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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.

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Running headline: Condition of *Luciobarbus sclateri* in the Guadiamar River.

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.

Key-words: Freshwater fish; mass-length relationships; environmental assessment; habitat differences.

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., 2007). 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 Nieker y 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 & 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 & 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 & 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 & 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 & 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 & 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 & Austin, 1996).

The southern Iberian barbel, *Luciobarbus sclateri* (Günther, 1868), is an endemic fish in the ecosystems of the central-southern Iberian Peninsula (Doadrio, 2002; Kottelat & 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 & Drake, 2008), and its reproductive migration usually occurs within the same catchment (Herrera & Fernández-Delgado, 1992; Rodríguez-Ruiz & 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.

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).

Monitoring

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 & Fernández-Delgado, 1994; Encina & 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 & 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 & Moreno-Amich, 2001).

Each sampling site was characterized by the following fifteen environmental variables: conductivity ($\mu S\ cm^{-1}$), 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 & 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 & 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).

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 & 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 2001, 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 & 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 & Omland 2004). 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 & 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).

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 Figure 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). 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).

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 & 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 & 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 & 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 & 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 & 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 & 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.

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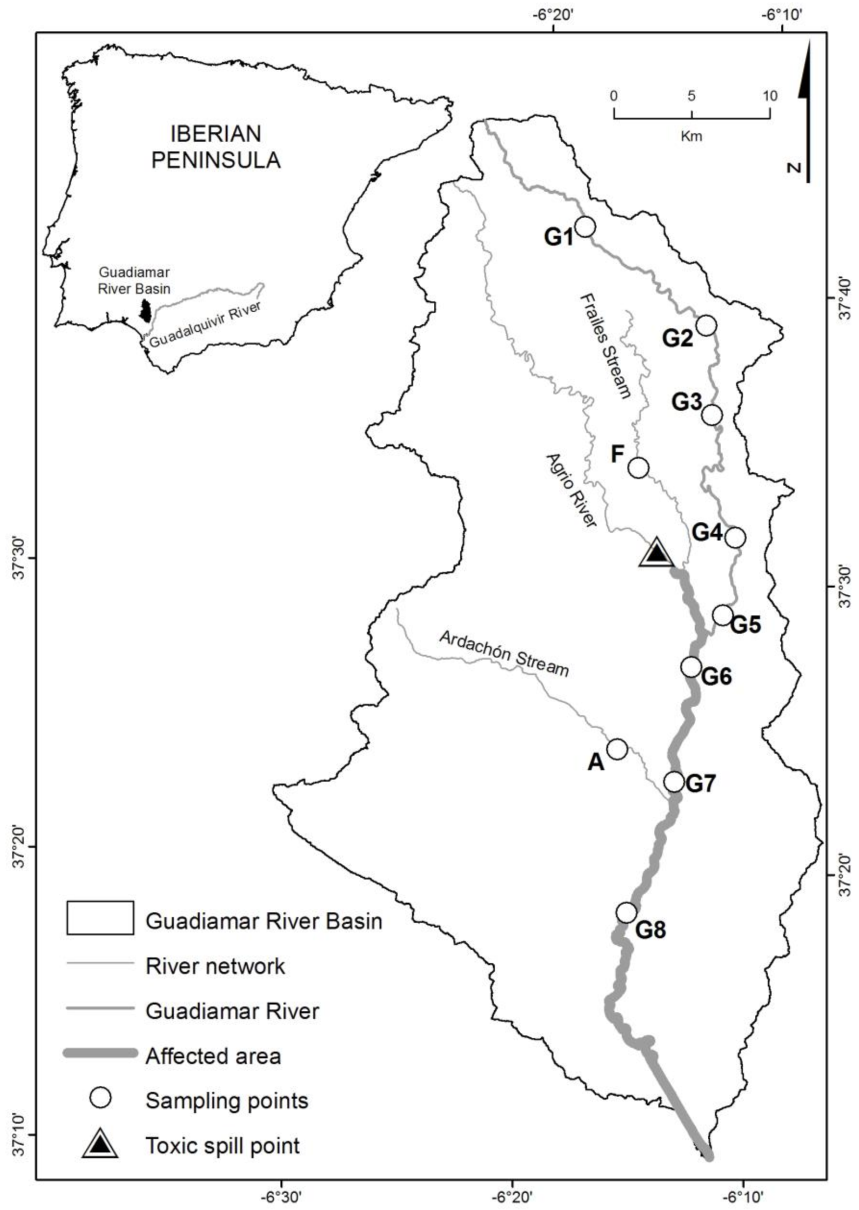

Fig.1. Sampling at sites at the Guadiamar River basin in the southern Iberian Peninsula. G1-G5: sampling sites located in the non-affected area of the Guadiamar River and G6-G8: sampling sites located in the affected area of the Guadiamar River; A and F: sampling sites located in the non-affected area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

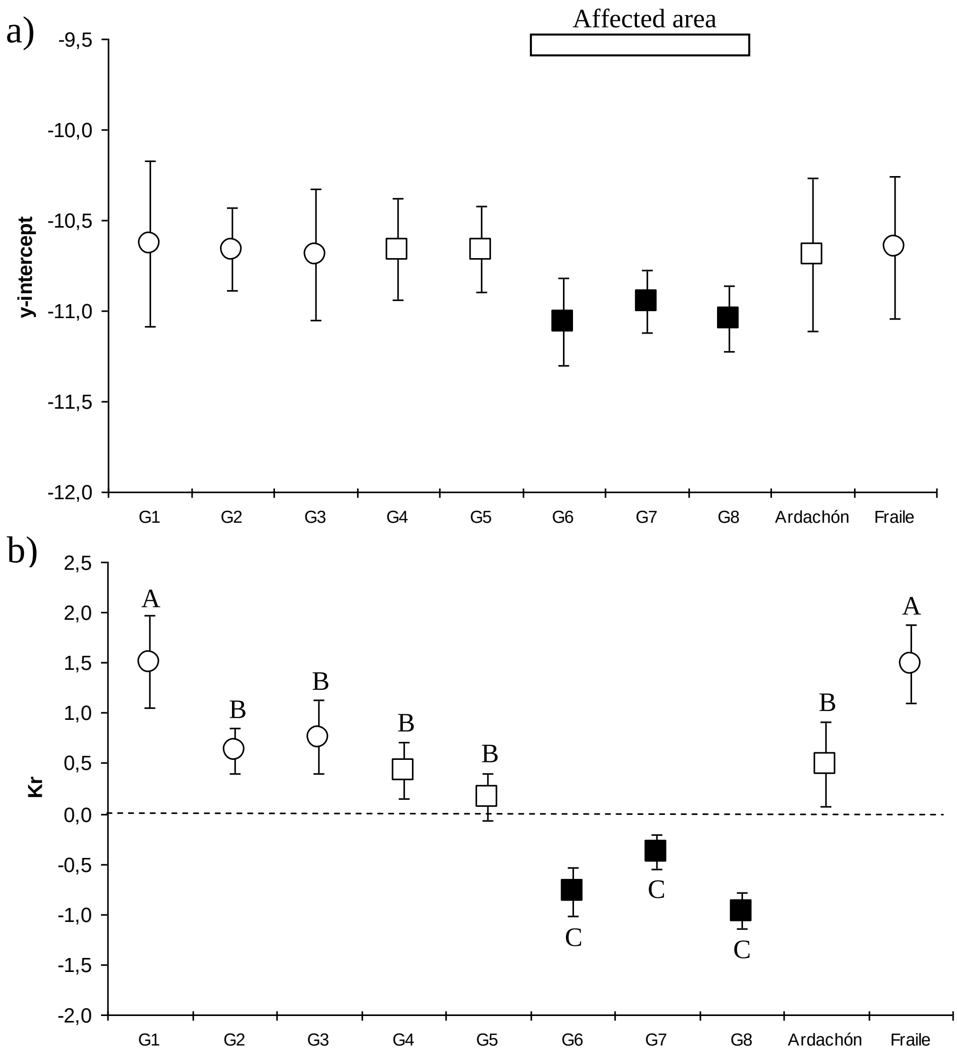

Fig.2. Mean fish condition estimated from the y-intercept of the mass-length relationships (a) and using residual values (Kr) (b) in each study site. Circles represent sites immersed in a forestry land use matrix, while squares are under agricultural land use (black squares are in the affected area). 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 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

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.

Variables	H'	S	Conductivity	Oxygen	T^a	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 (T^a)	-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

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

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|)
	pH (53%)			0.17760	0.02529	7.022	0.000416 ***
Model *a*	Seasonal Water Flow (35%)	0.96	0.00003	-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 **