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

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ABSTRACT

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

INTRODUCTION

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

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

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

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

METHODS

Study area

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

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

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

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

Fish and Habitat Data

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

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

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

Analysis overview

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

Predictive models

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

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

To validate the model, the expected species richness (sum of expected probabilities of each taxon) was compared to the observed richness in the validation reference sites (20 sites). If the model was valid, we would expect a 1:1 relation between the observed and expected taxa. Hence, a regression slope not different from 1 (t-test) and an intercept not different from 0 would be expected. An alternative measure was used to evaluate the precision of the model at species level, by establishing the best possible and the worst possible models (Van Sickle et al., 2005). The standard deviation of our model was compared to that derived from a model which took into account all possible non-sampled related variation which displayed the lowest possible SD (SD_R) and a null model which was assumed to be the worst possible model, with the highest standard deviation hence (SD_{null}). Our model would be classified as good if its standard deviation in the validation data ($SD_{O/E}$) improves SD_{null} and end close to SD_R. Additionally, the area under the curve (AUC) of the Reciever Operating Characteristic (ROC) for the same data set was assessed as a measure of prediction success (Fielding and Bell, 1997).

Assessment of Condition and Conservation Value

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

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

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

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

RESULTS

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

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

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

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

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

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

DISCUSSION

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

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

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

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

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

assessment, the concordance reinforces the result we present here, since the same area has been found to have the highest conservation values with two alternative methods.
River regulation may be behind the decrease of the OE50 and CV in many of the sub-basins we studied. The more time the sub-basin has been regulated, the higher biodiversity loss and the lower conservation value they displayed (Fig. 4.). Alqueva and Pedrogao were the last big reservoirs built in the Guadiana River basin, affecting the last big sub-basin which had not been regulated yet (Ardila River) and where the biodiversity priority areas are concentred. They have recently created more unsuitable habitat for most of native fish species by affecting their inter sub-basins movements and enhancing the population of exotic species as suggested Filipe et al. (2004) for this area and Clavero et al. (2004) found for the Iberian Peninsula. Thus, the establishment of discrete reserves, as would be the case in the Guadiana River due to the presence of multiple reservoirs in the basin, could not be enough to protect freshwater fishes (Meffe, 2002; Angermeier, 2000; Lindermayer et al., 2000) and must be deeply studied.
The actual reserve system seems to be the result of partial contributions of regional authorities instead of a global planned project. No significant relationship between the CV index and the *Natura 2000* network may suppose that some priority areas for conservation planning could be out of the final reserve system. This uncovers the need to review the current *Natura 2000* network applying complementarity criteria to check its competence to sustain all the Guadiana´s freshwater fish biodiversity in a whole basin context. However, the identification of biodiversity priority areas should imply neither the lack of active management regimes within them nor in off-priority areas (Cowling et al., 2003; Lindermayer et al., 2000). We highly recommended a mixed protection scheme where the conservation efforts are opened out to off-reserve

management (Linke et al., 2007; Margules & Pressey, 2000) especially in the control of exotic fish species populations that may affect the contiguous reserved areas.

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

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

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

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

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

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

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

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

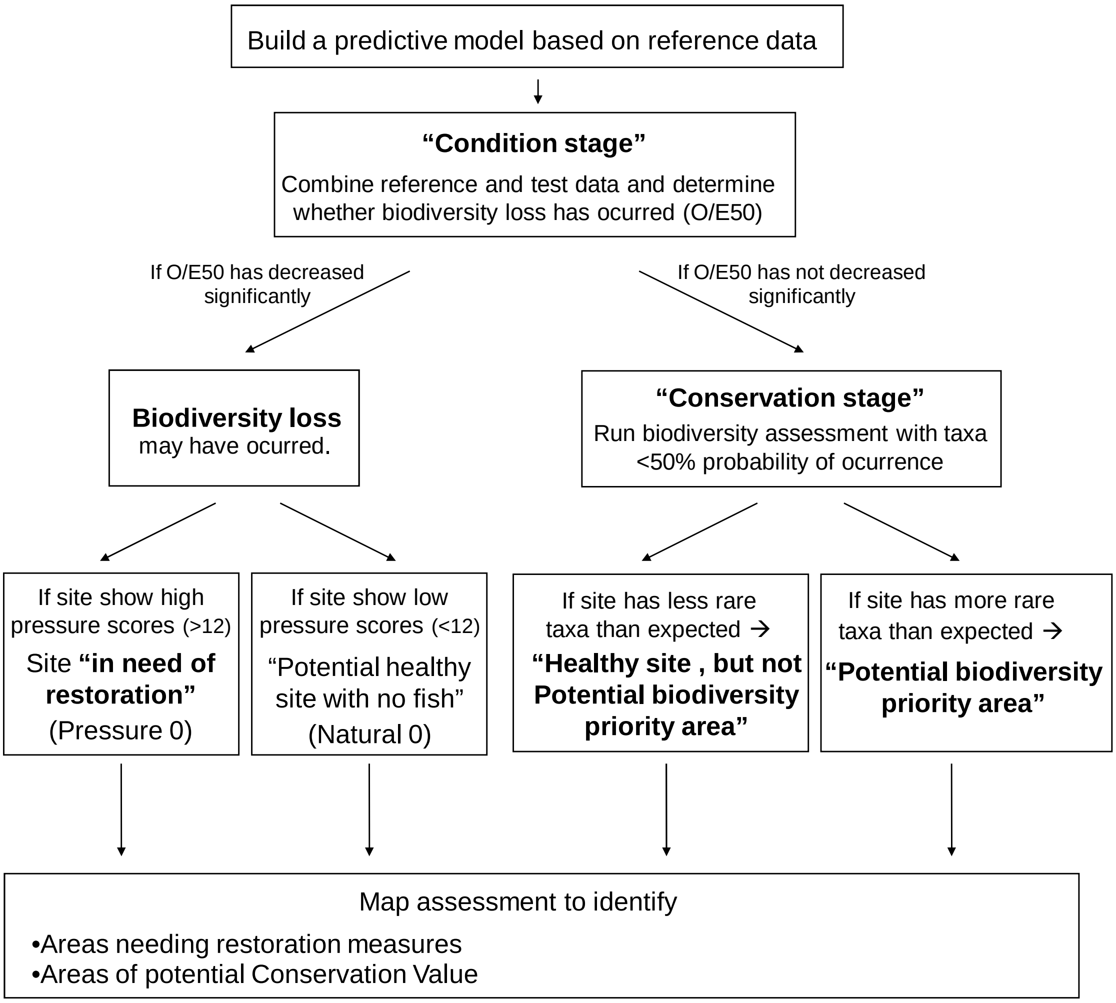

Fig. 2. A) Map of the index of Biodiversity loss for sampled sites (n=241). White dots denotes not significant biodiversity loss and hence sites that were considered in the second step. White triangles represent sites with significant loss of biodiversity, though any common native fish species was found.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

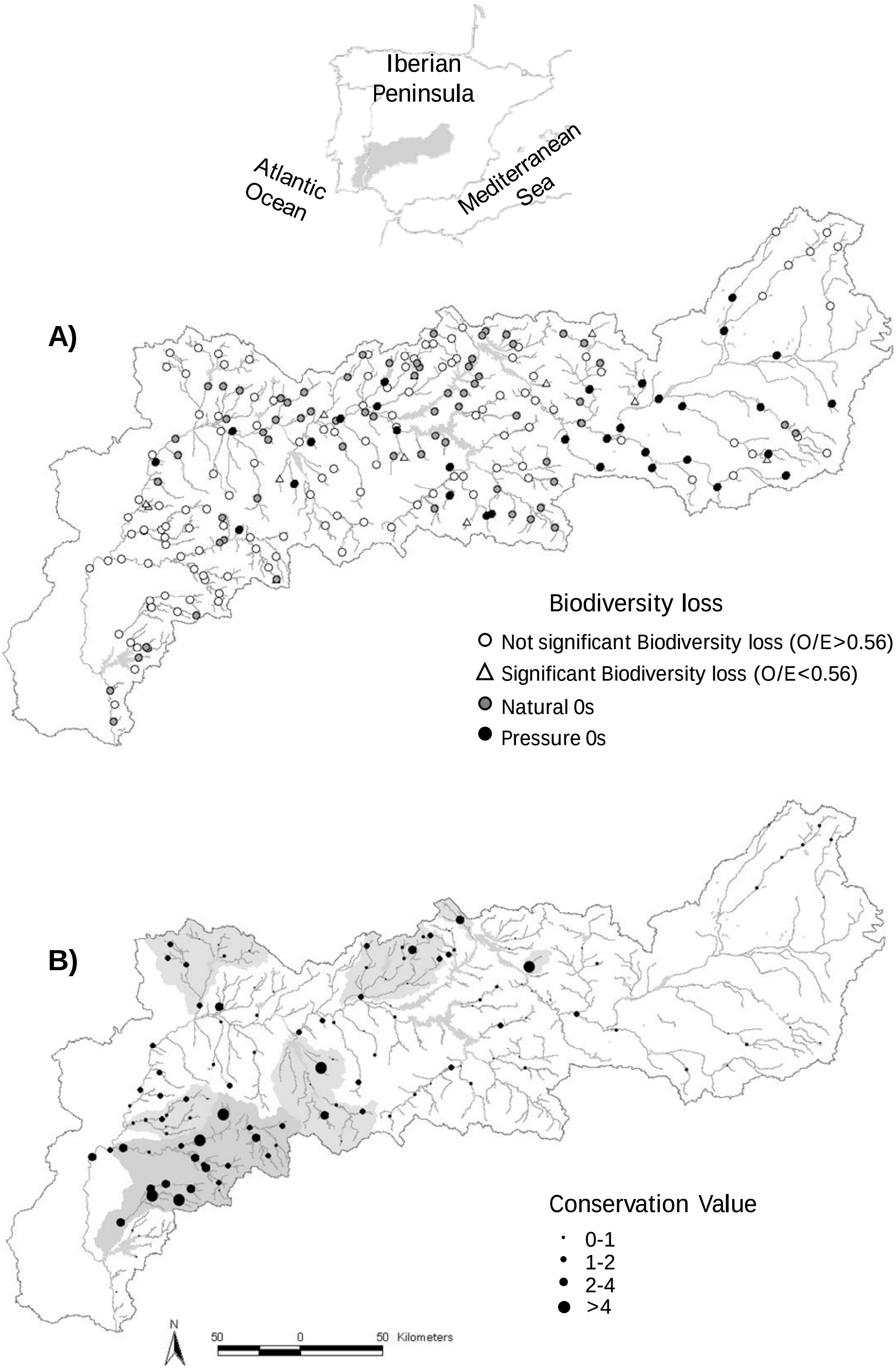

Fig. 3. Principal Component Analysis carried out on the environmental matrix of the 94 sites with 0 values for the OE50 index. Pearson correlation between PC1 and the Pressure index (Mean ± SE values for this index through the PC1 are also shown to visualise the difference between natural 0s -white dots-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

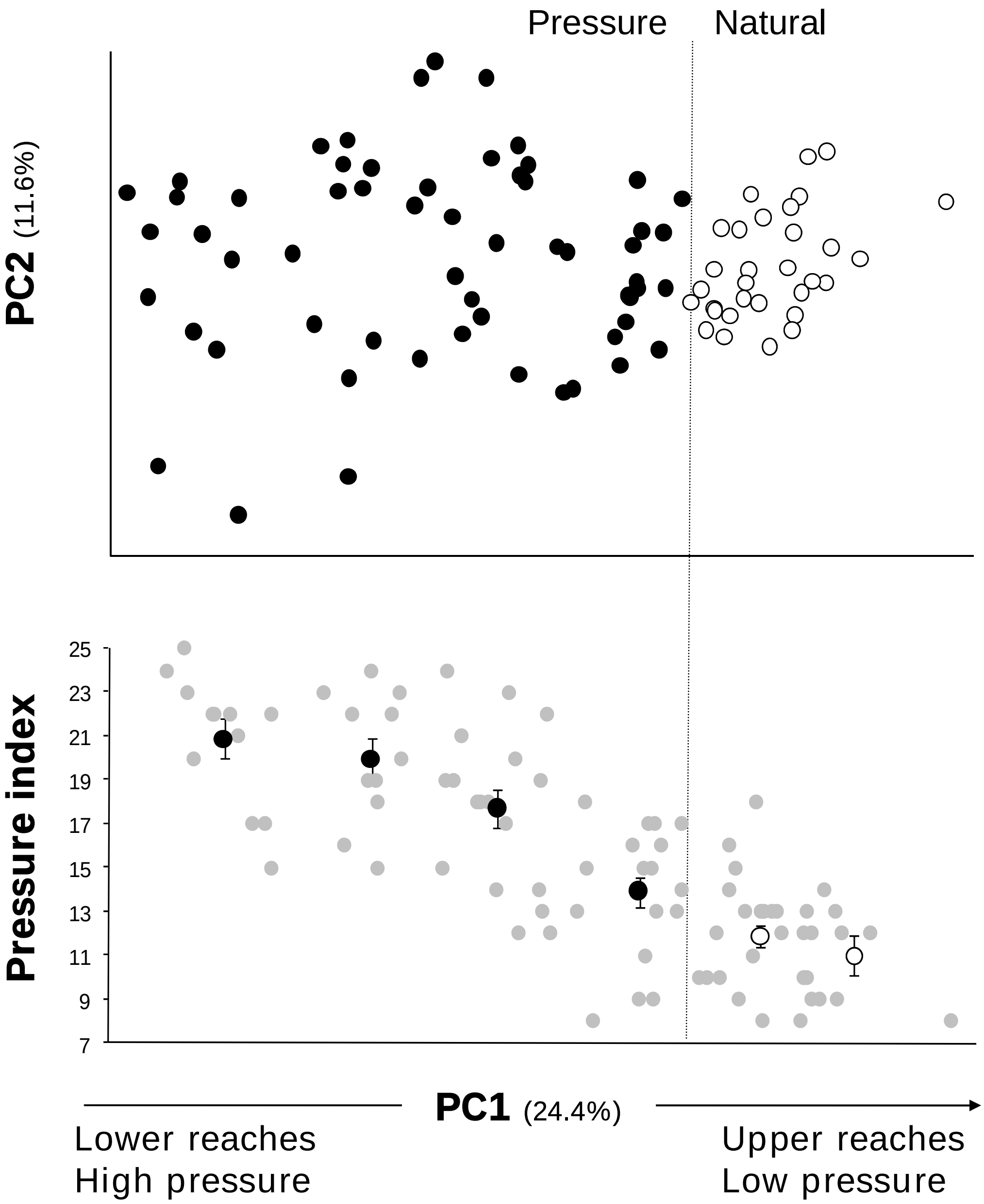

Fig. 4. Effect of basin regulation on the indices of Biodiversity loss and Conservation value through time. It is shown the scores (Mean ± ES) of the indices grouped in sub-basins regulated in the same decade. Only those sites located in a sub-basin containing a big reservoir (more than 100 Hm^3) were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