Fernández-Delgado. (2010). On the occurrence of Anaecypris hispanica, an extremely endangered Iberian endemism, in the Guadalquivir River basin. *Journal of Fish Biology* 76: 1454–1465.

De Silva, S.S. (1980). Biology of juvenile grey mullet: a short review. *Aquaculture*, 19, 21-36

Del Valls, A. and J. Blasco. (2005). *Integrated assessment and management of the ecosystems affected by the Aznalcóllar mining spill (SW, Spain)*. UNESCO Unitwin, Cádiz, Spain.

Delibes, M., S. Cabezas and B. Jimenez. (2009). Animal decisions and conservation: the recolonization of a severely polluted river by the Eurasian otter. *Animal Conservation* 12: 400-407.

Detenbeck, N.E., P.W. Devore, G.J. Niemi and A. Lima. (1992). Recovery of temperate-stream fish communities from disturbance: a review of case studies and synthesis of theory. *Environmental Management* 16: 33–53.

Dextrase, A. and N.E. Mandrak. (2005). Impacts of invasive alien species on freshwater fauna at risk in Canada. *Biological Invasions* 8: 13–24.

Díaz_Luna, J.L. and F. Gómez-Caruana. (1998). Una breve historia sobre propios y extraños. *Biológica* 24: 47.

Doadrio, I. (1990). Phylogenetic relationships and classification of Western Palearctic species of the genus Barbus (Osteichthyes, Cyprinidae). *Aquatic Living Resources* 3: 265–282.

Doadrio, I. (1996). *Estudio sobre la calidad de las aguas continentales destinadas a la vida piscícola en el ámbito de la cuenca del Guadalquivir*. INIMA. Ministerio de Medio Ambiente, Madrid.

Doadrio, I. (2001). *Atlas y Libro Rojo de los Peces Continentales de España*. Dirección General de Conservación de la Naturaleza, Madrid. 366 pp.

Doadrio, I. and J.A. Carmona. (2004). Phylogenetic relationships and biogeography of the genus Chondrostoma inferred from mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution* 33: 802–815.

Doadrio, I., J.A. Carmona and A. Machordom. (2002). Haplotype Diversity and Phylogenetic Relationships Among the Iberian Barbels (*Barbus*, Cyprinidae) Reveal Two Evolutionary Lineages. *The Journal of Heredity* 93: 140 - 147.

Doadrio, I., S. Perea, P. Garzón-Heydt and J.L. González. (2011). *Ictiofauna continental española. Bases para su seguimiento.* DG Medio Natural y Política Forestal. MARM. Madrid. 616 pp.

Domínguez, J., J.C. Pena, J. De Soto and E. Luis. (2002). Alimentación de dos poblaciones de perca sol (*Lepomis gibbosus*), introducidas en el Norte de España: Resultados preliminares. *Limnética* 21(1-2): 135-144.

Drake, P.; A.M. Arias y R.B. Rodríguez. (1984). Biología de los Mugílidos *(Osteichthies Mugilidae)* en los esteros de las salinas de San Fernando (Cádiz). I. Crecimiento en longitud y peso. *Investigación Pesquera,* 48: 27-43.

Drake, P., F. Baldo, J.A. Cuesta, D. García-González, A. Silva-García, A.M. Arias, A. Rodrıguez, I. Sobrino, and C. Fernández-Delgado. (1999). Initial effects of the toxic waste spill (Aznalcóllar mine accident) on the aquatic macrofauna of the Guadalquivir Estuary. *The Science of the Total Environment* Special Issue 242: 271-280.

EEA’s Corine land cover. (2009). Corine land cover 2000 (CLC2000) seamless vector database. Disponible en: http://www.eea.europa.eu/data-and-maps/data/corine-land-cover-2000-clc2000-seamless-vector-database. Consultado en mayo de 2010.

Elvira, B. (1984). First record of the North American catfish *Ictalurus melas* (Rafinesque, 1820) (Pisces, Ictaluridae) in Spanish waters. *Cybium* 8(1): 96-98.

Elvira, B. (1995). Conservation status of endemic freshwater fish in Spain. *Biological Conservation* 72: 129–136.

Elvira, B. (1998). Impact of introduced fish on the native freshwater fish fauna of Spain. In: I.G. Cowx (ed.) *Stocking and Introduction of Fish*, Fishing News Books, Oxford. pp. 186–190.

Elvira, B. y A. Almodóvar. (2001). Freshwater fish introductions in Spain: facts and figures at the beginning of the 21st century. *Journal of Fish Biology* 59: 323–331.

Encina, L. and C. Granado-Lorencio. (1997a). Food habits and food resource partitioning in three coexisting *Barbus* species. *Folia Zoologica* 46: 325–336.

Encina, L. and C. Granado-Lorencio. (1997b). Seasonal variations in the physiological status and energy content of somatic and reproductive tissues of chub. *Journal of Fish Biology* 50(3): 511-522.

Encina, L., A. Rodríguez-Ruiz and C. Granado-Lorencio. (2006). The Iberian ichthyofauna: Ecological contributions. *Limnetica* 25(1-2): 349-368.

Ensign, W.E., K.N. Leftwich, P.L. Angermeier and C.A. Dolloff. (1997). Factors influencing stream fish recovery following a large-scale disturbance. *Transactions of the American Fisheries Society* 126: 895–907.

Fernández, R.O., J.V.G. Cervera and K. Vanderlinden. (2007). Temporal and spatial monitoring of the pH and heavy metals in a soil polluted by mine spill. Post cleaning effects. *Water, Air, and Soil Pollution* 178: 229-243.

Fernandez-Delgado, C. (1989) Life-history patterns of the mosquitofish, *Gambusia affinis,* in the estuary of the Guadalquivir river of south-west Spain. *Freshwater Biology* 22: 395–404.

Fernández-Delgado C. (1990). Life history patterns of the common carp, *Cyprinus carpio,* in the estuary of the Guadalquivir River in the south west Spain. *Hydrobiología* 206(1): 19-28.

Fernández-Delgado, C. (coord). (2003). *Naturaleza de Andalucía*. Ed. Giralda, Sevilla. 339 pp.

Fernández-Delgado, C., F.J. Oliva-Paterna, P.A. Rincón, L. Gálvez-Bravo, R.J. De Miguel, A. Ramiro, E. Pino, J.P. Peña, F. Aranda, C. Arribas and R. Moreno (2007). *Informe final de la Primera Fase del Proyecto: Bases para la elaboración del Plan de Conservación de las Especies de Peces Continentales Autóctonas de Andalucía*. Consejería de Medio Ambiente. Junta de Andalucía.

Fernández-Delgado, C., P. Drake, A.M Arias, D. García. (2000). *Peces de Doñana y su entorno.* Organismo Autónomo Parques Nacionales. Ediciones del Ministerio de Medio Ambiente. Madrid. Spain. 271 pp.

Fernández-Delgado, C. and Drake, P. (2008). Efectos del accidente minero de aznalcóllar sobre la comunidad de peces del río Guadiamar y estuario del Guadalquivir. In: *La restauración ecológica del río Guadiamar y el proyecto del Corredor Verde*. Junta de Andalucía, Sevilla, pp. 263-281.

Fernández-Delgado, C, J.A. Hernando, M. Herrera and M. Bellido. (1988). Life-History patterns of the sandsmelt *Atherina boyeri* Risso, 1810 in the estuary of the Guadalquivir river, Spain. *Estuarine, Coastal and Shelf Science* 27: 697-706.

Fernández-Delgado, C., J. A. Hernando y M. Bellido. (1989). Age and growth of yellow eels, *Anguilla anguilla*, in the estuary of the Guadalquivir river (south-west Spain). *Journal of Fish Biology*, 3(4): 561-570.

Fernández-Delgado, C. and M. Herrera. (1994). Population structure, growth and reproduction of *Tropidophoxinellus alburnoides* (Steindachner, 1866) in an intermittent stream of the Guadalquivir River basin (Southern Spain). *Archiv für Hydrobiologie* 130: 359–369.

Fernández-Delgado, C. and M. Herrera. (1995a). Age structure, growth and reproduction of *Leuciscus pyrenaicus* in an intermittent stream in the Guadalquivir river basin, southern Spain. *Journal of Fish Bilogy* 46(3): 371-380.

Fernández-Delgado, C. and M. Herrera. (1995b). Age structure, growth and reproduction of *Rutilus lemmingii* in a intermittent stream of the Guadalquivir river basin, southern Spain. *Hydrobiologia* 299(3): 207-213.

Fernández-Delgado, C., F.J. Oliva-Paterna, P.A. Rincón, L. Gálvez-Bravo, R.J. De Miguel, A. Ramiro, E. Pino, J.P. Peña, F. Aranda, C. Arribas and R. Moreno (2010). Basis for the development of a conservation program for the freshwater fish in Andalusia. Junta de Andalucía, Sevilla. Disponible en: http://www.uco.es/investiga/grupos/aphanius/proyectodetalle.php?id_proyectos=7. Consultado en enero de 2011.

Fernández-Delgado, C., T. Saldaña, R. Pérez Alejandre, C. Arribas, J. Berná, A. Fernández-Borlán, R.J. De Miguel, M. Fernández, E. Pino y A. Ramiro. (2006). Seguimiento del efecto del vertido tóxico de las minas de Aznalcóllar sobre la comunidad de peces del río Guadiamar. Consejería de Medio Ambiente. Junta de Andalucía. Informe Final. Consejería de Medio Ambiente. Junta de Andalucía.

Ferreira, M.T., Aguiar, F.C. and Nogueira, C. (2005). Changes in riparian woods over space and time: influence of environment and land use. *Forest Ecology and Management* 212: 145–159.

Ferreira, M.T., L. Sousa, J.M. Santos, L. Reino, J. Oliveira, P.R. Almeida and R.V. Cortes. (2007). Regional and local environmental correlates of native Iberian fish fauna. *Ecology of Freshwater Fish* 16: 504-514.

Ferreras-Romero, M., F.J. Cano-Villegas and J.C. Salamanca-Ocaña. (2003). Valoración de la cuenca del río Guadiamar (sur de España), afectada por un vertido minero, en base a su odonatofauna. *Limnetica* 22(3-4): 53-62.

Field, A. (2005). *Discovering Statistics Using SPSS*. Second edition. SAGE, London. 779pp.

Filipe, A.F., I.G. Cowx and M.J. Collares-Pereira. (2002). Spatial modelling of freshwater fish in semi-arid river systems: a tool for conservation. *River Research and Applications* 18: 123–136.

Fox, M.G. (1994). Growth, density, and interspecific influences on pumpkinseed sunfish life histories. *Ecology* 75: 1157–1171.

Fox, M.G. and A.J. Crivelli. (2001). Life history traits of pumpkinseed (*Lepomis gibbosus*) populations introduced into warm thermal environments. *Archiv für Hydrobiologie* 150: 561–580.

Gallart, F., G. Benito, J.P. Martín-Vide, A. Benito, J.M. Prió and D. Regüés. (1999). Fluvial geomorphology in the dispersal and fate of pyrite mud particles released by the Aznalcóllar mine tailings spill. *The Science of the Total Environment* Special Issue 242: 13-26.

García-Berthou, E. (1991). Ontogenetic diet shifts and interrupted piscivory in introduced largemouth bass (Micropterus salmoides). *International Review of Hydrobiology*, 258(1): 24-33.

García-Berthou, E. (1999). Food of introduced mosquitofish: ontogenetic diet shift and prey selection. *Journal of Fish Biology* 55: 135–147.

García-Berthou, E. (2001a). On the misuse of residuals in ecology: testing regression residuals vs. the analysis of covariance. *Journal of Animal Ecology* 70: 708–711.

García-Berthou, E. (2001b). Size- and depth-dependent variation in habitat and diet of the common carp (*Cyprinus carpio*). *Aquatic sciences* 63 (4): 466-476.

García-Berthou, E. and R. Moreno-Amich. (1993). Multivariate analysis of covariance in morphometric studies of the reproductive cycle. *Canadian Journal of Fisheries and Aquatic Sciences* 50: 1394–1399.

García-Berthou, E. and R. Moreno-Amich. (2000a). Introduction of exotic fish into a Mediterranean lake over a 90-year period. *Archiv für Hydrobiologie* 149: 271–284.

Garicía-Berthou, E. and R. Moreno-Amich. (2000b). Food of introduced pumpkinseed sunfish: ontogenetic diet shift and seasonal variation. *Journal of Fish Biology* 66 (2): 315-326.

García de Jalón, M., Mayo-Rustarazo, B. Gallego and F. Hervella. (1993). Las comunidades piscícolas de los embalses de Madrid. Directrices para su gestión. *Ecologia* 7: 467-485.

Garralon, A., P. Gomez, M.J. Turrero, A. Benito, J.M. Prio and D. Regues. (1999). The geochemical aspects of toxic waters retained in the Entremuros area (Spain). *The Science of the Total Environment* Special Issue 242: 27-40.

Gasith, A. and V.H. Resh. (1999). Streams in Mediterranean climate regions: abiotic influences and biotic responses to predictable seasonal events. *Annual Review of Ecology and Systematics* 30: 51–81.

Gido, K.B. and J.H. Brown. (1999). Invasion of North American drainages by alien fish species. *Freshwater Biology* 42: 387–399.

Gillette, D.P., A.M. Fortner, N.R. Franssen, S. Cartwright, C.M. Tobler, J.S. Wesner, P.C. Reneau, F.H. Reneau, I. Schlupp, E.C. Marsh-Matthews, W.J. Matthews, R.E. Broughton and C.W. Lee. (2012). Patterns of change over time in darter (Teleostei: Percidae) assemblages of the Arkansas River basin, northeastern Oklahoma, USA. *Ecography* 35: 855–864.

Gisbert, E. L. Cardona and F. Castello (1995a). Competition between mullet fry. *Journal of Fish Biology* 47: 414–420.

Gisbert, E., L .Cardona and F. Castello (1995b). Alimentación de los alevines de mugílidos en el Delta del Ebro. *Miscellanea Zoológica* 18: 145-151.

Gisbert, E. L. Cardona and F. Castello (1996). Resource partitioning among planktivorous fish larvae and fry in a mediterranean coastal lagoon. *Estuarine, Coastal and Shelf Science* 43: 723–735.

Giudicelli, J., M. Dakki and A. Dia. (1985). Caractéristiques abiotiques et hydrobiologiques des eaux courantes méditerranéennes. *Verhandlungen Internationale Vereinigung Limnologie* 22: 2094-2101.

Glasby, T.M. and A.J. Underwood. (1996). Sampling to differentiate between pulse and press perturbations. *Environmental Monitoring and Assessment* 42: 241–252.

Godinho, F.N. and M.T. Ferreira. (1996). The application of size-structure indices to Micropterus salmoides (Lacépède, 1802) and Lepomis gibbosus (L., 1758) populations as a management tool for southern Iberian reservoirs. *Publicaciones Especiales, Instituto Español de Oceanographía* 21: 275–281.

Godinho, F.N. and M.T. Ferreira. (1998). The relative influence of exotic species and environmental factors on an Iberian native fish community. *Environmental Biology of Fish* 51: 41–51.

Godinho, F. N. and M. T. Ferreira. (2000). Composition of endemic fish assemblages in relation to exotic species and river regulation in a temperate stream. *Biological Invasions* 2: 231–244.

Godinho, F. N., M. T. Ferreira and R. V. Cortes. (1997a). The environmental basis of diet variation in pumpkinseed sunfish, *Lepomis gibbosus*, and largemouth bass, *Micropterus salmoides*, along an Iberian river basin. *Environmental Biology of Fishes* 50: 105–115.

Godinho, F. N., M. T. Ferreira and R. V. Cortes. (1997b) Composition and spatial organization of fish assemblages in the lower Guadiana basin, southern Iberia. *Ecology of Freshwater Fish* 6: 134–143.

Goede, R.W. and B.A. Barton. (1990). Organismic indices and an autopsy-based assessment as indicator of health and condition of fish. *American Fisheries Society Symposium* 8: 93–108.

Gomes-Ferreira, A., F. Ribeiro, L. Moreira Da Costa, I. G. Cowx and M. J. Collares-Pereira. (2005). Variability in diet and foraging behaviour between sexes and poliploidy forms of the hybridogenetic *Squalius alburnoides* complex (Cyprinidae) in the Guadiana River basin, Portugal. *Journal of Fish Biology* 66: 454-467.

González-Carmona, J. A. (1997) *Evolución y modelos de reproducción en un organismo unisexual de la península Ibérica: El complejo Tropidophoxinellus alburnoides*. Tesis doctoral. Universidad Complutense de Madrid.

Grigg, N.S. (1996). *Water resources management. Principles, regulations, and cases.* McGraw-Hill, New York.

Grimalt, J.O. and E. Macpherson. (1999). The environmental impact of the mine tailing accident in Aznalcóllar (South-west Spain). *The Science of the Total Environment* Special Issue 242 (1-3): 1-337.

Gutiérrez, J. L. (1997). *Estructura por edades, crecimiento y biología reproductiva del pez sol (Lepomis gibbosa L. 1758) en el río Guadiato (Córdoba)*. Tesis Doctoral. Universidad de Córdoba.

Gutiérrez-Estrada, J.C., I. Pulido-Calvo and C. Fernandez-Delgado. (2000). Age-structure, growth and reproduction of the introduced pumpkinseed (*Lepomis gibbosus*, L. 1758) in a tributary of the Guadalquivir River (Southern Spain). *Limnetica* 19: 21–29.

Harris, J.H. (1995). The use of fish in ecological assessments. *Australian Journal of Ecology* 20: 65-80.

Harrison, T.D., A.E.L. Ramm, E.C. Cerff. (1986). A low-cost effective trap for use in sampling aquatic fauna. *Aquaculture* 58: 145-149.

Harvey, B.C. (1987). Susceptibility of Young-of-the-Year fishes to downstream displacement by flooding. *Transactions of the American Fisheries Society* 116: 851-855.

Harvey, B.C. (1991). Interaction of abiotic and biotic factors influences larval fish survival in an Oklanoma Stream. *Canadian Journal of Fisheries and Aquatic Sciences* 48.

He, Y.F., Wang, J.W., Lek-Ang, S. and Lek, S. (2010). Predicting assemblages and species richness of endemic fish in the upper Yangtze River. *The Science of the Total Environment* 408: 4211-4220.

Hellawell, J.M. (1978). *Biological Surveillance of Rivers*. Water Research Center, Stevenage. 332 pp.

Hermoso V, M. Clavero, F. Blanco-Garrido and J. Prenda. (2009). Assessing freshwater fish sensitivity to different sources of perturbation in a Mediterranean basin. *Ecology of Freshwater Fish* 18: 269-281.

Hermoso, V., M. Clavero, F. Blanco-Garrido and J. Prenda. (2010). Assessing the ecological status in species-poor systems: A fish-based index for Mediterranean Rivers Guadiana River, SW Spain. *Ecological Indicators* 10: 1152-1161.

Hernández, L.M., B. Gómara and M. Fernández. (1999). Accumulation of heavy metals and As in wetland birds in the area around Doñana National Park affected by the Aznalcóllar toxic spill. *The Science of the Total Environment* Special Issue 242: 293-308.

Herrera, M. and C. Fernández-Delgado. (1992). The life-history patterns of *Barbus bocagei sclateri* (Günther, 1868) in a tributary stream of the Guadalquivir River basin, southern Spain. *Ecology of Freshwater Fish* 1: 42-51.

Herrera, M. and C. Fernández- Delgado. (1994). The age, growth and reproduction of *Chondrostoma polylepis willkommii* in a seasonal stream in the Guadalquivir River basin (southern Spain). *Journal of fish Biology* 44(1): 11-22.

Herrera, M., J.A. Hernando, C. Fernández-Delgado and M. Bellido. (1988). Age, growth and reproduction of the barbel, *Barbus sclateri* (Günther, 1868), in a first-order stream in southern Spain. *Journal of Fish Biology* 33: 371-388.

Hladík, M. and J. Kubecka. (2003). Fish migration between a temperate reservoir and its main tributary. *Hydrobiologia* 504: 251–266.

Hoey, A.S. and M.I. McCormick. (2004). Selective predation for low body condition at the larval-juvenile transition of a coral reef fish. *Oecologia* 139: 23-29.

Hubbs, C.L. (1940). Speciation of fishes. *American Naturalist* 74: 198–211.

Hudson-Edwards, K.A., M.G. Macklin, H.E. Jamieson, P.A. Brewer, T.J. Coulthard, A.J. Howard and J.N. Turner. (2003). The impact of tailings dam spills and cleanup on sediment and water quality in river systems: the Ríos Agrio-Guadiamar, Aznalcóllar, Spain. *Applied Geochemistry*. 18: 221–239.

Hughes, S.J., J.M. Santos, M.T. Ferreira, R. Caraça and A.M. Mendes. (2009). Ecological assessment of an intermittent Mediterranean river using community structure and function: evaluating the role of different organism groups. *Freshwater Biology* 54: 2383–2400.

Hughes, S., J. Santos, T. Ferreira and A. Mendes. (2010). Evaluating the Response of Biological Assemblages as Potential Indicators for Restoration Measures in an Intermittent Mediterranean River. *Environmental Management* 46: 285-301.

Hynes H.B.N. 1995. *The ecology of running waters*. Liverpool University press, Liverpool.

ICOLD. (2001). *Tailings dams—risk of dangerous occurrences, lessons learnt from practical experiences*. United Nations Environmental Programme (UNEP) Division of Technology, Industry and Economics (DTIE) and International Commission on Large Dams (ICOLD), Paris.

IUCN. (2012). IUCN Red List of Threatened Species. Version 2012.2. <www.iucnredlist.org>. Consultado en enero de 2013.

Jakob, E.M., S.D. Marshall and G.W. Uetz. (1996). Estimating Fitness: A Comparison of Body Condition Indices. *Oikos* 77(1): 61-67.

Johnson, J.B. and K.S. Omland. (2003). Model selection in ecology and evolution. *Trends in Ecology and Evolution* 19: 101-108.

Junk, W.J., P.B. Bayley and R.E. Sparks. (1989). The flood pulse concept in river-floodplain systems. *Canadian Special Publication of Fisheries and Aquatic Sciences* 106: 110–127.

Kaiser, H.F. (1960). The application of electronic computers to factor analysis. *Educational and Psychological Measurement* 20: 141-151.

Karr, J.R. (1991). Biological integrity: a long-neglected aspect of water resource management. *Ecological Applications* 1: 66–84.

Kennedy, R.J., R. Rosell and J. Hayes. (2012). Recovery patterns of salmonid populations following a fish kill event on the River Blackwater, Northern Ireland. *Fisheries Management and Ecology* 19: 214–223.

Kieffer, J.D. and P.W. Colgan. (1993). Foraging flexibility in pumpkinseed (*Lepomis gibbosus*): influence of habitat structure and prey type. *Canadian Journal of Fisheries and Aquatic Sciences* 50: 1699–1705.

Kopp, D., J. Syvaranta, J. Figuerola, A. Compin, F. Santoul, and R. Céréghino. (2009). Environmental effects related to the local absence of exotic fish. *Biological Conservation* 142: 3207-3212.

Kottelat, M. and J. Freyhof. (2007). *Handbook of European Freshwater Fishes*. Kottelat, Cornol, Switzerland and Freyhof, Berlin. 646 pp.

Koutrakis, E.T., N.I. Kamidis and I.D. Leonardos. (2004). Age, growth and mortality of a semi-isolated lagoon population of sand smelt, Atherina boyeri (Risso, 1810) (Pisces: Atherinidae) in an estuarine system of northern Greece. *Journal of Applied Ichthyology* 20: 382–388.

Kruskal, J.B. (1964a). Multidimensional scaling by optimizing goodness of fit to a nonmetric hypothesis. *Psychometrika*, 29: 1–27.

Kruskal, J.B. (1964b). Nonmetric multidimensional scaling: a numerical method. *Psychometrika* 29: 115–129.

Kruskal, J.B. and Wish M. (1978). *Multidimensional Scaling*. Sage Publications, Beverly Hills, CA.

Kubach, K.M., M.C. Scott and J.S. Bulak. (2011). Recovery of a temperate riverine fish assemblage from a major diesel oil spill. *Freshwater Biology* 56: 503–518.

Lake, P.S. (2000). Disturbance, patchiness, and diversity in streams. *Journal of the North American Benthological Society* 19(4): 573–592.

Lake, P.S. and L.A. Barmuta. (1986). Stream benthic communities: persistent presumptions and current speculations. In: *Limnology in Australia.* P. De Deckker and W. D. Williams (editors). CSIRO, Melbourne, and Dr. W. Junk Publishers, Dordrecht, The Netherlands. pp. 263–276.

Lammert, M. and J.D. Allan. (1999). Assessing biotic integrity of streams: effects of scale in measuring the influence of land use/cover and habitat structure on fish and macroinvertebrates. *Environmental Management* 23: 257–270.

Larimore, R.W. and P.W. Smith. (1963). The fishes of Champaign County, Illinois, as affected by 60 years of stream changes. *Bulletin of the Illinois Natural History Survey* 28(2): 299–382.

Leonardos, I.D. and A. Sinis. (2000). Age, growth and mortality of Atherina boyeri Risso,1810 (Pisces: Atherinidae) in the Mesolongi and Etolikon lagoons (W. Greece). *Fisheries Research* 45: 81-91.

Leunda, P.M., B. Elvira, F. Ribeiro, R. Miranda, J. Oscoz, M.J. Alves and M.J. Collares-Pereira. (2009). International Standardization of Common Names for Iberian endemic Freshwater Fishes. *Limnetica*, 28(2): 189-202.

Leunda, P.M., J. Oscoz, B. Elvira, A. Agorreta, S. Perea, R. Miranda. (2008). Feeding habits of the exotic black bullhead *Ameiurus melas* (Rafinesque) in the Iberian Peninsula: first evidence of direct predation on native fish species. *Journal of Fish Biology* 73: 96-114.

Lobón-Cerviá, J. (1991). *Dinámica de poblaciones de peces. Pesca eléctrica y los métodos de capturas sucesivas en la estima de abundancias*. Monografías del Museo Nacional de Ciencias Naturales, Barcelona.

Lobón-Cerviá, J. y A. De Sostoa. (1987). El crecimiento del cacho (L*euciscus cephalus pyrenaicus* Günther , 1862) en el río Jarama (cuenca del Tajo). *Doñana Acta Vertebrata* 14: 41-51.

Lonzarich, D.G., M.L.Jr. Warren and M.R.E. Lonzarich. (1998). Effects of habitat isolation on the recovery of fish assemblages in experimentally defaunated stream pools in Arkansas. *Canadian Journal of Fisheries and Aquatic Sciences* 55: 2141–2149.

López-Pamo, E., D. Barettino, A. Pacheco, C. Ortiz, G. Arránz, J.C. Gumiel, B. Martínez-Pledel, M. Aparicio and O. Montouto. (1999). The extent of the Aznalcóllar pyrite sludge spill and its effects on soils. *The Science of the Total Environment* Special Issue 242: 57-88.

Lorenzoni, M., A.J. Martin Dörr, R. Erra, G. Giovinazzo, M. Mearelli and S. Selvi. (2002). Growth and reproduction of largemouth bass (*Micropterus salmoides* Lacépède, 1802) in Lake Trasimeno (Umbria, Italy). *Fisheries Research*, 56(1): 89-95.

Lorenzoni, M., M. Corboli, L. Ghetti, G. Pedicillo and A. Carosi. (2007). Growth and reproduction of the goldfish Carassius auratus: a case study from Italy. Invading Nature - Springer Series In: *Invasion Ecology*, vol. 2. Chapter 13.

Lucas, M. and E. Baras. (2000). Methods for studying spatial behaviour of freshwater fishes in the natural environment. *Fish and Fisheries* 1: 283-316.

Lucena, J., M. Blasco and I. Camacho. (1979). Estudio del crecimiento en peso y logitud del *Barbus barbus sclateri* Günther, del embalse de Cubillas. *Boletín de la Real Sociedad Española de Historia Natural* (Biología) 77: 479-488.

Lucena, J. y I. Camacho. (1978). Variaciones estacionales de algunos parámetros en el barbo de sclater (*Barbus barbus sclateri* Günther). *Boletín de la Real Sociedad Española de Historia Natural (Biología)* 76: 243-251.

Luque, G.M., J. Reyes-Lopez and J. Fernández-Haeger. (2007). Recovery of ground ant (Hymenoptera: Formicidae) communities six years after a major environmental disaster. *Environmental Entomology* 36: 337-347.

Macklin, M.G., P.A. Brewer, D. Balteanu, T.J. Coulthard, B. Driga, A.J. Howard, S. Zaharia. (2003). The long term fate and environmental significance of contaminant metals released by the January and March 2000 mining tailings dam failures in Maramures County, upper Tisa Basin, Romania. *Applied Geochemistry* 18: 241–257.

Macklin, M.G., P.A. Brewer, K.A. Hudson-Edwards, G. Bird, T.J. Coulthard, I.A. Dennis, P.J. Lechler, J. R. Miller and J. N. Turner. (2006). *Geomorphology* 79: 423.

Macklin, M.G., K.A. Hudson-Edwards, H.E. Jamieson, P. Brewer, T.J. Coulthard, A.J. Howard and V.H. Renenda. (1999). Physical stability and rehabilitation of sustainable aquatic and riparian ecosystems in the Río Guadiamar, Spain, following the Aznalcóllar mine tailings dam failure. *Mine, Water and Environment*. International Mine Water Association Congress, Seville, Spain. pp. 271-278.

Madejón, P., M.T. Dominguez and J.M. Murillo. (2010). Seasonal and temporal evolution of nutrient composition of pastures grown on remediated and non remediated soils affected by trace element contamination (Guadiamar Valley, SW Spain). *Spanish Journal of Agricultural Research* 8: 729-740.

Magalhaes, M.F. (1993a). Effects of season and body-size in the distribution and diet of the Iberian chub *Leuciscus pyrenaicus* (Gunther) in a lowland river. *Journal of Fish Biology* 42: 875–888.

Magalhaes, M.F. (1993b). Feeding of an Iberian stream cyprinid fish assemblage: seasonality of resource use in a highly variable environment. *Oecologia* 96: 253–260.

Magalhâes, M.F., D.C. Batalha and M.J. Collares-Pereira. (2002a). Gradients in stream fish assemblages across a Mediterranean landscape: contributions of environmental factors and spatial structure. *Freshwater Biology* 47: 1015–1031.

Magalhâes, M.F., P. Beja, C. Canas and M.J. Collares-Pereira. (2002b). Functional heterogeneity of dry-season fish refugia across a Mediterranean catchment: the role of habitat and predation. *Freshwater Biology* 47: 1919–1934.

Manzano, M., C. Ayora, C. Domenech, P. Navarrete, A. Garralon, and M.J. Turrero. (1999). The impact of the Aznalcóllar mine tailing spill on groundwater. *The Science of the Total Environment* Special Issue 242: 189-209.

Marquez-Ferrando, R., J.M. Pleguezuelos and X. Santos. (2009). Recovering the Reptile Community after the Mine-Tailing Accident of Aznalcollar (Southwestern Spain) *Restoration Ecology* 17: 660-667.

Mather, P.M. (1976). *Computational Methods of Multivariate Analysis in Physical Geography*. J. Wiley and Sons, London. pp. 532.

Matthews, W.J. (1986). Fish faunal structure in an Ozark stream: stability, persistence and a catastrophic flood. *Copeia* 1986: 388–397.

Matthews, W.J. (1998). *Patterns in Freshwater Fish Ecology*. Chapman and Hall, New York.

Matthews, W.J. and E. Marsh-Matthews. (2003). Effects of drought on fish across axes of space, time and ecological complexity. *Freshwater Biology* 48: 1232–1253.

McArthur, R.H. and Wilson, E.O. (1967). *The theory of island biogeogeography*. Princeton University Press, Princeton, New Jersey.

McCune, B. and J. Grace. (2002). *Analysis of Ecological Communities, MjM Software Design*. Gleneden Beach, OR. pp. 300.

Meade, R. (2004). Fish and invertebrate recolonization in a Missouri prairie stream after an acute pollution event. *North American Journal of Fisheries Management* 24: 7–19.

Meharg, A.A., D. Osborn, D.J. Pain, A. Sánchez, and M.A. Naveso. (1999). Contamination of Doñana food-chains after the Aznalcóllar mine disaster. *Environmental Pollution* 105(3): 387-390.

Mehner, T., J. Ihlau, H. Dorner and F. Holker. (2005). Can feeding of fish on terrestrial insects subsidize the nutrient pool of lakes? *Limnology and Oceanography* 50: 2022-2031.

Montes, C. (2003). La investigación en el desarrollo del Corredor Verde del Guadiamar. *II Jornadas sobre la Restauración del Río Guadiamar después del vertido minero de Aznalcóllar. Resumen de ponencias y conclusiones*. Sevilla.

Moran-López, R., E. Da Silva, J.L. Pérez Bote and C. Corbacho. (2006). Associations between fish assemblages and environmental factors for Mediterranean-type rivers during summer. *Journal of Fish Biology* 69: 1552–1569.

Moreno-Rojas, R., M.J. Gordillo-Otero, A. Sánchez-Palenzuela, T. Saldaña, C. Arribas, and C. Fernández-Delgado. (2005). Effect of the toxic waste spill (Aznalcóllar mine accident) on the levels of heavy metals in different organs of the Guadiamar River fish. In: Del Valls, A. and J. Blasco, eds. *Integrated assessment and management of the ecosystems affected by the Aznalcóllar mining spill (sw, Spain).* UNESCO, pp. 125-139.

Morgan, M.J. (2004). The relationship between fish condition and the probability of being mature in American plaice (*Hippoglossoides platessoides*). *ICES Journal of Marine Science* 61(1): 64-70.

Moyle, P.B. (1986). Fish introductions into North America: patterns and ecological impact. In: *Ecology of Biological Invasions of North America and Hawaii.* Mooney, H.A. and Drake, J.A. (eds). Ecological Studies, vol 58. Springer, New York. pp. 27–43.

Moyle, P.B. and Leidy, R.L. (1992). Loss of biodiversity in aquatic ecosystems: evidence from fish faunas. In: *Conservation Biology: The Theory and Practice of Nature Conservation, Preservation and Management.* Fiedler PL, Jain SK (eds). Chapman and Hall, London, pp. 127–170.

Moyle, P.B. and Light, T. (1996a). Biological invasions of fresh water: empirical rules and assembly theory. *Biological Conservation* 78: 149–161.

Moyle, P.B. and T. Light. (1996b). Fish invasions in California: do abiotic factors determine success?. *Ecology* 77: 1666–1760.

Munné, A., C. Solá and N. Prat. (1998). QBR: Un Índice rápido para la evaluación de la calidad de los ecosistemas de ribera. *Tecnología del Agua* 175: 20–37.

Murillo, J.M., T. Marañón, F. Cabrera and R. López. (1999). Accumulation of heavy metals in sunflower and sorghum plants affected by the Guadiamar spill. *The Science of the Total Environment* Special Issue 242: 285-296.

Murphy, B.R., M.L. Brown and T.A. Springer. (1990). Evaluation of the Relative Weight (Wr) Index, with New Applications to Walleye. *North American Journal of Fisheries Management* 10: 85-97.

Nájera, L. (1943). Datos sobre distribución de la *Gambusia holbrooki* en la provincia de Córdoba. *Actualidad Médica* 19: 210-216.

Nájera, L. (1945). Consideraciones sobre la *Gambusia holbrooki* y método para conseguir la repoblación autobiológica de los ríos. *Revista Ibérica de Parasitología* 4.

Nelva, A., M.J. Collares-Pereira et M. Coelho. (1988). Systemetique et repartition du genre *Chondrostoma* Agassiz, 1835 (Pisces, Cyprinidae). *Archiv für Hydrobiologie* 113(1): 93-112.

Nicola, G. G., A. Almodóvar and B. Elvira. (1996). The diet of introduced largemouth bass, *Micropterus salmoides*, in the Natural Park of the Ruidera Lakes, central Spain. *Polskie Archiwum Hydrobiologii* 43: 179–184.

Niemi, G.J., P. Devore, N. Detenbeck, D. Taylor, A. Lima, J. Pastor, J.D. Yount and R.J. Naiman. (1990). Overview of case studies on recovery of aquatic systems from disturbance. *Environmental Management* 14: 571–587.

Nikolsky, G.V. (1963). *The Ecology of Fishes*. Academic Press, London

Norris, R.H. and Hawkins, C.P. (2000). Monitoring river health. *Hydrobiologia* 435: 5–17.

Oksanen, J., F.G. Blanchet, R. Kindt, P. Legendre, P.R. Minchin, R.B. O'Hara, G.L. Simpson, P. Solymos, M. Henry, H. Stevens and H. Wagner. (2011). *vegan: Community Ecology Package*. R package version 2.0-1. Disponible en: http://CRAN.R-project.org/package=vegan, Consultado en junio de 2012.

Olías, M., J.C. Cerón, I. Fernández, F. Moral and A. Rodríguez-Ramírez. (2005). State of contamination of the waters in the Guadiamar valley five years after the Aznalcóllar spill. *Water, Air, and Soil Pollution* 166: 103–119.

Olías, M., J.C. Cerón, F. Moral and F. Ruiz. (2006). Water quality of the Guadiamar River after the Aznalcóllar spill (SW Spain). *Chemosphere* 62: 213–225.

Oliva-Paterna, F.J., A. Andreu and M. Torralva. (2003a). Water quality affects the Condition of *Barbus sclateri* Günther, 1868 (Pisces, Cyprinidae) in semi-arid reservoirs from the Iberian Peninsula. *Anales de Biología* 25: 3-11.

Oliva-Paterna, F.J., P.A. Miñano and M. Torralva. (2003b). Habitat quality affects the conditions of *Barbus sclateri* in Mediterranean semi-arid stream. *Environmental Biology of Fishes* 67: 13-22.

Oliva-Paterna, F.J., M.M. Torralva and C. Fernández-Delgado. (2002). Age, growth and reproduction of *Cobitis paludica* in a seasonal stream. *Journal of Fish Biology* 60: 389–404.

Oliva Paterna, F.J., A. Vila-Gispert and M. Torralva. (2003c). Condition of *Barbus sclateri* from semi-arid aquatic systems: effects of habitat quality disturbances. *Journal of Fish Biology* 23: 699-709.

Olson, M.H., G.G. Mittelbach and C.W. Osenberg. (1995). Competition between predator and prey: Resource-based mechanisms and implications for stage-structured dynamics. *Ecology* 76: 1758–1771.

Pena, J.C. y J. Domínguez. (1985). Contribución al conocimiento de la ictiofauna leonesa: La Gambusia (*Gambusia affinis holbrooki* Girard 1859). 61: 103-114.

Peres-Neto, P.R., P. Legendre, S. Dray and D. Borcard. (2006). Variation Partitioning of Species Data Matrices: Estimation and Comparison of Fractions. *Ecology* 87: 2614–2625.

Pérez-Alejandre, R. (2009). *Biología y ecología de las larvas de peces del río Guadiamar en zonas afectadas y no afectadas por el vertido tóxico de las monas de Aznalcóllar*. Tesis Doctoral. Universidad de Córdoba.

Pérez-Bote, J.L., M.C. Soriguer and A.J. Rodríguez-Jiménez. (2001). Características de los nidos y áreas de nidificación en el pez sol *Lepomis gibbosus* (L., 1758) (Osteychthyes, Centrarchidae) en la cuenca media del Guadiana: río versus embalse. *Zoologica Baetica* (12): 3-13.

Peterson, J.T. and P.B. Bayley. (1993). Colonization rates of fishes in experimentally defaunated warmwater streams. *Transactions of the American Fisheries Society* 122: 199–207.

Pinto, R., J.M. Arenas and F. Carrascal. (2008). El Corredor Verde del Guadiamar en el context de la RENPA. In: *La restauración ecológica del río Guadiamar y el proyecto del corredor verde*. Consejería de Medio Ambiente, Junta de Andalucía, Sevilla. pp. 65-87.

Pinto, P., M. Morais, M. Ilhéu and L. Sandin. (2006). Relationships among biological elements macrophytes, macroinvertebrates and ichthyofauna for different core river types across Europe at two different spatial scales. *Hydrobiologia* 566: 75–90.

Pires, A.M., I.G. Cowx and M.M. Coelho. (1999). Seasonal changes in fish community structure in the middle reaches of the Guadiana basin, Portugal. *Journal of Fish Biology* 54: 235–249.

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

Poff, N.L. and J.V. Ward. (1989). Implications of streamflow variability and predictability for lotic community structure: a regional analysis of streamflow patterns. *Canadian Journal of Fisheries and Aquatic Sciences* 46: 1805–1818.

Poff, N.L. and J.V. Ward. (1990). Physical habitat template of lotic systems: recovery in the context of historical pattern of spatiotemporal heterogeneity. *Environmental Management* 14: 629–645.

Pou-Rovira, Q. (2004). *Ecologia demográfica de la perca americana (Micropterus salmoides) a l´Estany de Banyoles*. Tesis Doctoral. Universidad de Girona.

Prat, N., C. Solà, S. Plans, J. Toja, M.D. Burgos and M. Rieradevalls. (1999). Effect of dumping and cleaning activities on the aquatic ecosystems of the Guadiamar River following a toxic flood. *The Science of the Total Environment* Special Issue 242: 231-248.

Prenda, J. and C. Granado-Lorencio. (1994). Estimas del espacio vital y calidad del habitat a lo largo del Invierno en tres especies de peces (Cyprinidae) de un rio de regimen Mediterraneo. *Doñana, Acta Vertebrata* 21: 61–77.

Pringle, C. (2003). What is hydrologic connectivity and why is it ecologically important? *Hydrological Processes* 17: 2685–2689.

Pyke, G.H. (2005). A review of the biology of *Gambusia affinis* and *G. holbrooki*. *Reviews in Fish Biology and Fisheries* 15: 339–365.

Quinn, G.P. and M.J. Keough. (2002). *Experimental Design and Data Analysis for Biologists.* Cambridge University Press, Cambridge. 322 pp.

R Development Core Team. (2012). *R: A language and environment for statistical computing.* R Foundation for Statistical Computing, Vienna. ISBN 3-900051-07-0. Disponible en: http://www.R-project.org/. Consultado en junio de 2012.

Rangel, T.F.L.V.B., J.A.F., Diniz-Filho and L.M. Bini. (2006). Towards an integrated computational tool for spatial analysis in macroecology and biogeography. *Global Ecology and Biogeography* 15: 321–327.

Resh, V H., A.V. Brown, A.P. Covich, M.E. Gurtz, H.W. Li, G.W. Minshall, S.R. Reice, A.L. Sheldon, J.B. Wallace and R. Wissmar. (1988). The role of disturbance in stream ecology. *Journal of the North American Benthological Society* 7: 433–455.

Ribeiro, F. and M.J. Collares-Pereira. (2010). Life-history variability of non-native centrarchids in regulated river systems of the lower River Guadiana drainage (south-west Iberian Peninsula). *Journal of Fish Biology* 76: 522–537.

Ribeiro, F., I.G. Cowx, P. Tiago, A.F. Filipe, L. Moreira Da Costa and M.J. Collares-Pereira. (2003). Growth and reproductive traits of diploid and triploid forms of the *Squalius alburnoides* cyprinid complex in a tributary of the Guadiana River, Portugal. *Archiv für Hydrobiology* 156: 471–484.

Ribeiro, F., B. Elvira, M.J. Collares-Pereira and P.B. Moyle. (2008). Life-history traits of non-native fishes in Iberian watersheds across several invasion stages: a first approach. *Biological Invasions* 10: 89-102.

Ribeiro, F. and P.M. Leunda. (2012). Non-native fish impacts on Mediterranean freshwater ecosystems: current knowledge and research needs. *Fisheries Management and Ecology* 19(2): 142-156.

Rico, M., G. Benito, A.R. Salgueiro, A. Diezherrero and H.G. Pereira. (2008). Reported Tailings Dam Failures: A Review of the European Incidents in the Worldwide Context. *Journal of Hazardous Materials* 152(2): 846–852.

Robalo, J.I., V. Carvalho Almada, A. Levy and I. Doadrio. (2007). Re-examination and phylogeny of the genus *Chondrostoma* based on mitochondrial and nuclear data and the definition of 5 new genera. *Molecular Phylogenetics and Evolution* 42: 362–372.

Robalo, J.I., C. Sousa Santos, A. Levy and V.C. Almada. (2006). Molecular insights on the taxonomic position of the paternal ancestor of the *Squalius alburnoides* hybridogenetic complex. *Molecular Phylogenetics and Evolution* 39: 276-281.

Robinson, B.W. and D.S. Wilson. (1996). Genetic variation and phenotypic plasticity in a trophically polymorphic population of pumpkinseed sunfish (*Lepomis gibbosus*). *Evolutionary Ecology* 10: 631–652.

Robinson, B.W., D.S. Wilson, A.S. Margosian and P.T. Lotito. (1993). Ecological and morphological differentiation of pumpkinseed sunfish in lakes without bluegill sunfish. *Evolutionary Ecology* 7: 451–464.

Rodríguez-Jiménez, A.J. (1987). Notas sobre la ecología de *Rutilus lemmingii* (Steindachner 1866) (Pisces: Cyprinidae). *Ecología* 1: 247-256.

Rodríguez-Jiménez, A.J. (1989). Hábitos alimenticios de *Micropterus salmoides*, *Lepomis gibbosus* y *Gambusia affinis* en las orillas del embalse de Proserpina (Extremadura, Spain). *Limnética* 5: 13–20.

Rodríguez-Ruiz, A. and C. Granado-Lorencio. (1992). Spawning period and migration of three species of cyprinids in a stream with mediterranean regimen (SW-Spain). *Journal of Fish Biology* 41: 545-556.

Roghair, C.N. and C.A. Dolloff. (2005). Brook trout movement during and after recolonization of a naturally defaunated stream reach. *North American Journal of Fisheries Management* 25: 777–784.

Roth, N.E., J.D. Allan and D.L. Erickson. (1996). Landscape influences on stream biotic integrity assessed at multiple spatial scales. *Landscape Ecology* 11: 141–156.

Ruiz, A.R. (1998). Fish species composition before and after construction of a reservoir on the Guadalete River SW Spain. *Archiv für Hydrobiologie* 142: 353–369.

Ryon, M.G. (2011). Recovery of fish communities in a warmwater stream following pollution abatement. *Environmental Management* 47: 1096–1111.

Saldaña, T. (2006). *Edad, crecimiento y reproducción del barbo común (Barbus sclateri, Günther, 1868) en una zona del río Guadiamar no afectada por el vertido tóxico de las minas de Aznalcóllar (Sevilla).* Tesis de Licenciatura. Universidad de Córdoba.

Schlosser, I.J. (1987). The role of predation in age- and size-related habitat use by stream fishes. *Ecology* 68: 651–659.

Schlosser, I.J. (1990). Environmental variation, life history attributes, and community structure in stream fishes: implications for environmental management and assessment. *Environmental Management* 14: 621–628.

Schulte-Hostedde, A.I., B. Zinner, J.S. Millar, and G.J. Hickling. (2005). Restitution of mass/size residuals: Validating body condition indices. *Ecology* 86: 155-163.

Shannon, C.E. and Weaver, W. (1949). *The Mathematical Theory of Communication.* University of Illinois Press, Urbana, Illinois.

Sheldon, A.L. (1988). Conservation of stream fishes: patterns of diversity, rarity and risk. *Conservation Biology* 2: 149–156.

Sheldon A.L. and Meffe G.K. (1995). Short-term recolonization by fishes of experimentally defaunated pools of a coastal plain stream. *Copeia* (4): 828–837.

Silva, E.B., J.C. Franco, T. Vasconcelos and M. Branco. (2010). Effect of ground cover vegetation on the abundance and diversity of beneficial arthropods in citrus orchards. *Bulletin of Entomological Research* 100: 489–499.

SAIH (Sistema Automático de Información Hidrológica de las Cuencas del GuadalquiviR). (2012). Disponible en:

http://www.juntadeandalucia.es/agenciadelagua/saih/DatosHistoricos.aspx. Consultado en septiembre de 2012.

Solà, C., M. Burgos, A. Plazuelo, J. Toja, M. Plans and N. Prat. (2004). Heavy metal bioaccumulation and macroinvertebrate community changes in a Mediterranean stream affected by acid mine drainage and an accidental spill (Guadiamar River, SW Spain). *The Science of the Total Environment* 333: 109–126.

Soriguer, M. C., R. Bravo, C. Vallespín, C. Gómez-Cama and J. A. Hernando. (2000a). Reproductive strategies of two species of cyprinids in a stream with Mediterranean regimen (SW Spain). *Archiv für Hydrobiologie* 148(1): 119-134.

Soriguer, M. C., C. Vallespín, C. Gomez-Cama and J. A. Hernando. (2000b). Age, diet, growth and reproduction of a Population of *Cobitis paludica* (de Buen, 1930) in the Palancar Stream (southwest of Europe, Spain) (Pisces: Cobitidae). *Hydrobiologia* 436: 51–58.

Sponseller, R.A., E.F. Benfield and H.M. Valett. (2001). Relationships between land use, spatial scale and stream macroinvertebrate communities. *Freshwater Biology* 46: 1409–1424.

Sutton, S.G., T.P. Bult and R.L. Haedrich. (2000). Relationships among fat weight, body weight, water weight and condition factors in wild salmon parr. *Transactions of the American Fisheries Society* 129: 527–538.

Tolonen, K.T., H. Hamalainen, I.J. Holopainen, K. Mikkonen and J. Karjalainen. (2003). Body size and substrate association of littoral insects in relation to vegetation structure. *Hydrobiologia* 499: 179–190.

Tomecek, J, V. Kovác and S. Katina. (2007). The biological flexibility of the pumpkinseed: a successful colonizer throughout Europe. *Invading Nature - Springer Series in Invasion Ecology* vol. 2. Chapter 16.

Torralva, M., M.A. Puig and C. Fernández-Delgado, (1997). Effect of river regulation on the life-history patterns of *Barbus sclateri* in the Segura river basin (south-east Spain). *Journal of Fish Biology* 51: 300-311.

Townsend, C.R. and A.G. Hildrew. (1994). Species traits in relation to a habitat templet for river systems. *Freshwater Biology* 31: 265–275.

Turner, J.N. (2003). *A geomorphological–geochemical assessment of the impact of the Aznalcóllar tailings dam failure on the Río Guadiamar southwest Spain*. PhD Thesis. University of Wales Aberystwyth.

Valls, A. and J. Blasco. (2005). *Integrated assessment and management of the ecosystems affected by the Aznalcóllar mining spill (SW, Spain).* UNESCO Unitwin, Cádiz.

Van den Brink, P.J., N.W. van den Brink and C.J.F. ter Braak. (2003). Multivariateanalysis of ecotoxicologicaldata using ordination: Demonstrations of utility on the basis of various examples. *Australasian Journal of Ecotoxicology* 9: 141–146.

Van Geen, A., R. Takesue and Z. Chase. (1999). Acid mine tailings in southern Spain. *The Science of the Total Environment* Special Issue 242: 221-230.

Van Niekerk, H.J. and M.J. Viljoen. (2005). Causes and consequences of the Merriespruit and other tailings-dam failures. *Land degradation and development* 16: 201-212.

Velasco, J.C., P.A. Rincón and J. Lobón-Cerviá. (1990). Age, growth and reproduction of the cyprinid *Rutilus lemmingii* (Steindachner, 1866) in the River Huebra, central Spain. *Journal of Fish Biology* 36(4): 469-480.

Verdiell-Cubedo, D., F.J. Oliva-Paterna and M. Torralva. (2006a). Condition of *Salaria pavo* in the Mar Menor coastal lagoon (SE Iberian Peninsula): potential influence of environmental variables on juveniles. *Journal of Applied Ichthyology* 22(5): 407-413.

Verdiell-Cubedo, D., F.J. Oliva-Paterna and M. Torralva. (2006b). Condition of *Gobius cobitis* (Pallas, 1811) juveniles in the Mar Menor coastal lagoon (SE Iberian Peninsula): Effects of inter- and intraspecific fish competition. Scientia Marina 70(2).

Verneaux, J., P. Galmiche, F. Janier, et A. Monnot (1982). Une nouvelle méthode pratique d'evaluation de la qualité des eaux courantes. Un indice biologique de qualité générale (BIG). *Annales Scientifiques de l'Université de Franche-Comté Besanqon, Biologie animale 4 ème série* 3(2): 11-21.

Vila-Gispert, A., M.G. Fox, L. Zamora and R. Moreno-Amich. (2007). Morphological variation in pumpkinseed *Lepomis gibbosus* introduced into Iberian lakes and reservoirs; adaptations to habitat type and diet? *Journal of Fish Biology* 71(1): 163-181.

Vila-Gispert. A. and R. Moreno-Amich. (2000). Fecundity and spawning mode of three introduced fish species in Lake Banyoles (Catalunya, Spain) in comparison with other localities. *Aquatic Sciences*, 62 (2): 154-166.

Vila-Gispert, A. and R. Moreno-Amich. (2001). Mass-length relationship of Mediterranean barbel as an indicator of environmental status in South-west European stream ecosystems. *Journal of Fish Biology* 59 (4): 824-832.

Vila-Gispert, A., L. Zamora and R. Moreno-Amich. (2000). Use of the condition of Mediterranean barbel (*Barbus meridionalis*) to assess habitat quality in stream ecosystems. *Archiv für Hydrobiologie* 148: 135–145.

Vinyoles, D., J.I. Robalo, A. de Sostoa, A. Almodóvar, B. Elvira, G.G. Nicola, C. Fernández-Delgado, C.S. Santos, I. Doadrio, F. Sardà-Palomera and V.C. Almada. (2007). Spread of the alien bleak *Alburnus alburnus* (Linnaeus, 1758) (Actinopterygii, Cyprinidae) in the Iberian Peninsula: The role of reservoirs. *Graellsia* 63(1): 101-110.

Vizzini, S. and A. Mazzola. (2005). Feeding ecology of the sand smelt Atherina boyeri (Risso 1810) (Osteichthyes, Atherinidae) in the western Mediterranean: evidence for spatial variability based on stable carbon and nitrogen isotopes Environmental. *Biology of Fishes* 72: 259–266.

Wang, L., J. Lyons and P. Kanehl. (2001). Impacts of urbanization on stream habitat and fish across multiple spatial scales. *Environmental Management* 28: 255–266.

Wasson, J.G., B. Villeneuve, A. IItal, J. Murray-Bligh, M. Dobiasova, S. Bacikova, H. Timm, H. Pella, N. Mengin, and A. Chandesris. (2010). Large scale relationships between basin and riparian land cover and ecological status of European rivers: examples with invertebrate indices from France, Estonia, Slovakia and United Kingdom. *Freshwater Biology* 55: 1465–148.

Winston, M.R., C.M. Taylor and J. Pigg. (1991). Upstream extirpation of four minnow species due to damming of a prairie stream. *Transactions of the American Fisheries Society* 120: 98–105.

Wootton, R.J. (1998). *The ecology of teleost fishes.* Fish and Fisheries Series, no. 24. Kluwer Academic Publishers, Dordrecht.

Wright, J.F. (1995). Development and use of a system for predicting the macroinvertebrate fauna in flowing waters. *Australian Journal of Ecology* 20: 181–197.

Yamanaka, H., Y. Kohmatsu and M. Yuma. (2006). Difference in the hypoxia tolerance of the round crucian carp and largemouth bass: implications for physiological refugia in the macrophyte zone. *Ichthyological research*, 54(3): 308-312.

Zamora-Muñoz, C., C.E. Sainz-Cantero, A. Sánchez-Ortega and J. Alba-Tercedor. (1995). Are biological indices BMWP' and ASPT' and their significance regarding water quality seasonality dependent? Factors explaining their variations. *Water Research* 29: 285-290.

Zardoya, R. y I. Doadrio. (1999). Molecular evidence on the evolutionary and biogeographical patterns of European cyprinids. *Journal of Molecular Evolution* 49: 227– 237.

APÉNDICES

APÉNDICE 1

Variables ambientales registradas en el apartado II.2

Appendix 1. Environmental variables (71) registered at the site and catchment scale. The first column at each scale shows the variables not summarized in PCAs. *Water quality: 1(high quality) – 4(low quality). ** See details about specific methods in Fernández-Delgado *et al*. 2010.

SITE SCALE				CATCHMENT SCALE	
Not included in PCAs	**Included in PCA1 (Habitat characteristics)**	**Included in PCA2 (bank stability)**	**Included in PCA3 (Land uses)**	**Not included in PCAs**	**Included in PCA4 (Land uses)**
Mean channel width (m)	Surface occupied by each type of mesohabitat (m^2)	Runoff (0-1)	% Native forests	Drainage area (km^2)	% Native forests
Reach length (m)	% each type of inorganic substrate in riffle, run or pool.	Livestock access (0-1)	% Low disturbed natural areas	Mean catchment slope (%)	% Native shrub or grassland
Mean valley width (m)	% organic substrate in runs and pools.	Human access (0-1)	% Non-irrigated crops		% Grazed fields
Riparian vegetation width (m)	% of the reach occupied by riffles, run or pool.	Ford, culvert or bridge (0-1)	% Irrigated crops		% Wetlands
Canopy cover (m^2)	Surface area occupied by riffles and runs (m^2)	Clearing of vegetation (0-1)	% Forestry		% Recreational areas
Distance to source (m)	Number of rapids	Water extraction (0-1)	% Recreational areas		% Crops
River length covered by reservoirs upstream (m)	Number of runs	Gravel or sand extraction (0-1)	% Urban, industrial, intensive agriculture		% Irrigated crops
No. obstacles downstream	Number of pools	Drain pipes (0-1)			% Urban areas
No. reservoirs upstream	Mean depth in runs (m)	Others (0-1)			% Industrial
Distance from mouth (m)	Mean pool depth (m)	% Embeddedness in rifles			% Mining
Distance to the nearest reservoir upstream (m)	Emerged aquatic vegetation (%)	Bank stability Index **			
No. reservoirs downstream	Underside black in color (0-1)				
Distance to the nearest reservoir downstream (m)	Submerged aquatic vegetation (%)				
Altitude (m.a.s.l.)	Floating aquatic vegetation				
Upstream order (Strahler)	Conductivity ($\mu S\ cm^{-1}$)				
Water quality(1-4)*	Cover of shelters (m^2)				

APÉNDICE 2

Ictiofauna de la cuenca del río Guadiamar

Familia Cyprinidae

BARBO COMÚN - *Luciobarbus sclateri* (Günther, 1868)

Pez fusiforme y alargado, con dos pares de barbillones, uno en el maxilar superior y otro en la comisura labial, que se exhiben como su característica más evidente y por la cual recibe su nombre. Boca ínfera y protráctil, con labios carnosos y un hocico redondeado claramente diferenciado. El labio inferior (sin lóbulo) recubre el extremo de la mandíbula. La longitud de la cabeza representa un 24-29 % de su longitud estándar (Lst).

Kottelat y Freyhof (2007) lo diferencian de otros barbos en base principalmente a las siguientes características: Línea lateral con 43-50 escamas, pedúnculo caudal es robusto y el tercer radio duro de su aleta dorsal esta densamente denticulado en 1/2-3/4 de su longitud.

La longitud máxima se encuentra en torno a los 620 mm LF (hembras) y 420 mm LF (machos) (Datos propios no publicados).

Su distribución se ciñe al sur de la Península Ibérica. En España se ubica en las cuencas del Guadalquivir, tramo bajo del Guadiana, Segura y cuencas de menor entidad que vierten directamente al Mediterráneo y el Atlántico (Doadrio, 2001).

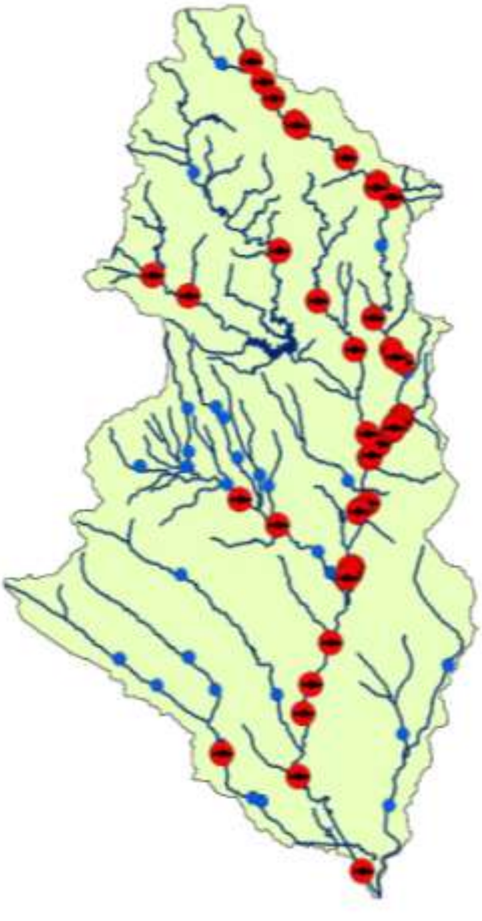

Figura 1. Distribución del barbo común en la cuenca del río Guadiamar. En rojo, puntos de muestreo donde se capturó la especie; en azul, puntos donde no se capturó.

En la cuenca del Guadiamar la especie se distribuye ampliamente (Fig. 1), con capturas en los tramos alto, medio y bajo; desde las zonas bien conservadas a las más degradadas.

El crecimiento del barbo en el Guadiamar sigue un patrón similar al de otros cursos fluviales de régimen mediterráneo (Saldaña, 2006). La madurez sexual se alcanza al tercer año de vida para los machos (60 a 90 mm LF) y al quinto o sexto (130 mm LF) para las hembras (Herrera y Fernández-Delgado, 1992). Al ser una especie potádroma, los machos y hembras listos para el desove, realizan migraciones río arriba hacia tramos fluviales de menor entidad (arroyos de 1er o 2º orden), buscando fondos pedregosos de aguas claras y bien oxigenadas. Una vez alcanzado un lugar apropiado para el desove, éste se realiza como mínimo en dos tandas entre marzo y julio (Lucena y Camacho, 1978; Herrera *et al.*, 1988; Herrera y Fernández-Delgado, 1992; Rodríguez-Ruiz y Granado-Lorencio 1992; Torralva *et al*. 1997; Soriguer *et al*. 2000a). Terminado el desove, retornan aguas abajo hacia sus lugares habituales de residencia. Su dieta es omnívora, principalmente larvas de quironómidos y detritus (Encina y Granado-Lorencio, 1997).

El barbo común, al igual que el resto de especies de barbo presentes en Andalucía, ha estado hasta hace pocos años incluido dentro del género *Barbus* (Gunther, 1868; Steindachner, 1864; Almaça, 1967). Sin embargo, recientes estudios moleculares (Zardoya y Doadrio, 1999) han dividido a los barbos europeos en dos géneros, *Barbus* y *Luciobarbus*, quedando los andaluces dentro de esta última categoría (Doadrio *et al.*, 2002). La diferencia entre géneros, tiene bases morfológicas, genéticas y biogeográficas. Entre las primeras se encuentran la estructura labial y los tubérculos nupciales, que en *Luciobarbus* son menos numerosos y más alargados. Respecto a las bases biogeográficas, el género *Barbus* ocupa la zona centro-oriental de Europa y el *Luciobarbus* queda relegado a zonas periféricas del Mediterráneo y norte de África (Doadrio, 1990; Bianco, 1989, 1990).

Tubérculos nupciales, dispersos y alargados en un macho de barbo común.

Estado de Conservación. La especie es endémica de la Península Ibérica. Su gran resistencia a diferentes grados de alteración en el hábitat (Oliva-Paterna *et al.*, 2003a), le hace ser la más ubicua de entre los peces epicontinentales de Andalucía, tal como un reciente estudio ha detectado (Fernández-Delgado *et al.*, 2007).

Siguiendo los criterios de la UICN, la especie a nivel mundial se encuentra catalogada como **LC** “Preocupación menor” (UICN, 2006). A nivel nacional, se encuadra como **LR, nt** “Riesgo menor: casi amenazada” de extinción (Doadrio, 2001). En Andalucía está catalogada como **LR, nt** “Riesgo menor: casi amenazada” de extinción (CMAJA, 2001).

BOGA – *Pseudochondrostoma willkommii* (Steindachner, 1866).

Pez fusiforme, esbelto y alargado. Su característica más notoria es una boca ínfera de perfil recto en visión ventral, lo cual está causado por una lámina córnea en el labio inferior. Este tipo de boca crea una prolongación del hocico a modo de "nariz", rasgo distintivo del género *Chondrostoma* (género al que anteriormente pertenecía, ver más abajo).

El borde de la mandíbula inferior ligeramente arqueado. Provista de 6-7 dientes faríngeos dispuestos en una sola fila y 26-35 branquiespinas. Línea lateral con 56-71 escamas. Posee 9-10 ½ radios ramificados en su aleta anal (Kottelat y Freyhof, 2007).

La longitud máxima alcanzada en la cuenca del Guadalquivir ha sido 290 mm (LF) (Datos propios no publicados).

La especie se distribuye por las cuencas del suroeste de la Península Ibérica. En España se localiza en las cuencas del Guadalquivir, Guadiana, Odiel y ríos del sur hasta la cuenca del río Vélez en Málaga (Doadrio, 2001).

En la cuenca del Guadiamar, la especie se encuentra muy localizada en el cauce principal, si bien está presente en varios tributarios como el río Frailes y aguas arriba del embalse del

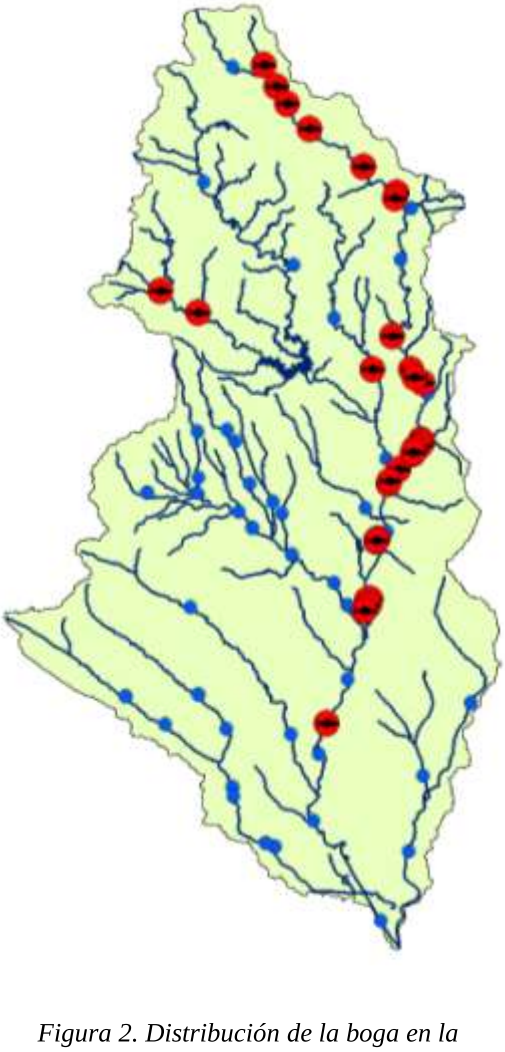

Figura 2. Distribución de la boga en la cuenca del río Guadiamar. En rojo, puntos de muestreo donde se capturó la especie, en azul, donde no se encontró.

Agrio en los arroyos Cañaveroso y de Vistahermosa.

Tanto el macho como la hembra adquieren su madurez sexual en el segundo año de vida. A esta edad, cuando los machos alcanzan los 75 mm (LF) y las hembras 90 mm (LF), entre marzo y mayo, remontan los ríos hasta los desovaderos con fondo de grava donde las hembras dejan, en al menos dos veces, su única puesta del año (Herrera y Fernández-Delgado, 1994). Tras la freza, se dirigen aguas abajo donde cursos de mayor entidad ofrecen mejores condiciones para permanecer el resto del año.

La obtención de su dieta, en su mayoría detritívora (Bellido *et al.*, 1989) la lleva a cabo raspando la superficie de las piedras con el labio córneo.

Hasta hace muy pocos años, el género *Chondrostoma* (Agassiz, 1835) abarcaba un conjunto de especies muy amplio (Nelva *et al*., 1988). Sin embargo, recientes estudios moleculares (Doadrio y Carmona, 2004; Robalo *et al.*, 2007) han reclasificado las especies europeas en seis géneros: *Chondrostoma*, *Pseudochondrostoma*, *Parachondrsotoma*, *Achondrostoma*, *Iberochondrostoma* y *Protochondrostoma*. Según Robalo *et al.* (2007), las especies andaluzas quedarían incluidas en los géneros *Pseudochondrostoma* (boga) e *Iberochondrostoma* (pardilla). A pesar de haberse encontrado claras diferencias a nivel molecular entre géneros, algunas especies no logran discernirse de forma fehaciente a nivel morfológico, lo que deja a la localización geográfica como único criterio de clasificación determinante.

Estado de Conservación. La especie es endémica de la Península Ibérica. Los requerimientos específicos de alimentación, sustrato y migración a lo largo del río (Bellido *et al.*, 1989), hacen que este pez que antaño fuera muy común en las aguas del sur de la Península Ibérica, hoy debido a la contaminación, alteración y fragmentación del hábitat ha disminuido sus poblaciones a escasas localidades (Fernández-Delgado, 2003).

A nivel mundial, la última actualización sobre el estado de conservación de la especie la cataloga como **VU** “Vulnerable a la extinción” bajo criterios **A 3 c e + 4 c e** (UICN, 2006). A nivel nacional, se encuadra como **VU** “Vulnerable a la extinción” bajo criterios **A 2 c e** (Doadrio, 2001). En Andalucía está catalogada como **VU** “Vulnerable a la extinción” bajo criterios **A 1 c e** (CMAJA, 2001).

CALANDINO - complejo *Squalius alburnoides* (Steindachner, 1866).

La especie se caracteriza por mostrar una franja negra que recorre el flanco desde el hocico hasta la base de la aleta caudal. El dorso es oscuro y el vientre plateado. Posee un escalón en el perfil dorso-ventral que provoca un pronunciado estrechamiento caudal. La boca es súpera. Posee 5 dientes faríngeos dispuestos en una sola fila.

Línea lateral con 42 escamas. Aleta anal de perfil cóncavo, con 8 ½ radios ramificados mientras la dorsal posee 7. (Kottelat y Freyhof, 2007).

La longitud máxima alcanza los 140 mm (LF) (hembras) (Fernandez-Delgado *et al.*, 2007) y 51 mm (LF) (machos) (Fernandez-Delgado y Herrera, 1994).

La especie se distribuye por la mitad occidental de la Península Ibérica. En España ocupa las cuencas de los ríos Duero, Tajo, Sado, Guadiana, Odiel y Guadalquivir (Doadrio, 2001).

En la cuenca del Guadiamar, la especie se encuentra ampliamente distribuida en los cursos que componen los tramos alto y bajo de la cuenca (Fig. 3).

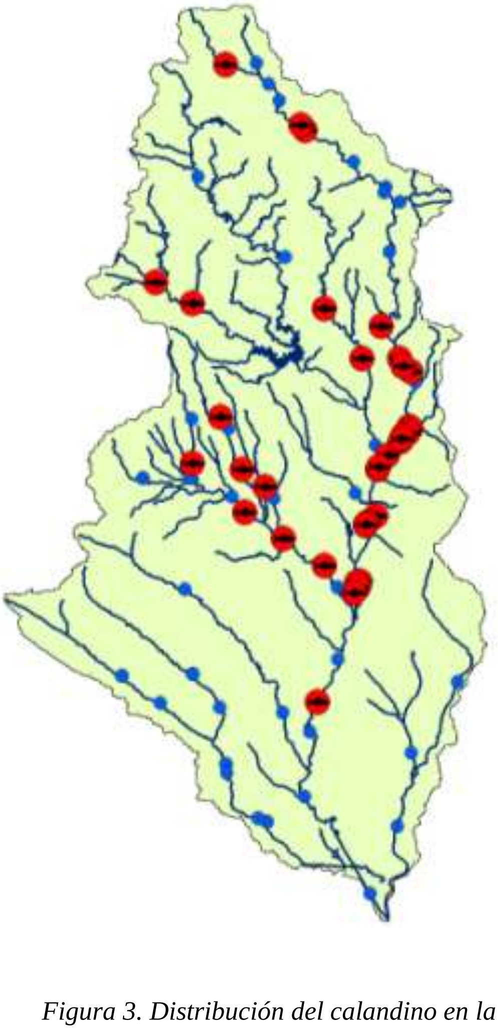

Figura 3. Distribución del calandino en la cuenca del río Guadiamar. En rojo, puntos de muestreo donde se capturó la especie, en azul, donde no se encontró.

Posiblemente, la peculiaridad más destacada del calandino sea su reproducción, que transcurre entre marzo y julio cuando ambos sexos alcanzan los dos años de edad (Fernández-Delgado y Herrera, 1994; Ribeiro *et al.*, 2003). Ésta se lleva a cabo mediante hibridogénesis. Las hembras depositan unos óvulos en los que ha sido eliminado el genoma paterno, y se sirven de esperma de la misma u otra especie para fecundar los huevos que darán nuevos individuos diploides, triploides e incluso tetraploides (Alves *et al.*, 1998). Si el esperma paterno ha sido de la misma especie, la descendencia será pura, pero al tener en cuenta que el número de machos en la mayoría de las poblaciones es muy inferior al de hembras (Fernández-Delgado y Herrera, 1994; Ribeiro *et al.*, 2003), hay una alta probabilidad de que el esperma utilizado sea de otras especies, normalmente de cacho, el cual suele compartir los mismos tipos de hábitat que el calandino, ríos y arroyos de aguas claras con algo de corriente y abundante vegetación (Coelho, 1997). En lugares donde le presencia de cacho es muy reducida, se han dado casos en los que ha utilizado esperma de boga. En estos últimos casos, los nuevos individuos serán híbridos y compartirán caracteres de ambas especies, siendo éstos de mayor tamaño que los puros (González-Carmona, 1997). Gracias a esta estrategia reproductiva, el calandino es considerado la segunda especia más ubicua de entre los peces continentales andaluces (Fernández-Delgado *et al.*, 2007).

En poblaciones con individuos de varias ploidías, los machos diploides restringen su alimentación a insectos de la superficie, las hembras diploides a la vegetación de fondo y los individuos triploides a ambos nichos (Gomes-Ferreira *et al.*, 2005).

Recientemente (Robalo *et al.*, 2006; Kottelat y Freyhof, 2007) se ha recurrido al género *Iberocypris* para albergar a dos especies endémicas de la Península Ibérica, el calandino *Iberocypris alburnoides* y la bogardilla *Iberocypris palaciosi* (Doadrio, 1980). Estas especies, ubicadas anteriormente dentro de los géneros *Rutilus*, *Thropidophoxinellus* y recientemente *Squalius*, poseen una serie de características que los excluyen de estos taxones, como son, un menor número de escamas en la línea lateral, una franja oscura que recorre el cuerpo y una aleta anal con el borde exterior cóncavo (en vez de convexo). Sin embargo, al formar *Iberocypris* complejos híbridos con especies de *Squalius* y haber indicios de un ancestro común, se considera la especie como un genero monofilético llamado complejo *Squalius alburnoides* (Robalo *et al.*, 2006; Leunda *et al.*, 2009).

Estado de Conservación: La especie es un endemismo de la Península Ibérica. A nivel mundial, la última actualización sobre el estado de conservación de la especie (UICN, 2006) la cataloga como **VU** “Vulnerable a la extinción” bajo criterios **A 3 c e**. A nivel nacional, se encuadra como **VU** “Vulnerable a la extinción” bajo criterios **A 2 c e** (Doadrio, 2001). En Andalucía está catalogada como **VU** “Vulnerable a la extinción” bajo criterios **A 1 c e** (CMAJA, 2001).

CACHO - *Squalius pyrenaicus* (Günther, 1868).

Es un pez fusiforme y robusto con respecto a su corto tamaño. Sin ningún atributo especial que lo diferencie rápidamente del resto de las especies. Forma complejos híbridos con el calandino, por lo que suele ser erróneamente identificado como cacho lo que en realidad es un calandino con parte de genoma del anterior. Para esclarecer dicha identificación, las características que más ayudan a discriminar ambas especies, son que el cacho posee una boca terminal; unos huesos infraorbitales anchos, siendo el segundo y quinto 4-5 veces más anchos que el canal sensorial que los atraviesa (Kottelat y Freyhof, 2007) y los dientes faríngeos dispuestos en dos filas 5-2. La aleta anal expone un perfil distal convexo y la dorsal posee 8 radios ramificados. La línea lateral alberga 37-42 escamas con una marca oscura en la raíz y una longitud corporal similar a la profundidad.

La longitud máxima alcanza los 180 mm LS (hembras) (Lobón-Cerviá y Sostoa, 1987) y 160 mm LF (machos) (Fernández-Delgado y Herrera, 1995a)

La especie se distribuye ampliamente por la Península Ibérica. En España se encuentra en las cuencas de los ríos Tajo, Guadiana, Guadalquivir, Guadalfeo, Barbate, Guadalmina, Segura, Vega, Velez, Guadalcacín, Odiel,

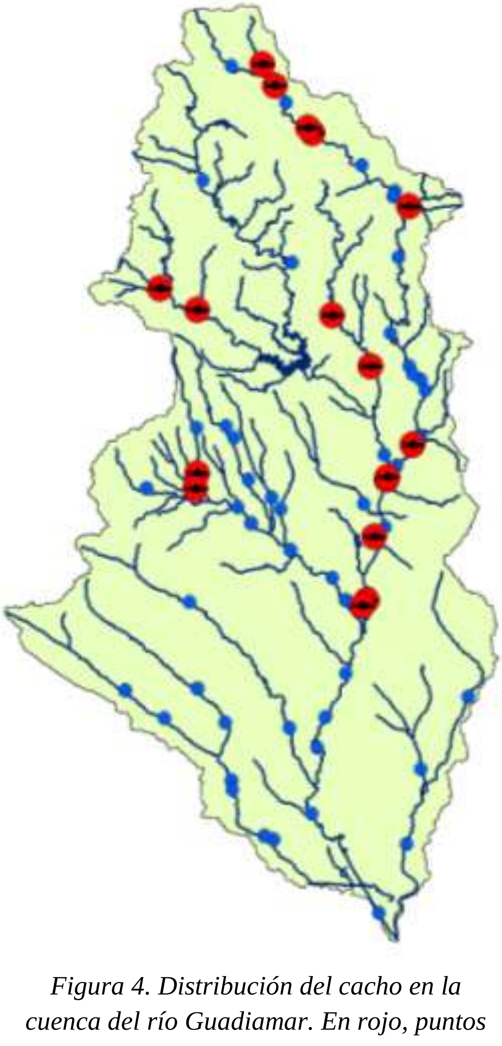

Figura 4. Distribución del cacho en la cuenca del río Guadiamar. En rojo, puntos de muestreo donde se capturó la especie, en azul, donde no se encontró.

Vélez, Guadalhorce, Benahavis, Guadairo, Jara, Turia, Jucar, Mijares, Guadalest, Albufera de Valencia, Bullent, Verde y Serpis. Posiblemente introducido en el río Matarraña, cuenca del Ebro (Doadrio, 2001).

En la cuenca del Guadiamar, la especie ha sido capturada en arroyos de cabecera y en la sección media-alta del curso principal (Fig. 4).

Las hembras alcanzan la madurez sexual en su 4º año de edad (70 mm LF) y los machos en el 3º (60 mm LF) (Fernández-Delgado y Herrera, 1995a). El período reproductivo va de abril a julio. Con muestreos mensuales, Soriguer *et al*. (2000a) afirman que las hembras que no han llegado a su 5º año desovan una vez y las mayores varias veces, datos que no concuerdan con los de Fernández-Delgado y Herrera (1995a), donde muestreos semanales informan de sólo dos desoves en todas las clases de edad. Generalmente se alimentan de pequeños artrópodos acuáticos, aunque en verano la escasez de éstos los lleve a consumir principalmente restos vegetales (Magalhaes, 1993; Coelho *et al.*, 1997).

Estado de Conservación: La especie es endémica de la Península Ibérica. A nivel mundial, la última actualización sobre el estado de conservación de la especie la cataloga como **NT** (UICN, 2006). A nivel nacional, se encuadra como **VU** “Vulnerable a la extinción bajo criterios **A 2 c e** (Doadrio, 2001). En Andalucía está catalogada como **VU** “Vulnerable a la extinción” bajo criterios **A 1 c e** (CMAJA, 2001).

PARDILLA - *Iberochondrostoma lemmingii* (Steindachner, 1866).

La especie es fusiforme y algo robusta, con un pedúnculo caudal relativamente alto y un cierto moteado oscuro por todo el cuerpo. Sus rasgos más significativos son una boca subterminal y arqueada carente de lámina córnea, que provoca una leve prominencia redondeada del hocico.

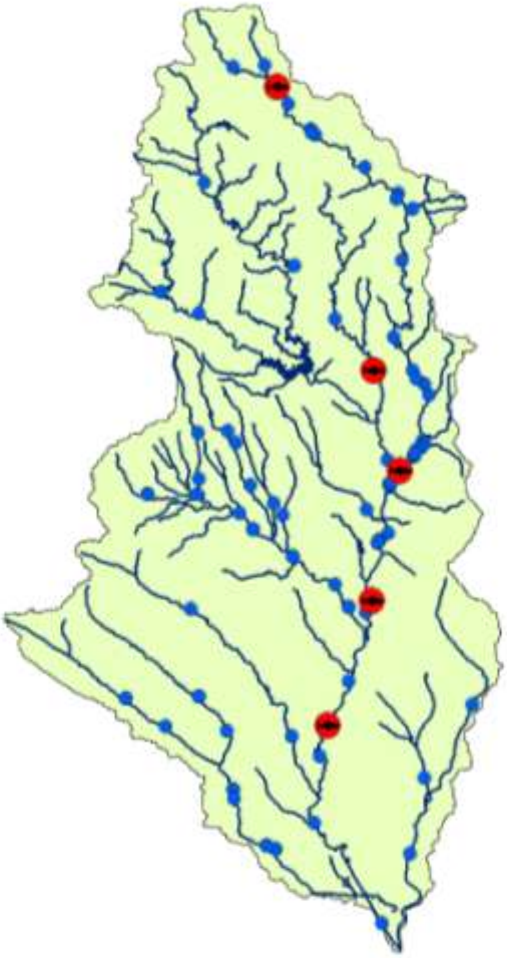

Figura 5. Distribución de la pardilla en la cuenca del río Guadiamar. En rojo, puntos de muestreo donde se capturó la especie, en azul, donde no se encontró.

Posee 49-59 escamas en la línea lateral, 4 ½- 7 filas de escamas entre ésta y el origen pélvico, y un diámetro ocular 1-1.5 veces la longitud del hocico (Kottelat y Freyhof, 2007). La longitud máxima registrada para la especie ha sido 144 mm LF (hembras) y 114 mm LF (machos) (Fernández-Delgado y Herrera, 1995b).

La especie se distribuye por la mitad occidental de la Península Ibérica. En España, se sitúa en las cuencas de los ríos Tajo, Guadiana, Guadalquivir, Odiel y los afluentes del suroeste del Duero (Doadrio, 2002

En la cuenca del Guadiamar, las escasas poblaciones que se han detectado de la especie se distribuyen a lo largo del cauce principal, si bien hay una que se adentra levemente en el río Frailes (Fig. 5).

En la especie, el período reproductivo ocupa los meses comprendidos entre febrero y mayo (Rodríguez-Jiménez, 1987), donde las hembras reparten su freza en dos puestas sobre macrófitos de aguas poco profundas (Fernández-Delgado y Herrera, 1995b). Tanto los machos como las hembras alcanzan su madurez sexual en su segundo año de vida (Velasco, 1990), ya que en su primer año el crecimiento es muy acelerado y llegan a un 65% de su longitud total alcanzando los 60 mm LF, (Rodríguez-Jiménez, 1987; Fernández-Delgado y Herrera, 1995). Se alimentan principalmente de detritus, algas y zooplancton en zonas de aguas no muy contaminadas (Fernández-Delgado, 2003).

Como ya se ha indicado, la pardilla se incluía hasta hace poco dentro del género *Chondrostoma* sin embargo, sus características moleculares y morfológicas han favorecido la creación de un nuevo género *Iberochondrostoma* (Robalo *et al*., 2007). En éste se incluyen cuatro especies de las que la pardilla es la más abundante y mejor distribuida, mientras que las tres restantes se encuentran aisladas en localidades muy reducidas. Todas estas especies tienen en común un pequeño tamaño, una boca pequeña y sin labio inferior corneo, y habitar en arroyos de velocidad reducida con vegetación acuática sumergida (Kottelat y Freyhof, 2007).

Estado de Conservación: La especie es un endemismo de la Península Ibérica. A nivel mundial, la última actualización sobre el estado de conservación de la especie la cataloga como **VU** “Vulnerable a la extinción” bajo criterios **A 2 c e** (UICN, 1996). A nivel nacional, se encuadra como **VU** “Vulnerable a la extinción” bajo criterios **A 2 c e** (Doadrio, 2001). En Andalucía está catalogada como **VU** “Vulnerable a la extinción” bajo criterios **A 1, 2 c** (CMAJA, 2001).

Familia Cobitidae

COLMILLEJA - *Cobitis paludica* (de Buen, 1930).

Especie caracterizada por su forma alargada o tubular, ligeramente comprimida por los flancos y de perfil dorso-ventral recto. Boca ínfera con tres pares de barbillones. Posee ojos pequeños con una espina subocular para la defensa, de donde probablemente le viene el nombre común. Además, presenta un llamativo colorido con ocelos oscuros sobre un fondo amarillento de escamas muy pequeñas, casi inapreciables.

Varía de otros cobítidos de la Península Ibérica principalmente por tener 6-7 radios ramificados en la aleta dorsal; los machos poseen en la base del segundo radio de las aletas pectorales una lámina circular que se denomina escama de canestrini (Kottelat y Freyhof, 2007).

La longitud máxima que alcanza la especie son 99 mm LS (hembras) y 90 mm LS (machos) en su 5º y 4º año, respectivamente (Oliva-Paterna *et al.*, 2002).

La especie se distribuye ampliamente por la Península Ibérica. En España, ocupa las cuencas de los ríos Ebro, Tajo, Guadiana, Guadalquivir, Guadalete, Guadalhorce, Guadalmedina, Barbate, Jara, Piedras, Vega, Peñíscola, Odiel, Júcar, Turia, Mijares, Bullent, Racons, Albufera de Valencia, afluentes de la

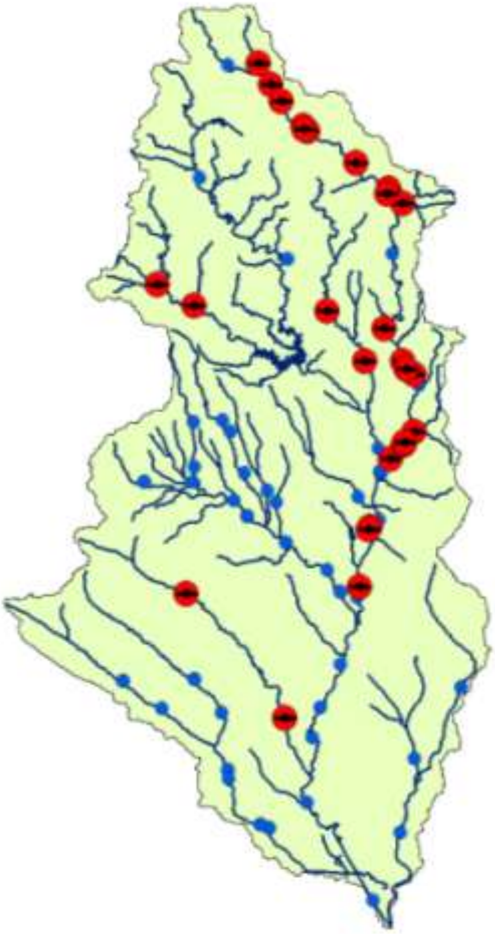

Figura 6. Distribución de la colmilleja en la cuenca del río Guadiamar. En rojo, puntos de muestreo donde se capturó la especie, en azul, donde no se encontró.

margen izquierda del Duero y probablemente introducido en el Miño y Nalón (Doadrio, 2001).

En la cuenca del Guadiamar, la especie se encuentra fundamentalmente distribuida por el cauce principal y algunos tributarios como el Agrio o el Alcarayón (Fig. 6).

En su primer año, la especie alcanza el 65-70% de su longitud total. Ambos sexos alcanzan la madurez sexual a principios de su segundo año. El periodo reproductivo va de marzo a julio, en el que desovan como mínimo dos veces sobre zonas con abundante vegetación (Oliva-Paterna *et al.*, 2002). Tras el desove vuelven a zonas con lecho de arena o grava, donde gracias a la carencia de una vejiga natatoria, pueden permanecer bajo el sustrato durante el día y alimentarse en él por la noche (Clavero *et al.*, 2005), principalmente de larvas de ostrécodos y quironómidos, a veces filtrando detritus (Soriguer, 2000b).

Especie cuyo mayor requisito sería un sustrato adecuado, ya que es muy resistente al estiaje y a la contaminación, pudiendo sobrevivir en pequeños charcos y tomar el oxigeno directamente del aire mediante un mecanismo de absorción intestinal (Kottelat y Freyhof, 2007). Es por ello, que junto al barbo y el calandino sea una de las especies más abundantes en Andalucía (Fernández-Delgado *et al.*, 2007).

Estado de Conservación: La especie es un endemismo de la Península Ibérica. A nivel mundial, la última actualización sobre el estado de conservación de la especie la cataloga como **VU** “Vulnerable a la extinción” bajo criterios **A 2 c e + 3 c e** (UICN, 2006). A nivel nacional, se encuadra como **VU** “Vulnerable a la extinción” bajo criterios **A 2 c e** (Doadrio, 2001). En Andalucía está catalogada como **VU** “Vulnerable a la extinción” bajo criterios **A 1 c e** (CMAJA, 2001).

Familia Anguilidae

ANGUILA - *Anguilla anguilla* (Linnaeus, 1758).

Cuerpo serpentiforme, cilíndrico y muy alargado; aletas dorsal, caudal y anal, alargadas y unidas entre sí. Pez ápodo, sin aleta pelvianas. Ojos muy pequeños con respecto a la cabeza, unas escamas diminutas embebidas en la piel y una mucosidad muy abundante por todo el cuerpo.

El tamaño común en ríos suele encontrarse entre 400-700 mm, llegando incluso a 2 m (Kottelat y Freyhof, 2007); si bien en el Guadalquivir la longitudes máximas registradas son de 541 mm (hembras) y 391 mm (machos) (Fernández-Delgado *et al.*, 1989).

La especie se distribuye por el océano Atlántico, mar Mediterráneo, Báltico, del Norte y Negro, además de adentrarse por los ríos que en ellos desembocan (Doadrio, 2001).

En la cuenca de Guadiamar, se ha localizado en el tramo medio del cauce principal y tramo bajo del arroyo de Tejada (Fig. 7).

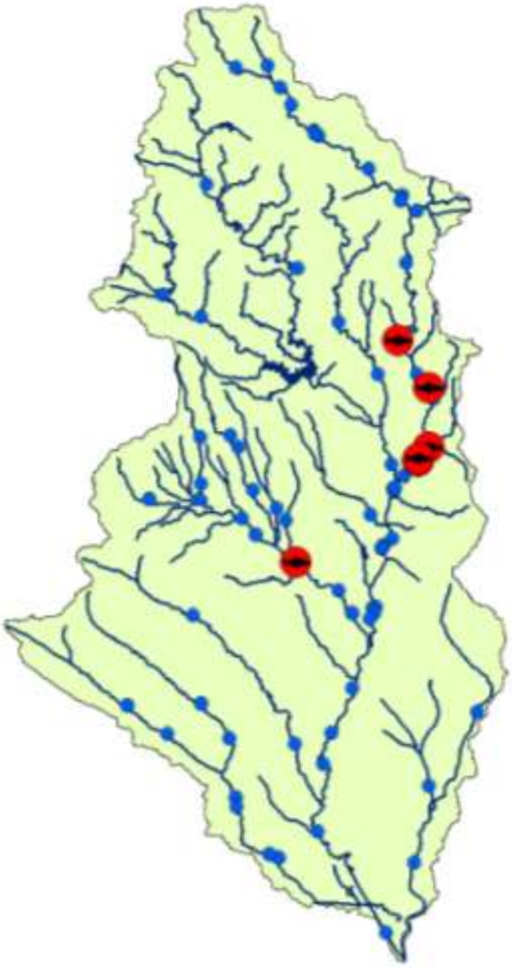

Figura 7. Distribución de la anguila en la cuenca del río Guadiamar. En rojo, puntos de muestreo donde se capturó la especie, en azul, donde no se encontró.

Es una especie catádroma con un ciclo de vida muy complejo. Éste empieza con el desove en el Mar de los Sargazos, zona oceánica cercana a Cuba, entre los meses de marzo y julio. Las larvas resultantes, denominadas "leptocéfalos", están aplanadas lateralmente y viajan durante 12-18 meses desde su zona de nacimiento a las costas europeas y norte de África. Una vez próxima a la plataforma continental, sufre una metamorfósis y su cuerpo se torna cilíndrico y apigmentado, es la fase de "angula". Siguiendo el rastro del agua dulce proveniente de los estuarios, penetran en ellos y comienza su pigmentación hasta completarla con tonos verde-amarillentos que le hacen llamarlo "angulón". Los machos no remontarán más de 200 Km., mientras que las hembras suben hacia las cabeceras. En este remonte, que puede durar de unos 8 a 18 años, los individuos siguen creciendo y adquieren su colorido definitivo con el dorso grisáceo y el vientre plateado, ahora sí, llegando al estadio de "anguila plateada". Una vez desarrollados, vuelven río abajo donde de nuevo en el estuario permanecen hasta terminar su desarrollo. De aquí, parten hacia el mar y una vez hayan desovado, morirán (Fernández-Delgado, 2003).

En este ciclo, cabe destacar la gran plasticidad de la especie en cuanto a la alimentación, ingiriendo todo tipo de material orgánico abundante en el medio donde se encuentre, y a la salinidad, pasando de aguas salobres a ríos de cabecera con poca conductividad. Por último, su capacidad para arrastrarse fuera del agua y respirar por la piel, culminan la lista de peculiaridades de esta especie migradora (Fernández-Delgado, 2003).

La cuenca del Guadiamar es el último aporte importante de agua que llega al Guadalquivir. Esto la convierte en la única subcuenca con presencia natural de la especie, ya que el primer obstáculo que impediría su remonte desde el estuario sería la presa de Alcalá del río (Sevilla), situada río arriba (Fernández-Delgado *et al.*, 2007). Esto pone de manifiesto lo vulnerable que es esta especie, a pesar de sus múltiples cualidades descritas anteriormente, a las barreras que imposibilitan completar su ciclo, encontrándose por ello sus poblaciones en declive (Doadrio, 2001).

Estado de Conservación: A nivel mundial, la especie ha reducido sus efectivos en más de un 95% en los últimos 25 años (Arribas, 2009) por lo que esta catalogada como **CR** "En Peligro Crítico" bajo criterios **A 2 b d + 4 b d** (UICN, 2012). A nivel nacional está incluida en el Libro Rojo como **VU** "Vulnerable a la extinción" bajo criterios **A 2 c e** (Doadrio, 2001). En Andalucía está registrada como **LR, nt** "Riesgo menor: casi amenazada" de extinción (CMAJA, 2001).

Familia Atherinidae

PEJERREY - *Atherina boyeri* (Risso, 1810).

Es un pez pequeño y estilizado, con una inconfundible banda longitudinal plateada, que va desde la cabeza hasta la cola. Equipado con un par de aletas dorsales, la primera alineada con las pelvianas y la segunda con la anal. Las aletas pectorales tiene forma de espátula .La boca es terminal, ligeramente súpera, y sus ojos son grandes y cercanos al perfil dorsal.

Según Kottelat y Freyhof (2007), las particularidades que ofrece con respecto a otras especies de *Atherinidae* son 39-49 escamas en la línea lateral; 23-31 branquiespinas y 13-15 ½ radios en la aleta anal.

El tamaño máximo registrado para la especie ha sido de 116 mm LT (hembras) y 98 mm LT (machos), ambos en su 4º año (Bartulovic *et al.*, 2004).

La especie se extiende por los litorales del mar Mediterráneo y el mar Negro, además de poblaciones aisladas en el Atlántico. En España, la especie se encuentra en las desembocaduras de los ríos de vertiente sur y levantina, así como poblaciones aisladas en la cuenca del Guadalquivir (Doadrio, 2001).

En la cuenca del Guadiamar existe una pequeña población muy localizada y aislada en el tramo medio del cauce principal (Figura 8).

Figura 8. Distribución del pejerrey en la cuenca del río Guadiamar. En rojo, puntos de muestreo donde se capturó la especie, en azul, donde no se encontró.

El período reproductivo de la especie va de marzo a junio. Tanto machos como hembras maduran al superar el primer año de vida, en el que alcanzan en torno a un 56-65 % del tamaño total (Fernández-Delgado *et al.*, 1988; Andreu-Soler *et al.*, 2003a y 2003b; Leonardos y Sinis, 2000). Para la freza, los individuos migran hacia el estuario para dejar su puesta sobre la vegetación sumergida, repitiéndolo varias veces en la época de reproducción. Una vez terminado el desove, pueden distribuirse tanto por zonas hipersalinas, como por aguas dulces río arriba, en poblaciones con una proporción de sexos desde 1:1 a 1:2.5 (machos:hembras) (Fernández-Delgado, 2003; Koutrakis *et al.*, 2004). Su dieta es fundamentalmente zooplanctívora (Vizzini y Mazzola, 2005).

El pejerrey, es una especie gregaria y estuárica, pero al igual que las especies migradoras, las barreras en el cauce impiden que se distribuya aguas arriba, así, como ocurría con la anguila, el Guadiamar es la única subcuenca del Guadalquivir a la que llegan algunas poblaciones, alcanzando incluso el tramo medio-alto.

Estado de Conservación: A nivel mundial, la especie no se encuentra catalogada en lista roja de especies amenazadas (UICN, 2008). A nivel nacional sin embargo, la especie esta incluida en el Libro Rojo como **VU** “Vulnerable a la extinción” bajo criterios **A 1 c** (Doadrio, 2001). En Andalucía, tampoco se encuentra catalogada en el Libro Rojo de los Vertebrados Amenazados de Andalucía (CMAJA, 2001).

Familia Mugilidae

ALBUR - *Liza ramada* (Risso, 1826).

Es un pez alargado y fusiforme, de cabeza aplanada dorso-ventralmente y boca terminal algo arqueada. A diferencia de otros mugílidos, muestra una mancha negra en la base de la aleta pectoral y al abatir ésta hacia delante no llega al ojo.

Posee dos aletas dorsales, de posición algo retrasadas con respecto al eje central. Entre el dorso oscuro y el vientre plateado, los flancos ofrecen varias bandas oscuras dispuestas longitudinalmente, aspecto típico de los mugílidos.

Posee 41-46 escamas en la línea lateral; ranuras simples y longitudinales en las escamas predorsales; 24 filas de escamas circumpedunculares; el ángulo posterior del hueso preorbital es redondeado; y un tamaño máximo de 540 mm. (Kottelat y Freyhof, 2007).

La especie se distribuye por las costas mediterráneas y del atlántico occidental, desde Cabo Verde y Senegal hasta las Islas Británicas y el sur del Báltico, el Mar Negro y el Mar de Azov(Kottelat y Freyhof, 2007). .

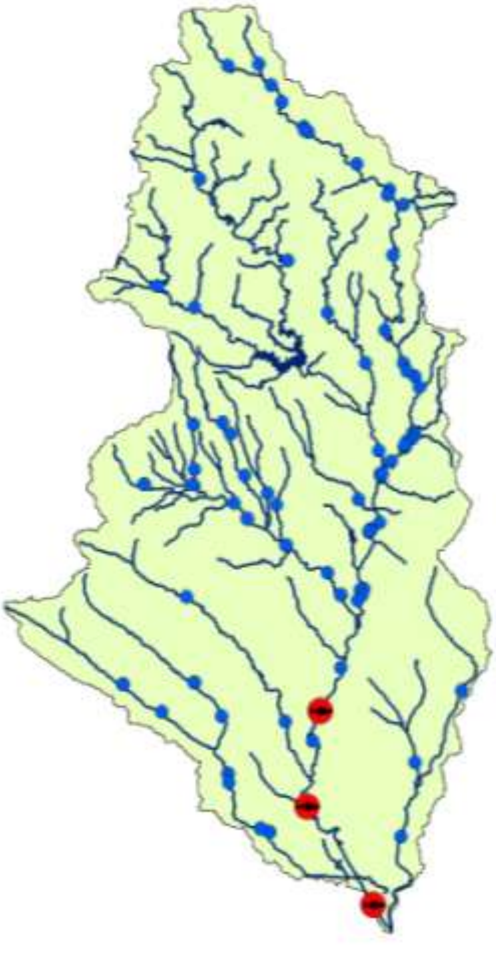

Figura 9. Distribución del albur en la cuenca del río Guadiamar. En rojo, puntos de muestreo donde se capturó la especie, en azul, donde no se encontró.

En la cuenca del Guadiamar, la especie se adentra sólo hasta el tramo bajo del curso principal (Fig. 9).

El período reproductivo para la especie se comprende entre octubre y diciembre. Los machos alcanzan su madurez sexual a los 2-3 años de vida, las hembras a los 4. Es una especie catádroma (migra al mar para desovar). Los juveniles viajan al estuario, donde se alimentan principalmente de zooplancton inicialmente, y de pequeños animales bentónicos posteriormente. Los adultos, al terminar la época de reproducción y volver a las zonas bajas de los ríos y lagunas costeras, se alimentan principalmente de crustáceos del zooplancton, quironómidos adultos, y en menor medida, removiendo el fondo y filtrando algas y detritus (Drake *et al.*,1984; Gisbert *et al.*, 1995a, 1995b y 1996; Almeida, 2003; Fernández-Delgado, 2003).

Estado de Conservación: Tanto a nivel mundial, nacional y regional, la especie no se encuentra catalogada en ninguna de sus correspondientes listas de especies amenazadas (UICN, 2008) (Doadrio, 2001) (CMAJA, 2001).

CAPITÁN - *Mugil cephalus* (Linnaeus, 1758).

Como el resto de los mugílidos, posee un dorso aplanado, unas listas oscuras sobre los flancos plateados y dos aletas dorsales, pero su peculiaridad con respecto a esta familia es que está dotado de un parpado adiposo que cubre casi la totalidad del ojo, siendo su cabeza también la más redondeada.

La especie se distribuye por los mares tropicales y subtropicales, el Mediterráneo, Mar Negro y las costas del sur del Atlántico hasta el Golfo de Vizcaya (Kottelat y Freyhof, 2007).

En la cuenca del Guadiamar, la especie se adentra en el tramo bajo del cauce principal, incurriendo incluso en zonas del tramo medio (Fig. 10).

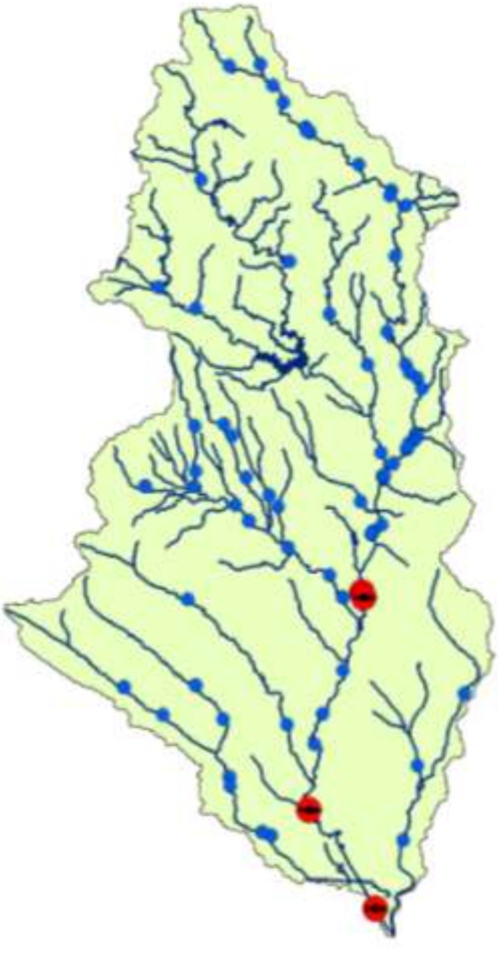

Figura 10. Distribución del capitán en la cuenca del río Guadiamar. En rojo, puntos de muestreo donde se capturó la especie, en azul, donde no se encontró.

Es una especie catádroma con una biología muy similar a la del albur. Los machos llegan a su madurez sexual a los 2 años y las hembras a los 3. La migración hacia el mar para el desove se produce entre julio y octubre (Kottelat y Freyhof, 2007) y vuelven a las zonas bajas de los ríos y lagunas salinas para alimentarse principalmente de algas y detritus removiendo el fondo, mientras que los juveniles, al llegar al estuario prefieren zooplancton (Cardona, 2000; Cardona *et al.*, 2007; De Silva, 1980; Drake *et al.*,1984;

Fernández-Delgado, 2003: Gisbert *et al.*, 1995a y 1995b).

Estado de Conservación: Tanto a nivel mundial, nacional y regional, la especie no se encuentra catalogada en ninguna de sus correspondientes listas de especies amenazadas (UICN, 2008) (Doadrio, 2001) (CMAJA, 2001).

ESPECIES EXÓTICAS

Familia Cyprinidae

CARPA - *Cyprinus carpio* (Linnaeus, 1758).

La especie se diferencia de otros ciprínidos presentes en la península por una aleta dorsal larga que llega casi a la caudal; dos pares de barbillones, uno en el maxilar superior y otro en la comisura de la boca; y por último, un cuerpo no muy alargado, comprimido por los flancos y bastante curvo en el perfil dorsal.

Original de la cuenca del Danubio, cuencas norte del Mar Negro, Mar Caspio y oriente próximo. (Kottelat y Freyhof, 2007). Introducida en España en el siglo XVI durante la dinastía de los Habsburgo con fines ornamentales, donde hoy se encuentra ampliamente distribuida (Fernández-Delgado, 2003).

En la cuenca del Guadiamar, la especie se ha instaurado en el tramo medio y bajo del cauce principal, sin embargo no se ha extendido a ninguno de los tributarios (Fig. 11).

En nuestras latitudes, las cálidas aguas de los embalses, lagunas y cursos bajos de los ríos propician el rápido crecimiento de los individuos. Tanto los machos como las hembras

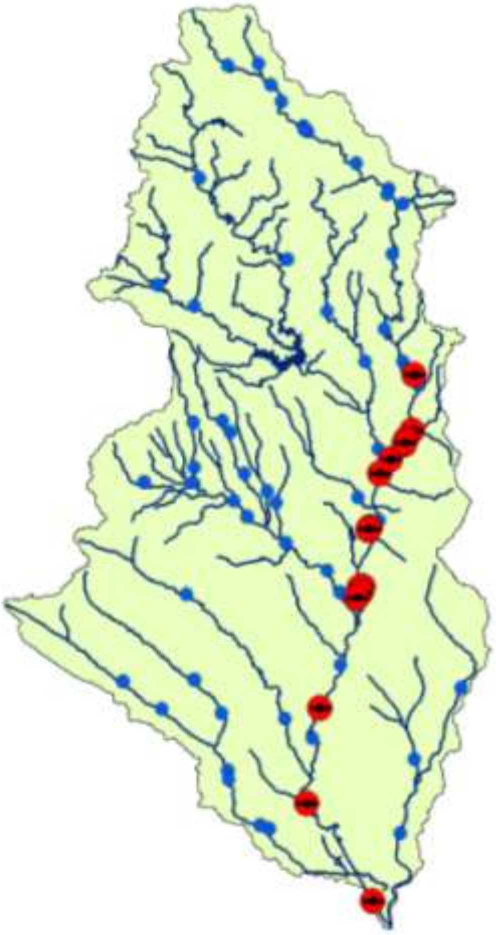

Figura 11. Distribución de la carpa en la cuenca del río Guadiamar. En rojo, puntos de muestreo donde se capturó la especie, en azul, donde no se encontró.

alcanzan su madurez sexual al 2º año de vida. El período reproductivo varía en función de la Latitud, siendo el más largo conocido de marzo a agosto, mientras lo más usual en nuestras aguas es en mayo y junio. Para el desove, utilizan aguas someras con abundante vegetación donde dejar la puesta aprovechando las primeras horas del día. Los alevines se alimentarán principalmente de zooplancton, mientras los adultos son omnívoros, incluyendo detritus, plantas, semillas y crustáceos e insectos bentónicos (Crivelli, 1981; Fernández-Delgado, 1990, 2003; García-Berthou, 2001b).

En la Península Ibérica se encuentran dos variedades, la “carpa común”, totalmente cubierta de escamas y la “carpa de espejos”, que tan solo tiene algunas dispersas e irregulares. Ambas son muy resistentes a bajas concentraciones de oxígeno, altas temperaturas y cantidades importantes de contaminación orgánica (Fernández-Delgado *et al.*, 2007).

Estado de Conservación: **Exótica, Invasora.**

CARPÍN - *Carassius gibelio* (Bloch, 1782).

Éste se caracteriza por su forma comprimida por los flancos y poseer una aleta dorsal alargada similar a la carpa, pero a diferencia de ésta, carecer de barbillones.

El género *Carassius* tiene una alta similitud morfológica entre sus especies (Kalous *et al*., 2012), por lo que existe una gran confusión a la hora de identificar los individuos del género presentes en la Península Ibérica. *Carassius auratus* fue introducida en la antigüedad desde Asia con fines ornamentales (Fernández-Delgado, 2003) mientras que *Carassius gibelio* ha sido introducido más recientemente desde Europa central, al incorporarse en los lotes de carpas transportados por el resto de Europa para la introducción principalmente en embalse (Kottelat y Freyhof, 2007). En esta tesis, a falta de un estudio genético, hemos decidido aceptar la especie como *C. gibelio*, ya que al estar presente tan sólo en tramos bajo el principal embalse y aparecer junto a la carpa, vemos más probable que el origen de éstos individuos haya venido por las vías descritas por Kottelat y Freyhof (2007) para *C. gibelio*.

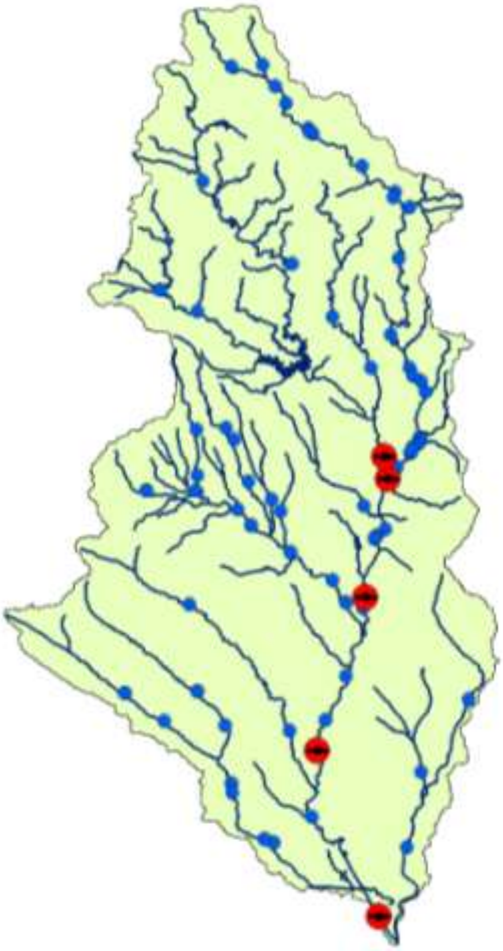

Figura 12. Distribución del carpín en la cuenca del río Guadiamar. En rojo, puntos de muestreo donde se capturó la especie, en azul, donde no se encontró.

Diferenciado de otras especies de *Carassius* por tener un color dorado-bronce-parduzco; 26-31 escamas en la línea lateral completa; el último radio duro de la aleta dorsal y anal denticulado; el borde de la aleta dorsal cóncavo o recto; normalmente 5 radios ramificados en la aleta anal; 38-47 branquiespinas; peritoneo negro (Kottelat y Freyhof, 2007). La longitud máxim alcanza los 450 mm, aunque no suele sobrepasar los 20 mm (Lorenzoni *et al.*, 2007).

En la cuenca del Guadiamar, la especie no es muy abundante, encontrándose algunos individuos dispersos en el tramo medio y bajo del cauce principal. Sin embargo, se adentra levemente en el río Agrio. (Fig. 12).

Su biología es muy similar a la de la carpa. En aguas tranquilas como embalses, estanques y cursos bajos de los ríos, se alimentan de detritus e invertebrados que encuentran en el fango. Allí, a los 2 años, entre abril y agosto, llevan a cabo el desove sobre la vegetación sumergida. Su estrategia reproductiva comprende tanto reproducción sexual para los individuos diploides como reproducción por ginogénesis (mediante división mitótica del embrión) para las hembras triploides, por lo que en Europa, sus poblaciones suelen tener una sex ratio tendente claramente hacia las hembras (Lorenzoni *et al.*, 2007).

Su gran potencial como especie invasora es debido a que soporta altas temperaturas en el agua, baja concentración de oxígeno, cantidades importantes de contaminación orgánica y una un amplio rango de tolerancia con respecto al pH, de 4.5 a 10.5 (Yamanaka, 2006).

Estado de Conservación: **Exótica, Invasora.**

ALBURNO – *Alburnus alburnus* (Linnaeus, 1758).

Ciprínido muy similar a los individuos autóctonos del género *Squalius*, especialmente parecido al calandino, con el que comparte una boca súpera, un ojo relativamente grande con respecto a la cabeza, la disposición de las aletas y entre individuos de poca talla, el aspecto general. No obstante, sus características más discriminantes son una aleta caudal alargada que casi alcanza a la caudal y la ausencia parcial de escamas en la quilla, si bien, en individuos adultos es fácil de apreciar un cuerpo aplanado por los flancos de un llamativo brillo plateado.

Según Kottelat y Freyhof (2007), difiere de otras species de *Alburnus* en Europa principalmente por poseer una quilla desprovista de escamas desde el ano hasta la base de las aletas pélvicas.

Las longitudes encontradas en la cuenca del Gudalquivir alcanzan los máximos registrados para la especie, cercanos a los 160 mm de longitud furcal (Fernandez-Delgado *et al.*, 2007).

Especie originaria de Europa central, desde los Pirineos hasta los Urales, ausente en la mitad norte de Escandinavia y la cuenca mediterránea excepto en Francia y parte de Turquía). La primera introducción constatada en España fue en

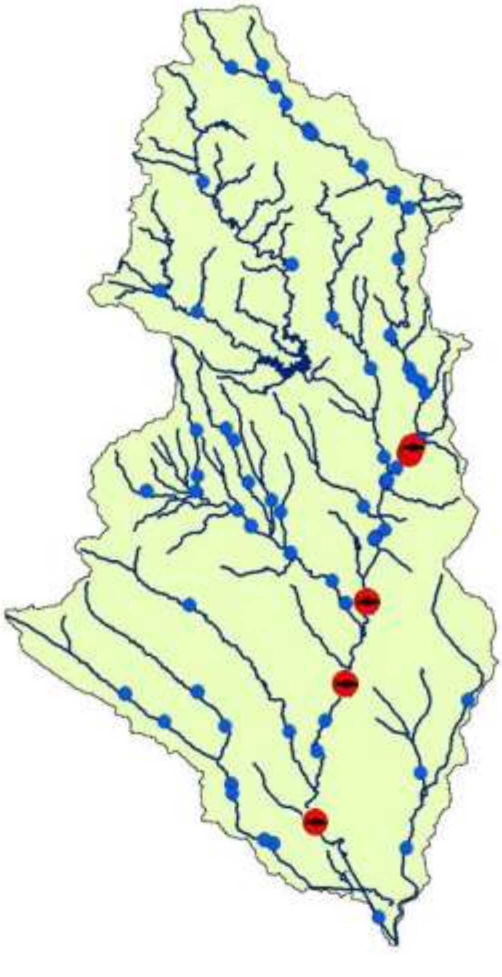

Figura 13. Distribución del alburno en la cuenca del río Guadiamar. En rojo, puntos de muestreo donde se capturó la especie, en azul, donde no se encontró.

el lago Banyoles (Girona) a principios del siglo XX (Díaz-Luna and Gómez-Caruana, 1998), pero su expansión empezó a partir de la década de los 90, cuando fue introducida por los pescadores como cebo vivo o pez pasto para especies piscívoras tales como el Lucio (Esox lucius) y el Siluro (Silurus glanis) (Doadrio *et al*., 2011).

En la cuenca del Guadiamar, la especie ha establecido poblaciones muy numerosas en el tramo medio y bajo del cauce principal (Fig. 13).

En la Península Ibérica, la especie se ha expandido desde los embalses a los ríos colindantes, vive cerca de la superficie y su dieta se compone de zooplancton, crustáceos, insectos y pequeños peces. Los individuos alcanzan la madurez sexual a los dos años, si bien algunos lo hacen en el primero. En estas latitudes la reproducción se lleva a cabo entre los meses de noviembre y enero (Doadrio *et al*., 2011).

La alta fecundidad (Hladík and Kubečka, 2003), plasticidad de dieta (Mehner *et al*., 2005) y gran tolerancia a los cambios de temperatura (Vinyoles *et al*., 2007), permiten a esta especie anteponerse a otras que compartan los mismos hábitat y recursos, principalmente, y en el caso del Guadiamar, al calandino y al cacho. Por otro lado, el parentesco común del alburno con dichas especies del género *Squalius* (Robalo *et al*., 2006) hace que sea fácil su hibridación y por tanto, se produzca la entrada de genes alóctonos en la población autóctona (Vinyoles *et al*., 2007; Almodóvar *et al*., 2012).

Estado de Conservación: **Exótica, Invasora.**

Familia Centrarchidae

PERCASOL - *Lepomis gibbosus* (Linnaeus, 1758).

La especie presenta una coloración de fondo verde-azulado con machas anaranjadas y un lóbulo oscuro con bordes llamativos en el opérculo. El cuerpo es aplanado por los flancos, con una aleta dorsal dividida en dos partes, la primera compuesta por radios duros y la segunda por blandos y ramificados. Además, posee un iris rojo y un pedúnculo caudal estrecho.

Según Kottelat y Freyhof (2007), difiere de otros centrarquidos introducidos en Europa por tener una pequeña solapa en el opérculo, tan ancha como el ojo y de color negro con el extremo verde, rojo o anaranjado; boca pequeña, con longitud de la mandíbula superior similar a la del ojo; 36-37 líneas en la línea lateral; 3-4 espinas en la aleta anal; y la parte posterior de las aletas dorsal y anal sin marcas oscuras.

La longitud máxima registrada para la especie en la cuenca del Guadalquivir ha sido 140 mm (Gutierrez, 1997).

Procedente de Norteamérica, fue introducida en el siglo XX en la Península Ibérica para su uso como cebo vivo y ornamental (Fernández-Delgado, 2003). Actualmente se ha extendido por todo el territorio nacional, y de

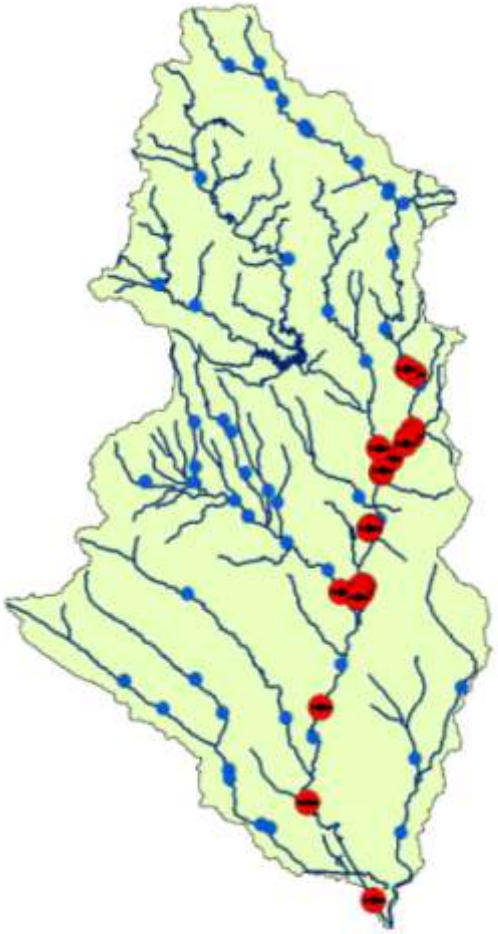

Figura 14. Distribución del percasol en la cuenca del río Guadiamar. En rojo, puntos de muestreo donde se capturó la especie, en azul, donde no se encontró.

manera más importante en la zona centro-sur del país, llegando a ser la especie dominante en numerosos tramos de algunas cuencas como es el caso del río Guadiato en Córdoba (Fernández-Delgado *et al.*, 2007) o la zona litoral del lago Banyoles en Girona (Garicía-Berthou y Moreno-Amich, 2000a).

En la cuenca del Guadiamar, la especie se ha extendido por el tramo medio y bajo del cauce principal, incluso se han capturado individuos que se adentran en los río Agrio y arroyo Ardachón (Fig. 14).

El período reproductivo para la especie en Europa va de marzo a septiembre en función de la Latitud (Tomecek *et al.*, 2007). Tanto machos como hembras maduran en su 2º año de vida. Las hembras dividen su puesta en al menos dos frezas (Gutierrez, 1997; Gutierrez *et al.*, 2000). Éstas depositan los huevos sobre un nido que el macho ha construido separando las piedras del fondo y del que será protector hasta la eclosión de los huevos (Pérez-Bote *et al.*, 2001). La especie se encuentra bien adaptada en aguas superficiales, de poca corriente y abundante vegetación, de hay su proliferación en embalses y orillas de ríos con caudal controlado (Tomecek *et al.*, 2007). Su alimentación varía en función de su edad y época del año. Los juveniles se alimentan principalmente de zooplancton, mientras que los adultos incrementan el tamaño de sus presas, prefiriendo generalmente larvas de quironómidos, moluscos y camarones, y especialmente en primavera y verano, huevos de peces y restos vegetales (Garicía-Berthou y Moreno-Amich, 2000b).

Su gran impacto se debe a su voracidad con respecto a alevines y su feroz territorialidad, desplazando así a los individuos autóctonos de la zona.

Estado de Conservación: **Exótica, Invasora.**

BLACK-BASS - *Micropterus salmoides* (Lacèpéde, 1802).

Como centrarquido, comparte el esquema de sus aletas y el cuerpo aplanado por los flancos con el percasol. Sin embargo, a diferencia de la anterior llamativa especie, ésta es mucho más críptica, de mayor tamaño y con pedúnculo caudal más alargado.

Según Kottelat y Freyhof (2007), sus características principales son: una línea lateral con 55-68 escamas; una prominente franja oscura o fila de manchas alineadas; 3 radios duros en la aleta anal; y una boca grande, con una longitud de mandíbula superior mayor que el diámetro del ojo.

Procedente de Norteamérica, fue introducida en España en 1955 para la práctica de pesca deportiva en los embalses (Fernández-Delgado, 2003).

En la cuenca del Guadiamar, la especie se ha extendido por el tramo medio del cauce principal, incluso se ha adentrado hasta el río Frailes (Fig. 15).

Los machos alcanzan la madurez sexual en su 2º año de vida (220 mm) y las hembras en su 3º (300 mm). La reproducción se lleva a cabo principalmente en el mes de mayo La especie tiene un comportamiento reproductivo similar al del percasol. El macho abre un hueco en el fondo

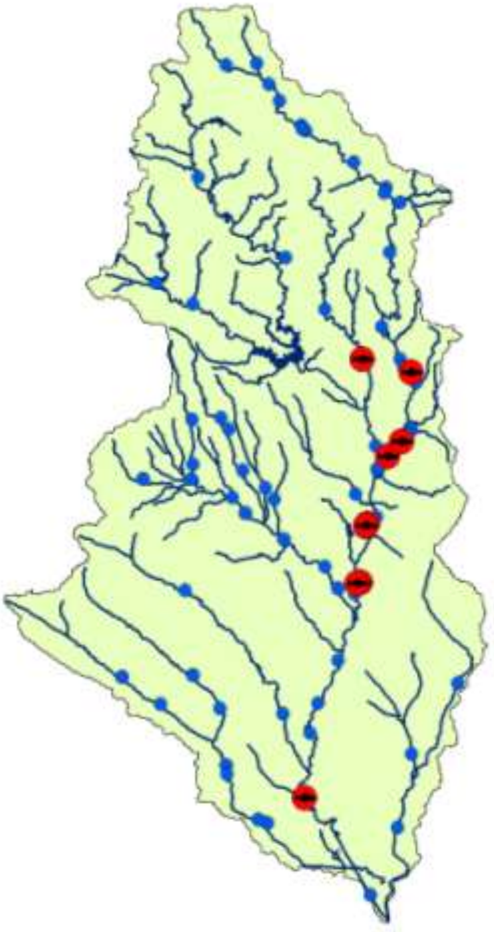

Figura 15. Distribución del black-bass en la cuenca del río Guadiamar. En rojo, puntos de muestreo donde se capturó la especie, en azul, donde no se encontró.

arenoso e incita a la hembra a que desove, éste vigilará la puesta e incluso los alevines hasta que decidan dispersarse (Lorenzoni *et al.*, 2002; Pou-Rovira, 2004). La especie varía su dieta en función de su edad y tamaño, así, los jóvenes de primer año se alimentan principalmente de microcrustáceos, los de un tamaño intermedio de anfípodos e insectos y finalmente los de gran tamaño ingieren cangrejos y peces, siendo su piscivoría menor que la que llevan a cabo en su zona de origen (Rodríguez-Jiménez, 1989; García-Berthou, 1991; Nicola *et al.*, 1996; Godinho *et al.*, 1997a, 1997b).

Estado de Conservación: **Exótica, Invasora.**

Familia Poeciliidae

GAMBUSIA - *Gambusia holbrooki* (Agassiz, 1859).

Pez de pequeño tamaño con acusado dimorfismo sexual. Los machos no suelen superar los 30 mm. y las hembras los 60 mm. Éstas, además poseen un perfil dorsal relativamente recto y uno ventral curvo que se vuelve recto en el pedúnculo caudal, mientras que los machos mantienen rectos ambos perfiles. Con respecto a las aletas, la dorsal se encuentra con un retraso similar en ambos sexos, mientras que la anal, alarga sus radios en los machos para dotarlos de un órgano copulador (gonopodio). Finalmente, con respecto al colorido, ambos mantienen tonos grisáceos, más apagados en los machos y más vivos en las hembras, en las que suele destacar una mancha oscura a cada flanco que toma mayor consistencia en el período reproductivo.

Procedente de Norteamérica, fue introducida en 1921 en España por las autoridades sanitarias para combatir el paludismo (De Buen, 1929; Nájera, 1943, 1945).

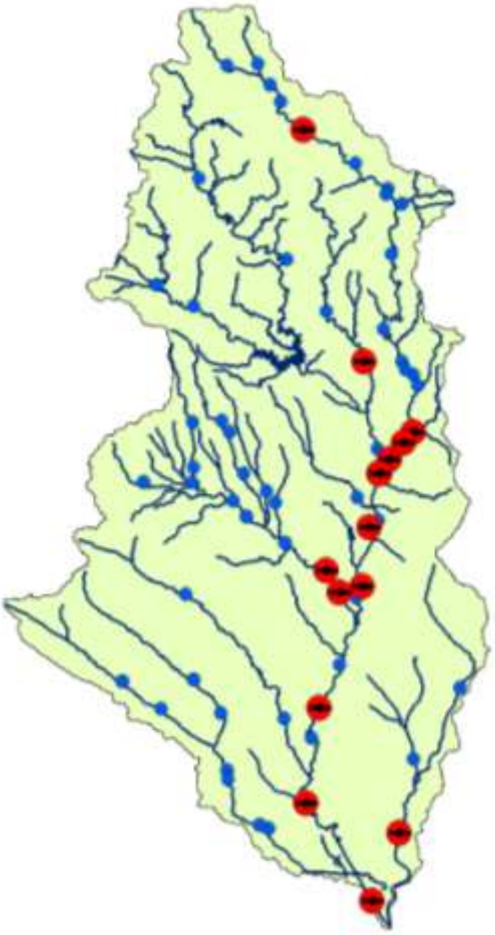

Figura 16. Distribución de la gambusia en la cuenca del río Guadiamar. En rojo, puntos de muestreo donde se capturó la especie, en azul, donde no se encontró.

En la cuenca del Guadiamar, la gambusia ha colonizado los tres tramos del cauce principal, el río frailes, el arroyo Ardachón e incluso es la única especie que persiste en las condiciones del arroyo de Majaberraque (Fig. 16).

Hembra de gambusia y su carga de embriones presionada hacia el exterior..

Su reproducción es muy peculiar. La fecundación, a diferencia de las anteriores especies descritas, es interna, ya que el macho se sirve de la prolongación de su aleta anal o gonopodio para introducir el esperma en la hembra, la cual irá desarrollando en sus ovarios sucesivos lotes de embriones, que irá expulsando cada 20-30 días. Los recién nacidos tardan alrededor de 8 semanas en madurar, lo que suscita la aparición de una tercera e incluso una cuarta generación en el mismo período reproductivo, que abarca de mediados de primavera a mediados de otoño, con un máximo de actividad en verano. Exhaustos por la reproducción, los individuos mueren. Si los nuevos individuos nacen a finales del período reproductivo, esperan al año siguiente para reproducirse, alcanzando un mayor tamaño y siendo por ende más fértiles (Busack y Gall, 1983; Pena y Domínguez, 1985; Fernández-Delgado, 1989; Pyke, 2005).

Prefieren aguas tranquilas, superficiales y con vegetación abundante en las orillas (Casterlin y Reynolds, 1977), aunque son capaces de adaptarse a bajas concentraciones de oxígeno, considerables niveles de contaminación y elevadas temperaturas, e incluso se extienden a zonas estuáricas con alta salinidad donde compiten ferozmente con los ciprinodóntidos autóctonos (Alcaraz y García-Berthou, 2007). Su dieta consta principalmente de cladóceros, quironómidos, larvas de insectos y huevos de otros peces (García-Berthou, 1999).

Su exitosa estrategia reproductiva y la alta tolerancia que demuestra a tan distintas aguas, son la clave del éxito como colonizador de esta especie invasora.

Estado de Conservación: **Exótica, Invasora.**

Familia Ictaluridae

PEZ GATO NEGRO – *Ameiurus melas* (Rafinesque, 1820).

Se diferencia principalmente por tener cuatro pares de barbillones, un cuerpo desprovisto de escamas, dos aletas dorsales, y unos radios espinosos muy robustos en las aletas pectoral y caudal. Según Kottelat y Freyhof (2007), difiere de otras especies de peces gato presentes en La Península Ibérica (como el siluro *Silurus glanis*) principalmente por ser la segunda aleta dorsal adiposa. En España, la longitud máxima conocida alcanza los 300 mm (Doadrio *et al*., 2011). Especie procedente del Centro-Este de Norte América, fue introducida en la Península Ibérica a principios del siglo XX en el Lago Banyoles (Girona), introduciéndose posteriormente por el resto de la cuencas de la península (Elvira, 1984, 1998).

En la cuenca del Guadiamar, la especie tan sólo se ha detectado visualmente mientras se llevaban a cabo los muestreos de este estudio en el tramo alto del cauce principal del Guadiamar en 2011 (Fig. 17).

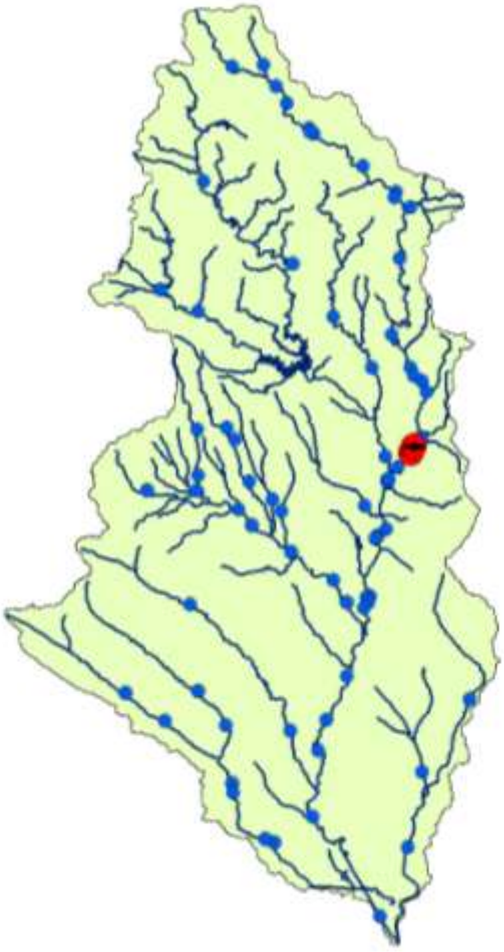

Figura 17. Distribución del pez gato negro en la cuenca del río Guadiamar. En rojo, puntos de muestreo donde se capturó la especie, en azul, donde no se encontró.

El hábitat común de la especie son pozas y aguas remansadas de los tramos bajos de los ríos, por lo que se adapta bien a los embalses (Kottelat y Freyhof, 2007). Es muy resistente a la contaminación, los bajos niveles oxígeno y las altas temperaturas del agua (Doadrio *et al*., 2011). De hábitos principalmente nocturnos, es una especie generalista y oportunista, cuya su dieta va desde insectos u otros peces hasta restos vegetales (Leunda *et al*., 2008). Los individuos comienzan a desovar a los tres años, dejando una sola puesta por año durante los meses de mayo-Julio, cuidando la puesta hasta que se liberan los embriones. Durante los primeros años se les suele ver en grupos nadando por la superficie (Kottelat y Freyhof, 2007)..

Al ser una especie piscívora, causa una amenaza para las especies autóctonas, además sus alta tolerancia a la contaminación del agua y su tamaño no demasiado grande que le permite habitar cursos de pequeño caudal, incrementan su amenaza de invasión (Doadrio *et al*., 2011).

Estado de Conservación: **Exótica, Invasora.**

Agrupando la distribución de las especies anteriores, se obtienen unos mapas que aportan una visión representativa de la situación actual de la comunidad de peces en la cuenca.

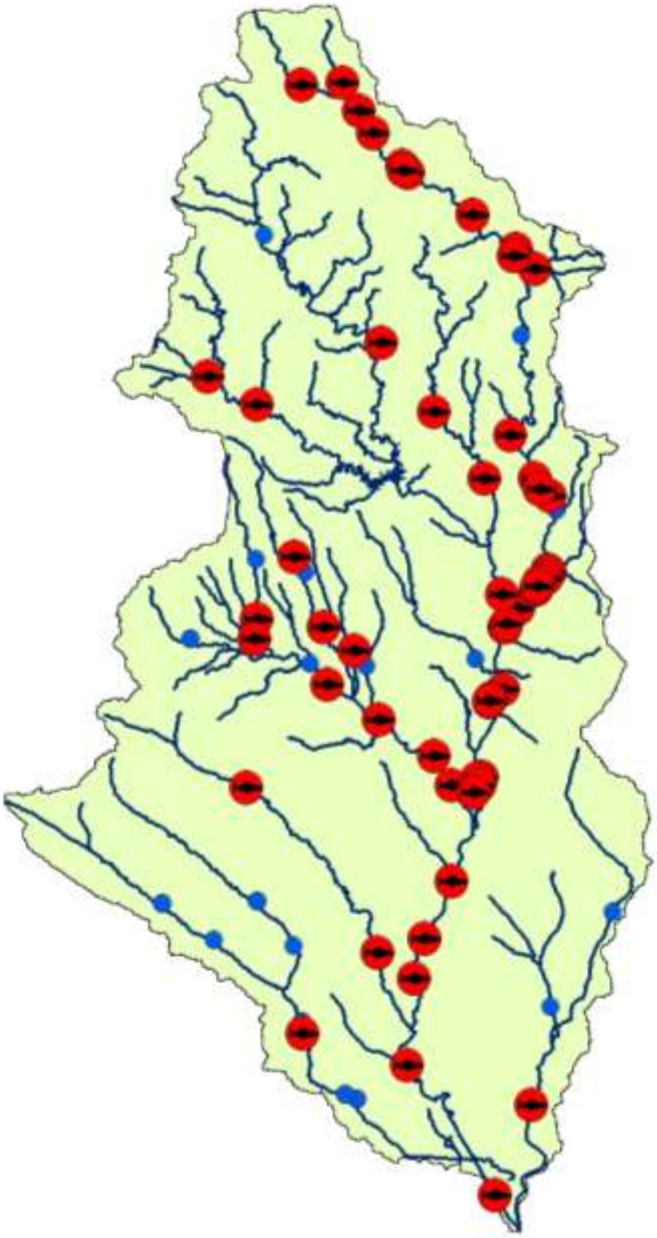

Figura 18. Puntos de muestreo en la cuenca del río Guadiamar donde se hallaron peces, tanto especies autóctonas como alóctonas.

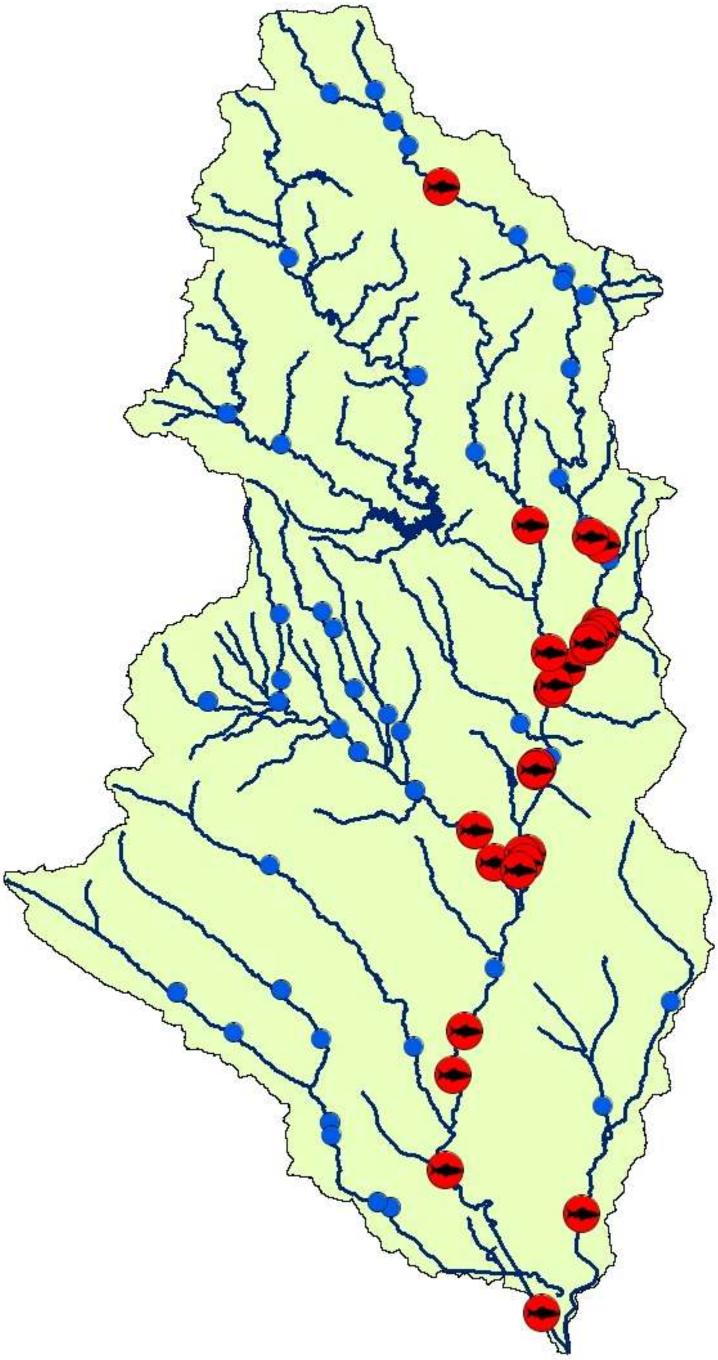

Figura 19 Dispersión de las especies exóticas en la cuenca del río Guadiamar.

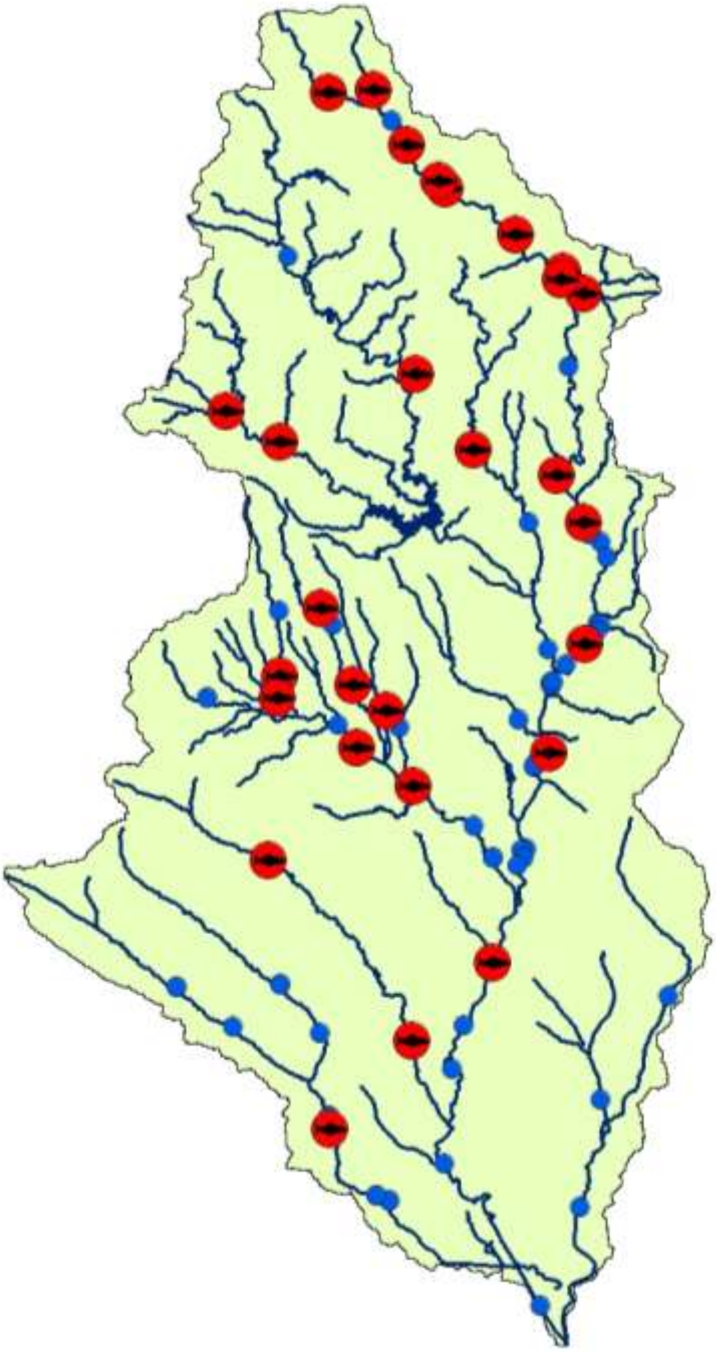

Figura 20. Puntos de muestreo en la cuenca del río Guadiamar donde sólo se encontraron especies autóctonas.

Nota: *Las fotos de peces pertenecientes a éste apéndice han sido tomadas en la cuenca del Guadalquivir y son propiedad del Grupo de investigación Aphanius del Departamento de Zoología de la Universidad de Córdoba.*