Journal of Applied Ecology

British Ecological Society

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|---|---|
| Journal: | *Journal of Applied Ecology* |
| Manuscript ID: | draft |
| Manuscript Type: | Standard Paper |
| Date Submitted by the Author: | n/a |
| Complete List of Authors: | Hermoso, Virgilio; University of Huelva, Biología Ambiental y SP<br>Clavero, Miguel; Centre Tecnològic Forestal de Catalunya, Grup d□Ecologia del Paisatge, Àrea de Biodiversitat; Universitat de Girona, Departament de Ciències Ambientals<br>Blanco-Garrido, Francisco; Mediodes, Consultoría Ambiental y Paisajismo S.L.<br>Prenda, Jose; Universidad de Huelva, Biologia amniental y SP |
| Key-words: | ANCOVA, driver, functional vs numerically mediated process, passenger, per capita effect, SEM, freshwater fish |
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# Invasive species and habitat degradation in Iberian streams: an explicit analysis of their role and interactive effects on freshwater fish biodiversity loss

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Running headline: Drivers of freshwater fish biodiversity loss.

Word count: 8430

ABSTRACT

1. The diversity of life on Earth is under the so called biodiversity crisis, which is specially pressing in freshwater ecosystems. Habitat loss and degradation and invasive species are commonly cited as the main causes. Distinguishing the role of each extinction driver and their potential interactions through a mechanistic understanding of impact is crucial for achieving conservation goals.

2. We analyze whether freshwater fish invasive species are mere passengers co-occurring in the biodiversity loss process driven by habitat degradation or as main drivers of the decline of native fish communities in an Iberian basin. Moreover, since few invaded ecosystems are free from habitat loss and degradation, we also tested whether native species simply responded to the abundance of invasive species or if habitat degradation modified the functional relationships between natives and invasive species.

3. We found invasive species to be leading the decline of freshwater fish native communities, while habitat degradation neither played an active role nor influenced invasive species *per capita* effect on natives. Lower reaches and areas close to reservoirs held the most seriously injured fish communities independently of their habitat degradation status. Then Mediterranean freshwater fish show some resilience to habitat perturbations while invasive species should be raised to the center of attention of conservation actions. Moreover, the essential ecological role that hydrologically stable reaches might play for native communities' persistence in highly fluctuating environments, such as the Mediterranean, is endangered by the proliferation of invasive species in those environments.

4. Synthesis and applications: Conservation efforts to reduce biodiversity loss among Mediterranean areas freshwater fish communities should focus on mitigating the effect of invasive species especially in better conserved areas. However, the high cost and low efficiency of management actions against invasive species may difficult the effective fight against invasive threats, while new tools such as harder legislation could help reduce the current introduction rates. The roles of different drivers leading the decay of native communities should not be directly extrapolated across taxonomic groups and/or environments, but be analyzed in different particular situations in order to tackle objective management plans facing the current biodiversity problem.

KEYWORDS: ANCOVA, driver, freshwater fish, functional vs numerically mediated process, passenger, per capita effect, SEM.

## INTRODUCTION

The diversity of life on Earth is rapidly dismissing under the so called biodiversity crisis (Olson et al., 2002). Extinction rates are 100-1000 times higher than pre-human levels in many different taxonomic groups from a wide range of environments (Pimm et al., 1995). There is a general agreement on the urgent need for management actions focused on conservation to face this problem (Olson et al., 2002). But efficient management programs must rely on the understanding of the mechanisms driving the processes of biodiversity lost. The study of the relationships between these extinction drivers and biodiversity loss transcend thus mere theoretical discussions, as it has clear implications for achieving conservation goals, ideally leading to an optimized use of the limited resources intended for conservation issues (Knight et al., 2007).

Many are the factors that have been cited as extinction drivers acting upon different organisms or in different regions, but habitat loss and degradation and invasive species are commonly cited as the main causes of biodiversity loss (Riccardi, 2004; Clavero & García-Berthou, 2005; Didham et al, 2007). However, due to the frequent spatial co-occurrence of habitat degradation, increases in the abundance of invasive species and native species' declines (Fig 1A), the ultimate mechanisms driving biodiversity loss often remain unclear (Guveritch & Padilla, 2004; Didham et al, 2007). The different visions on this issue go from the perception that invasive species are mere passengers (i.e. a co-occurring, though basically independent, phenomenon) of the biodiversity loss process driven by habitat degradation (Fig. 1C) to the designation of invasive species as main drivers of native species' decline (Fig. 1B) (Didham et al., 2005). Effective conservation action however demands a well-defined identification the relative roles of habitat degradation and invasive species in the processes of biodiversity loss. For example, eradication plans would be completely inefficient, and conservation budget wasted, if invasive species simply co-occurr with natives' declines driven by habitat degradation (Myers et al., 2000; Zavaleta et al., 2001).

Some efforts have been recently devoted to analyze explicitly the roles of habitat degradation and invasive species on native species decline and extinction (e.g., Marchetti et al., 2004; MacDougall & Turkington, 2005; Light & Marchetti, 2006). These works have allowed the introduction of hypothesis-testing reasoning in the debate on the role of invasive species on biodiversity loss, even though they have provided contradictory conclusions. MacDougall and Turkington (2005) argued that invasive species were mainly passengers in the decline of native species in an oak sabana herbaceous community in

Canada, suggesting that native species' recruitment limitations in degraded systems would be a consistent explanation for invasive species dominance. On the other hand, Light & Marchetti (2006) identified invasive freshwater fish species as the primary direct driver in the decline of native fish communities in Californian river basins.

Research on the two major recognized drivers of species decline is often approached as though they are independent single-factor problems (Fazey et al., 2005), although they can also act synergistically through different pathways of interaction between invasive species, habitat degradation and native decline (Didham et al, 2007). Habitat degradation may promote increases in the local abundance or regional distribution of invaders, with total invasive impact scaling in direct proportion to invader abundance (i.e. without changes in the *per capita* impact) (Fig. 1A). However, habitat degradation can also change the mode of action or functional response of invasive species, with total impact scaling disproportionately with invader abundance (i.e. with changes in the *per capita* interaction effects) (Fig. 1D). For example, habitat degradation implying natural refuge losses could expose native species to higher predation rates by invasives, resulting in an increased *per capita* effect of invasive species. It is important to discriminate between these two pathways because they stem from different mechanisms of action and have different consequences for conservation management strategies then (Didham et al., 2007).

In the present work we analyze the role of invasive species, different sources of habitat perturbation, natural environmental gradients and their possible interactions in the decay of native freshwater fish communities in a Mediterranean basin. Mediterranean freshwater ecosystems harbor a highly endemic freshwater fish fauna (Reyjol et al., 2007) featuring a large proportion of threatened species (Smith & Darwall, 2006). These systems have suffered a long history of habitat degradation, including modifications of flow regimes, urban and agricultural spills, dam construction and river channelization or destruction of riverine vegetation (Allan & Flecker, 1993; Cowx, 2002; Collares-Pereira & Cowx, 2004) and are at the same time among the most heavily invaded systems in the world (Leprieur et al., 2008). Mediterranean streams and rivers are thus an appropriate scenario to test some of the ideas about the roles of habitat destruction and invasive species in the process of biodiversity loss. With this aim, we first analyzed the likelihood that invasive species were acting as "driver" (Fig. 1B) or "passengers" (Fig. 1C) of native fish biodiversity loss at the reach scale. This supposes a refinement of these kinds of studies, since previous works such as Marchetti et al. (2004) were carried out at coarser scales. In a second approach we studied the nature of the interactions between the impacts of invasive fish species and native communities and habitat degradation. We tested whether native species simply responded to the abundance of invasive species (Fig. 1A-C) or if habitat degradation modified the functional relationships between natives and invasives (Fig. 1D). Furthermore, we also included in the analyses the effects of natural upstream-downstream gradient, which is one of the most important factors structuring stream fish communities (Angermeier & Schlosser, 1989; Matthews, 1998; Magalhães et al., 2002), and the role of reservoirs, which act as a center of fish introductions, facilitating their establishment and being the source of subsequent expansion within basins (Clavero et al., 2004; Havel et al., 2005; Johnson et al., 2008).

## METHODS

### *Study area*

The Guadiana River basin is located in the South-Western Iberian Peninsula draining a total area of 67,039 km$^2$ to the Atlantic Ocean. It features a typical Mediterranean climate, with high intra and inter-annual discharge variation, going from severe and unpredictable floods between autumn and spring to persistent summer droughts (Gasith & Resh, 1999). Mean air temperature ranges from 13 to 18.1 ºC, with a strong intra-annual variation in extreme temperatures. Mean annual precipitation ranges from 350 to 1200 mm (with a mean of 450 mm). Although it is not an overpopulated area (28 hab/km$^2$), the landscape has been deeply transformed during the last century by agricultural activities. Almost a half of the basin (49.1%) is currently under agriculture uses. As a consequence, about 11,000 hm$^3$ of water is retained in 88 large reservoirs (>1 hm$^3$) and more than 200 small ones (<1 hm$^3$) for water supply. Other common human perturbations are related to river channel modifications such as river channelization and degradation and even completely depletion of the riparian forest (Hermoso et al., in press-a).

Guadiana's freshwater fish fauna, with 14 native species found in this study (Table 1), is especially relevant within the circum-Mediterranean context and it was recently identified as an important hotspot (Smith & Darwall, 2006). However, almost two thirds (64.3%) of the total native species in the basin is currently threatened attending to IUCN criteria (Table 1).

### *Fish and habitat data*

Fish community was characterized in 152 localities (Fig. 2) through the whole basin, using electrofishing during spring (April-June) in 2002, 2005 and 2006. Sampling was conducted once at each location without block-nets along 100 m long stretches, covering all habitats available at this scale. This sampling effort has been proved to be sufficient to capture most species present, except for large rivers, as Filipe et al. (2004) suggest on a previous study in the same area. Alternative methodological approaches similar to that used in other European countries for these kinds of environments (Kestemont & Goffaux, 2002) were followed at those sites (<2% of total sites). All fish were identified to species level when possible and then returned to the water.

Habitat was characterized through 25 environmental variables, covering two different spatial scales: site and basin. Two approaches were used in this characterization: *in situ* measures, which described micro and mesohabitat characteristics at each locality, and remote GIS measures used to record variables from digital maps as described in Hermoso et al. (in press-a). All these environmental metrics could be split into two categories: a) variables that described the natural habitat variability in the basin and b) descriptors of human perturbations (Table 2). All variables were checked for normality and transformed when necessary prior to analysis (arcsine for land uses variables -expressed as %- and log (x+1) for the remaining).

*Definition of dependent and independent variables*

We used two different variables as descriptors of the status of native freshwater fish community: total native species richness and a measure of native communities' biotic integrity. Biotic integrity was assessed through an Index of Community Integrity (ICI), which measures the general deviation of the observed community composition from an expected community in absence of any source of perturbation (human or biotic) following the reference condition approach (Hughes et al., 1986; Reynoldson et al., 1997; Bailey et al., 1998). The reference community composition (probability of occurrence of each species) was obtained through an Assessment by Nearest Neighbour Analysis predictive approach (ANNA; Linke et al., 2005). In ANNA, sites are treated as a continuum avoiding artificial classifications, and predictions are derived from the most environmentally-similar reference sites. The ANNA model finds the set of most environmentally-similar reference sites for each target site, and predicts its community composition based on the community composition of those nearest neighbours (Linke et al., 2005). Given the difficulties to model rare specie's occurrence, only species with prevalence higher than 5% could be included. The model was built and validated in two independent sets of reference localities and only environmental variables not affected by human perturbations were used as predictors (Table 2).

Performance tests showed this model to be valid and accurate enough to be used in the index minimizing the probability of committing type I and II statistical errors (Hermoso et al., in press-b). The deviation of the observed presences-absences against the expected probabilities in absence of perturbations (O-E, henceforth residuals) was measured for each species in each site, thus obtaining ten different residuals for a given site. Negative values indicate species loss (the species was predicted to be present with a certain probability but it was absent). The lower the residuals, the higher the probability of presence unconfirmed hence. In the opposite extreme, positive residuals owe to observed presences with low predicted probabilities. These residuals were standardized to a (0,1) normal distribution (x-mean/SD in the reference data set) and then transformed into probabilities, which could be interpreted as probabilities of a certain site to be a reference site. Each partial species measure was then summed up in the final index score.

To reduce the dimensionality of our independent data set in order to simplify the analyses, we performed two principal component analyses (PCAs) in two different sub sets of environmental variables. A first PCA was carried out on variables related to human perturbations (Table 2) to obtain a reduced number of gradients (principal components or PCs) describing habitat degradation. The first two PCs accounted for 56.9% of the total variance in the perturbation variables included in the analysis. The first PC (denoted henceforth as $PC1_{deg}$) was mainly related to the general perturbation status related to land-uses, alteration of the riparian forest and degradation of water quality (Table 3). The second PC (henceforth $PC2_{deg}$) discriminated sites affected by agriculture from those with urban derived impacts (Table 3). A second PCA was similarly performed on variables not related to human perturbations, to extract the main patterns of natural variation in our study area. The first PC (henceforth $PC_{nat}$) retained more than a half of the original variance and was mainly related to the longitudinal natural upstream-downstream gradient (Table 3). These PCs would be later used as surrogates of human perturbations and natural variations respectively in the analyses. The abundance of exotic species [(*Log +1*) transformed] was used to account for the effect of exotics and the distance to the nearest reservoir (whether upstream or downstream) was also included as independent, given their special significance for the establishment and dispersion of exotics in freshwater ecosystems.

*Invasive species as drivers or passenger in natives declines*

The driver or passenger role of invasive species in the decay of native freshwater fish communities was explored through two different approaches. Firstly, we built all possible multiple regression models between our dependent variables (biotic integrity and native species richness) and the set of independents. These models included a full model with all the independents as predictors, single models for each independent and all possible combinations of multivariable models. Then we ranked all of them according to their Akaike's information criterion (AIC), and calculated the increase in AIC with respect to the top-ranked model (ΔAIC). AIC estimates the distance between a certain model and the (unknown) theoretical underlying mechanism generating the data, lower AICc values indicating a better fit (Burnham & Anderson 2002). We used the occurrence of each independent within the set of models with reasonable support ($\Delta AIC<7$, according to Bumham & Anderson, 2002) as an estimate of their importance explaining native decline. A high occurrence of exotics in the best models would be expected if exotics had an active role in native declines instead of being mere passengers.

Additionally, Structural Equation Modeling (SEM; Bollen, 1989) was used to allow considering some of our variables as independent and dependent at the same time (Gerbin & Anderson, 1988). This is a great advance with respect to the previous approach which can only analyze a single layer of linkages between dependent and independent variables at a time. The SEM approach allows testing multiple relationships between the set of variables under consideration, allocating more accurately our target variables (invasive species) within in the complex matrix of relationships where they are interacting. The aim of this analysis was not to check the strength of relationship between our variables, but testing the role of exotics as drivers or passengers in the decline of native fish communities, through the comparison of three alternative models (full, driver and passenger). The full model (Fig. 3A), which included all the reasonable relationships between our variables, was used as the baseline for the comparison with the other alternative hypotheses. The driver model (Fig. 3B) only considered direct effects of $PC_{nat}$ and invasive species' abundance on biotic integrity/native species richness, while habitat degradation ($PC1_{deg}$ and $PC2_{deg}$) and reservoirs had only indirect effects via exotics. The passenger model (Fig. 3C) assumed direct effects of habitat degradation and natural changes on biotic integrity/native species richness and excluded any effect of invasive species. Within SEM, hypotheses are translated into a series of regression equations that can be solved simultaneously to generate estimated covariance/correlation matrices. Then each estimated matrix can be evaluated against the observed sample covariance/correlation matrix by means of a goodness-of-fit index to determine whether the hypothesized model is an acceptable representation of the data. We used the likelihood ratio test which measures the probability that the observed and expected (under the models constrictions) covariance/correlation matrices differ by more than would be expected because of random sampling errors (Mitchell 1993; Shipley 2000). If the data is consistent with the model specified, no significant differences between the observed and expected covariance/correlation matrices are expected.

### *Invasive-native species relationships along environmental gradients*

To test whether functional relationships between native communities and invasive species changed along environmental gradients we carried out analyses of covariance (ANCOVA). We used the abundance of invasive species as covariate, testing their influence on biotic integrity/native species richness (dependent variables) along each perturbation ($PC1_{deg}$ and $PC2_{deg}$) and natural gradient ($PC_{nat}$) (factors). To allow the use of continuous variables as factors, PC gradients and distances to reservoirs were categorized into 4 equal-sized levels. The possible changes in the functional relationship between invasive species and native communities were tested through homogeneity of slopes tests. Significant results of the covariate × factor interaction terms would imply changes in the *per capita* impacts (i.e. slopes) of invasive fish, while non-significant results (i.e. constant slopes along environmental gradients) would denote simple numerically mediated responses of native communities to invasive species. Whenever the interaction term from the homogeneity of slopes analyses was not statistically significant ($P > 0.10$), it was deleted from the models, and standard ANCOVA analyses were run.

## RESULTS

### *Invasive species as drivers or passenger of natives declines*

The top-ranked multiple regression model for the biotic integrity included invasive species abundance as the only predictor. The remaining models with a reasonable support ($\Delta AIC<7$) were highly redundant and just variants of the top-ranked model including different combinations of the remaining predictor variables. Moreover, they represented all the possible combinations between invasives abundance and the other independent variables, though only invasives abundance had significant effects in all of them (Table 4). In the case of native species richness, the top-ranked model included exotic abundance as well as the natural and both perturbation gradients (full model). The abundance of Invasive species appeared with significant effects in all of the 10 models with a moderate support, while the natural upstream-downstream gradient appeared in 8 of them (Table 4).

The goodness of fit test showed the full model for both biotic integrity and native species richness to be consistent with the data, since no significant differences were found between the observed and the expected correlation matrices (Fig. 3). The driver model, which assumes only direct effects of exotics abundance on biotic integrity or native species richness (the effect of the remaining variables would be canalized indirectly by their relationship with exotics) showed to be also consistent. The driver model was the one that better fitted our data when analyzing biotic integrity, while the full model was the best model for species richness (Fig. 3). However, the passenger model, in which the effect of exotic abundance on native communities had not been included, was inconsistent (Fig. 3). These analyses also revealed a strong effect of exotic abundance on both biotic integrity and native species richness and a clear influence of the natural upstream-downstream gradient on both invasives abundance and native species richness. This latter effect was not detected for biotic integrity since it was previously accounted for in the predictive models used in the assessment of the ICI. The distance to the nearest reservoir also showed significant effects on invasive species abundance (and thus indirectly on natives), as well as the natural gradient on both perturbation gradients ($PC1_{deg}$ and $PC2_{deg}$). Moreover, $PC1_{deg}$ only had significant effects on native species richness while $PC2_{deg}$ showed no significant effects neither on native communities nor invasive abundance.

*Invasive-native species relationships along environmental gradients*

The results of the different ANCOVAs showed that the slope of the relationship between the abundance of exotics and both biotic integrity of native communities and native species richness was strikingly constant along the natural and perturbation gradients and the distance to the nearest reservoir (the interaction terms in the ANCOVA analyses were always >0.16; Fig. 4 and Table 5). When the interaction term was removed from the ANCOVA design, invasive abundance had a strong negative effect on both biotic integrity and species richness, again denoting the clear impact of invasive species on both variables (Fig. 4). $PC_{nat}$ and $PC2_{deg}$ had also significant effects on native species richness (Table 5), which tended to increase downstream localities and was higher in agricultural areas than in urbanized zones.

DISCUSSION

Few invaded ecosystems are free from habitat loss and degradation, introducing uncertainty when trying to discern the responsibility of these threats in the decay of native communities (Gurevitch & Padilla, 2004; MacDougal and Turkington, 2005; Didham et al., 2005). However, correctly establishing causality through a mechanistic understanding of impact is crucial for achieving conservation goals (Didham et al., 2007). With this respect, the identification of the driver or passenger role of invasive species on natives decline could not to be enough to face the problem of the current biodiversity crisis. Previous approaches have treated them as independent factors (Light & Marchetti, 2006; Godinho et al., 1997) while interactive effects between multiple causal agents are expected in complex systems rather than simple independent ones (Didham et al., 2007). So the understanding of how interactions between drivers might be mitigating or enhancing their net effects, is a crucial task to better understand the pathways leading to the observed situation and to correctly deal with biodiversity loss (Hulme, 2006).

The comparison of different SEM models based on quantitative data has been proposed as an optimal approach to exploring the role of invasive species on native communities (Didham et al., 2005) and as such has been used in recent studies (MacDougall & Turkington, 2005; Light & Marchetti, 2006; Harrison et al., 2006). Our results indicate that the role of invasive species in relation to biodiversity loss was closer to the driver than to the passenger hypothesis. The abundance of invasive species was a key variable explaining both native species richness and biotic integrity, and the driver model was the most parsimonious one explaining the biotic integrity of fish communities. This model assumed only direct effects of invasive species' abundance on natives, with habitat degradation or river damming having only indirect effects through their relationship with invasive species. The full model was which better fitted the data in the native species richness approach, although the driver was also consistent. Moreover, the passenger model, which did not consider the effects of invasive species on native communities and only included the effect of habitat degradation or natural gradients, did not fit our data. Our results highlight the primary direct role of invasive species on native fish decline in Iberian streams, discarding habitat degradation as a leading direct cause of fish biodiversity loss. However, although our results are clear and consistent, we cannot exclude the possibility that the fish biodiversity patterns observed could be related to sources of habitat degradation, such as increased effects of summer droughts due to water abstraction, which were not considered in our models (Shipley, 2000). The driver role of invasive species had been previously reported in California, which also features a Mediterranean climate regime, with invasive species being identified as the main factor leading to freshwater fish imperilment at the watershed scale (Light & Marchetti, 2006). Some other studies in the same area, although using different approaches, support the idea that modified habitats continue holding native species in the absence of invasions (Baltz & Moyle, 1993; Moyle 2002). Thus Mediterranean freshwater fish communities are apparently resilient to habitat perturbations, while invasive species would be the leading cause of their decline.

Further analysis gave light on potential interactive effects between habitat degradation and invasive species on native communities. As Didham et al. (2007) pointed out, the discrimination between different causal pathways of interaction between multiple drivers is essential for mitigating native species decline. However, we found that none of the habitat gradients used as factors showed to influence the relationship between invasive species abundance and native species richness or biotic integrity. Thus, the mechanism of action of invasive species on native communities in our study area can be interpreted as a numerically mediated process. However, this numerically pathway is not directly related to habitat degradation, as referred in Didham et al. (2007), and must be due to other drivers controlling invasive population dynamics leading to invasion success and/or abundance, such as time since introduction or invasion stage (With, 2002). As it has been reported previously, the association between establishment and spread of exotic species and habitat disturbance should not be assumed a priori, due to their lack of direct cause-effect relationship (Lozon & MacIsaac, 1997). In this sense, we found that the natural longitudinal gradient (upstream-downstream) and the distance to the nearest reservoir were the only environmental features with significant effects on the abundance of invasive species (see Fig. 3).

Our results strongly suggest that habitat stability, which is higher close to reservoirs and at lower reaches (Godinho et al., 1997; Magalhães et al.; 2002; Clavero et al., 2004), is the main environmental factor regulating the colonization success of invasive species. In fact, habitat stability is a critical factor structuring fish communities in highly fluctuating environments such as Mediterranean water courses, which experience extreme both intra and inter annual variations in water availability (Gasith & Resh, 1999). Permanent waters are essential refugia over the summer dry season, when small streams or headwaters become easily desiccated (Magalhães et al., 2002). Mediterranean freshwater fish, having evolved in such highly instable systems, tend to be habitat generalists very well adapted to survive in constantly changing environments (Clavero et al., 2004). There is however a clear natural pattern of native species richness and abundance change through the upstream-downstream gradient rising downstream in association with the increase in living space and environmental stability (Magalhães et al., 2002). But in our study area this pattern was blurred by the effect of invasive species and was only patent when their effects were accounted for in the analyses. Most of the invasive fish species introduced to Iberian freshwaters originally occupy much more stable habitats, often lentic systems (Elvira & Almodóvar, 2001; Ribeiro et al., 2008), and few of them are able to cope with the extreme flow fluctuations and harsh summer droughts that occur in small Mediterranean streams (Vila-Gispert et al., 2005). The milder environmental fluctuations that occur in the lower river reaches would favor the successful establishment of invasive species populations. Then, habitat stability seems to play an essential role for both native and invasive species populations, while the proliferation of the later in these environments may endanger the ecological service they were giving and the natural resilience of native communities.

Reservoirs do not seem to play a significant direct role in the decline of native fish communities, although they have indirect effects through their relationship with invasive species. Over the past century, human activity has promoted invasions both by creating new transport vectors and by changing natural habitats. Creation of impoundments is a clear example of this trend, promoting invasions by increasing colonization opportunities for non-indigenous taxa and by enhancing their subsequent establishment success (Shea & Chesson, 2002; Clavero et al., 2004; Havel et al., 2005; Johnson et al., 2008). Reservoirs entail a drastic reduction of habitat heterogeneity, converting extensive reaches of stream habitat into standing water at local scale, but also altering the downstream magnitude and timing of water flows, sediment load and creating barriers for fish migration (Malmqvist & Rundle 2002), which affect the whole basin. The favorable and more stable environment conditions in reservoirs facilitate the establishment of invasive species (Moyle & Light, 1996; Ribeiro et al., 2008), which are a common target of stocking practices, especially for angling purposes. Propagule pressure is a major factor for predicting the success of invaders in colonizing new ecosystems (Kolar & Lodge 2001) and reservoirs play an important role as center of introduction of invasive species (Clavero et al., 2004; Havel et al., 2005; Johnson et al, 2008). Increasing the amount of suitable habitat for invasive species raises total population size in the landscape which drives an increase in local density due to higher propagule pressure (Barlow & Kean, 2004). They also provide stepping-stones into new landscapes (Havel et al., 2005) favoring their dispersion through larger areas in the basin, which is facilitated by the habitat modifications commented above.

The driver and passenger models have different implications for conservation policies and practices. Our analyses showed invasive species to be the leading cause of native fish decline, while habitat degradation neither affected directly nor influenced the *per capita* effect of invasive species. In such a context, management plans should be focused on the control of invasive species. The most effective manner of addressing the invasion of non-native species to fresh waters is to actively prevent introductions and their negative effects, but little effort has been devoted to reducing the risk of new introductions. While human-mediated species introductions have occurred for centuries, the rate at which new introductions has increased dramatically during the last century (Lozon & MacIsaac, 1997), what makes this goal especially important. This could be mainly faced through proper legislation and active public awareness plans, although their efficiency has not been tested yet. Wherever invasive species have already become established, active management needs to be focused on reducing their harmful effects and preventing further spread (Saunders et al., 2002) especially in high sensitive areas holding healthy native communities. Different approaches can be followed in these areas: eradication or long-term control (Wittenberg & Cook, 2001). The first one is the most cost-effective way to tackle the problem, although it can only be recommended when it is ecologically feasible (high warranties of extirpation with low effects on native communities) and it has enough financial support. However, where eradication is not feasible (the species is highly widespread or the eradication methods can have severe consequences on natives), control is the next-best alternative. Invasive species control programs should focus on the areas of highest value for native biodiversity and those most at risk from non-native invaders (Saunders et al., 2002). Given the special role that reservoirs seem to play in the dispersion of exotics, these environments should be a focus of attention in future management programs. The application of any of the above commented management actions would be enhanced if applied in these focus areas.

Despite of the clear driver role of invasive species in our study area, this is not a constant pattern in other studies. MacDougal & Turkington (2005) or Harrison et al. (2006) reported invasive plant species to be mere passenger of habitat degradation in the decline of native communities. It seems then probable that the driver or passenger role of invasive species is dependent on the organisms and systems under analyses, a fact that should prevent from very categorical generalizations from results obtained in particular studies. Thus, we agree with Gurevitch & Padilla (2004) and Light & Marchetti (2006) in the recommendation that the effect of invasive species should be particularly studied in order to tackle objective management plans facing invasive species control.

Acknowledgements

The fieldwork was funded by the Confederación Hidrográfica del Guadiana through the project "Diseño y Explotación de la Red de Control Biológico de la Cuenca del Río Guadiana" and the Ministry of Science and Technology (REN2002-03513, CGL2005-02699). V. Hermoso held a pre-doctoral fellowship financed by the Spanish Ministry of Education (MEC) (AP-2004-1414). M.C. benefitted from a Juan de la Cierva contract also funded by MEC.

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Table 1. List of native freshwater fish species present in the Guadiana River basin. It is also given their prevalence within the 152 sampled sites, their threatened status according to IUCN (2008), and natural distribution area.

| Species | Distribution | Thtreatened status | Prevalence (%) |
|---|---|---|---|
| *Iberocypris alburnoides* | Iberian Peninsula | VU | 45 |
| *Cobitis paludica* | Iberian Peninsula | VU | 44 |
| *Squalius pyrenaicus* | Iberian Peninsula | NT | 22 |
| *Luciobarbus microcephaus* | Guadiana River | VU | 21 |
| *Iberochondrostoma lemmingii* | Iberian Peninsula | VU | 18 |
| *Luciobarbus comizo* | Iberian Peninsula | VU | 16 |
| *Pseudochondrostoma willkommii* | Iberian Peninsula | VU | 12 |
| *Salaria fluviatilis* | Circunmediterranean | LC | 9 |
| *Luciobarbus sclateri* | Iberian Peninsula | LC | 7 |
| *Anaecypris hispanica* | Guadiana River | EN | 5 |
| *Gobio lozanoi* | Iberian Peninsula | LC | 2 |
| *Luciobarbus guiraonis* | Iberian Peninsula | VU | 1 |
| *Anguilla anguilla* | North Atlantic | CR | 1 |
| *Alosa alosa* | Eastern Atlantic | LC | <1 |

CR: Critically Endangered, EN: Endangered, VU: Vulnerable, NT: Near Threatened, LC: Least Concern,

Table 2. Environmental variables used to characterize the sampled sites. * Denotes human potentially perturbed.

| Scale | Variable | Method | Code | Mean | Range |
|---|---|---|---|---|---|
| Site | Stream order (Strahler)2 | GIS | ORD | 2.1 | 1.0-6.0 |
| | Distance to headwater (km)2 | GIS | HED | 68.1 | 3.6-1,036.1 |
| | Distance to Guadiana River (km)2 | GIS | GUA | 58.2 | 0.0-196.0 |
| | River width (m) * | *In situ* | WID | 10.8 | 1.4-123.0-1.4 |
| | Riparian Quality Index (QBR, Munné et al., 2003) * | *In situ* | QBR | 61.8 | 0-100-0 |
| | $NH_4^+$ (mg/L) * | *In situ* | AMO | 1.38 | 0.02-51.60 |
| | $NO_2^-$ (mg/L) * | *In situ* | NTI | 0.10 | 0.01-2.00 |
| | $NO_3^-$ (mg/L) * | *In situ* | NTA | 4.09 | 0.50-55.90 |
| | $PO_5^{3-}$ (mg/L) * | *In situ* | PHS | 1.00 | 0.05-23.20 |
| | $SO_4^{2-}$ (mg/L * | *In situ* | SLF | 110.1 | 10.0-2380.0 |
| | $Cl^-$ (mg/L) * | *In situ* | CLR | 56.1 | 2.0-834.0 |
| | Conductivity (µS/cm) * | *In situ* | CND | 624.7 | 38.0-3230.0 |
| | Annual precipitation (mm/m$^2$)3 | GIS | PRE | 593.1 | 370.2-1114.5 |
| | Average annual air temperature (ºC)3 | GIS | ATEM | 15.85 | 13.0-18.0 |
| | Distance to the nearest reservoir upstream (km) 2* | GIS | DUP | 41.1 | 0.0-196.0 |
| | Distance to the nearest reservoir downstream (km) 2* | GIS | DWN | 25.9 | 0.2-115.8 |
| Basin | Basin area (Drainage surface in each site, 10$^3$ km$^2$) 1 | GIS | ARE | 260.1 | 0.9-5919.1 |
| | Gravelius index (Area/Perimeter)(m) 1 | GIS | GRA | 1.68 | 1.14-2.68 |
| | Land uses4 | | | | |
| | Urban/Industrial (%)* | GIS | BUI | 0.4 | 0.0-6.7 |
| | Intensive agriculture (%)* | GIS | BIA | 22.5 | 0.0-97.0 |
| | Extensive agriculture (%)* | GIS | BEA | 11.0 | 0.0-89.1-0.0 |
| | Natural (%)* | GIS | BNA | 65.8 | 0.9-100.0 |
| | Population density (Hab/Km$^2$)5* | GIS | POP | 21.0 | 0.0-459.3 |

Data sources
1 Digital Elevation Model 1:100.000. Confederación Hidrográfica del Guadiana.
2 Stream network provided by the Confederación Hidrográfica del Guadiana.
3 Atlas Climático Digital de la Península Ibérica (Ninyerola et al., 2005). Available at http://opengis.uab.es/wms/iberia/index.htm (May 2006).
4 CORINE Land-Cover 1:100.000. Confederación Hidrográfica del Guadiana.
5 Instituto Nacional de Estadística, available at www.ine.es (May 2006).

Table 3. Set of multivariate analysis used to define Environmental and Human Impairment gradients. Only loadings >0.34 are shown. Variable codes in Table 2.

| Aim | Variables | Extracted gradients | % expl. var. (Eigenvalue) | Negative extreme | Positive extreme |
|---|---|---|---|---|---|
| Extract a general human perturbation gradients | All the perturbation variables listed in Table 2 | $PC1_{deg}$ | 34.7 (3.13) | **NTOT** (-0.64), **CLR** (-0.67)<br>**SFL** (-0.55), **PHP** (-0.50)<br>**CND** (-0.71), **BUI** (-0.55)<br>**BIA**(-0.63), **POP**(-0.54) | **QBR** (0.48) |
| | | $PC2_{deg}$ | 22.1 (5.39) | **PHP** (-0.67), **SFL** (-0.57)<br>**POP**(-0.64), **QBR** (-0.35) | **SFL** (0.62), **BIA**(0.39)<br>**CND** (0.34) |
| Identify natural gradients | All the environmental variables listed in Table 2, not related to human perturbation | $PC1_{nat}$ | 50.5 (1.99) | **HED** (-0.96), **ARE** (-0.96)<br>**GRA** (-0.80), **ATEM** (-0.40)<br>**ORD** (-0.88) | |

*NTOT represents the sum of AMO, NTI and NTA.

Table 4. Summary of the set of multiple regression models with a reasonable support for biotic integrity and native species richness ($\Delta$AIC<7). The set of environmental gradients described in Table 3 as well as the distance to the nearest reservoir were used as predictors. * Denotes significant effects (P<0.01).

**Biotic integrity**

| Model rank | IS abundance | $PC1_{deg}$ | $PC2_{deg}$ | $PC1_{nat}$ | Dist Reservoir | $\Delta AIC$ |
|---|---|---|---|---|---|---|
| 1 | -0.432* | | | | | |
| 2 | -0.444* | | | | -0.117 | 0.92 |
| 3 | -0.438* | | -0.076 | | | 0.96 |
| 4 | -0.429* | 0.083 | | | | 1.04 |
| 5 | -0.453* | | | -0.069 | | 1.17 |
| 6 | -0.456* | 0.103 | | -0.093 | | 1.72 |
| 7 | -0.469* | | | -0.081 | -0.128 | 1.90 |
| 8 | -0.435* | 0.083 | -0.076 | | | 1.99 |
| 9 | -0.449* | | -0.071 | | -0.112 | 2.02 |
| 10 | -0.440* | 0.068 | | | -0.101 | 2.23 |
| 11 | -0.455* | | -0.068 | -0.059 | | 2.32 |
| 12 | -0.470* | 0.088 | | -0.099 | -0.110 | 2.77 |
| 13 | -0.458* | 0.100 | -0.064 | -0.082 | | 2.92 |
| 14 | -0.471* | | -0.060 | -0.071 | -0.122 | 3.20 |
| 15 | -0.445* | 0.069 | -0.071 | | -0.095 | 3.30 |
| 16 | -0.471* | 0.086 | -0.058 | -0.089 | -0.104 | 4.10 |

**Native richness**

| Model rank | IS abundance | $PC1_{deg}$ | $PC2_{deg}$ | $PC_{nat}$ | Dist Reservoir | $\Delta AIC$ |
|---|---|---|---|---|---|---|
| 1 | -0.720* | 0.304 | -0.194 | -0.509* | | |
| 2 | -0.711* | | -0.205 | -0.438* | | 1.49 |
| 3 | -0.726* | 0.298 | -0.191 | -0.513* | -0.047 | 1.93 |
| 4 | -0.713* | 0.312 | | -0.542* | | 2.72 |
| 5 | -0.725* | | -0.198 | -0.449* | -0.110 | 3.38 |
| 6 | -0.704* | | | -0.471* | | 4.25 |
| 7 | -0.722* | 0.303 | | -0.546* | -0.066 | 4.64 |
| 8 | -0.720* | | | -0.482* | -0.130 | 6.10 |
| 9 | -0.586* | | -0.270 | | | 6.28 |
| 10 | -0.579* | 0.197 | -0.269 | | | 6.72 |

Table 5. Results of partial ANCOVAS using biotic integrity or native species richness as dependent variables, the abundance of invasive species as co-variable and each environmental gradient as factors. To allow using the continuous gradients as factors they were divided in four categories (each category included an equivalent number of localities). The interaction term is also included between parenthesis when it was not significant and consequently removed from the final model.

**Biotic integrity**

| | *F* | *df* | *P* | *adj.* $R^2$ |
|---|---|---|---|---|
| IS abundance | 26.7 | 1 | < 0.001 | 0.13 |
| PC1deg | 1.2 | 3 | 0.32 | |
| interaction | (0.1) | (3) | (0.95) | |

| | *F* | *df* | *P* | *adj.* $R^2$ |
|---|---|---|---|---|
| IS abundance | 24.9 | 1 | < 0.001 | 0.13 |
| PC2deg | 1.3 | 3 | 0.29 | |
| interaction | (0.5) | (3) | (0.71) | |

| | *F* | *df* | *P* | *adj.* $R^2$ |
|---|---|---|---|---|
| IS abundance | 23.3 | 1 | < 0.001 | 0.12 |
| PC1nat | 0.9 | 3 | 0.46 | |
| interaction | (0.9) | (3) | (0.43) | |

| | *F* | *df* | *P* | *adj.* $R^2$ |
|---|---|---|---|---|
| IS abundance | 24.5 | 1 | < 0.001 | 0.13 |
| Dist. Reservoirs | 1.4 | 3 | 0.24 | |
| interaction | (1.7) | (3) | (0.16) | |

**Native richness**

| | *F* | *df* | *P* | *adj.* $R^2$ |
|---|---|---|---|---|
| IS abundance | 18.2 | 1 | < 0.001 | 0.08 |
| PC1deg | 1.0 | 3 | 0.41 | |
| interaction | (0.3) | (3) | (0.27) | |

| | *F* | *df* | *P* | *adj.* $R^2$ |
|---|---|---|---|---|
| IS abundance | 19.7 | 1 | < 0.001 | 0.14 |
| PC2deg | 5.0 | 3 | 0.002 | |
| interaction | (0.2) | (3) | (0.91) | |

| | *F* | *df* | *P* | *adj.* $R^2$ |
|---|---|---|---|---|
| IS abundance | 25.1 | 1 | < 0.001 | 0.13 |
| PC1nat | 4.2 | 3 | 0.007 | |
| interaction | (1.1) | (3) | (0.34) | |

| | *F* | *df* | *P* | *adj.* $R^2$ |
|---|---|---|---|---|
| IS abundance | 15.5 | 1 | < 0.001 | 0.09 |
| Dist. Reservoirs | 1.3 | 3 | 0.28 | |
| interaction | (1.3) | (3) | (0.28) | |

Figure 1. Different conceptual models explaining alternative pathways responsible for the decline of native communities. The two most commonly cited causes of biodiversity loss are included: habitat degradation and invasive species. A) Represents a full model, where both factors are responsible for the decline of native communities. B and C) Are two alternatives pathways, where invasive species act as drivers of native decline (only invasive species would have direct effects on natives) or passenger (habitat degradation would be the leading cause of natives decline). An additional interactive pathway can be also considered, where habitat degradation could be enhancing in different ways (numerically or functionally mediated processes, according to Didham et al., 2007) the *per capita* effect of invasive species (D). The study of this relationship is essential for facing the problem of biodiversity loss.

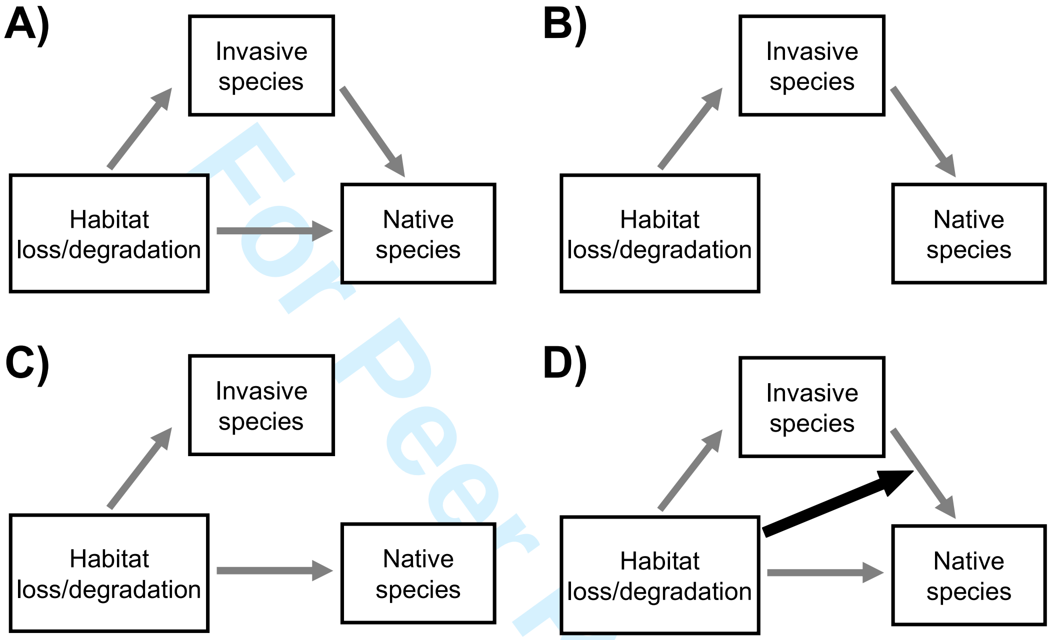


Figure 2. Study area with indication of the distribution of the sampling localities (n=152).

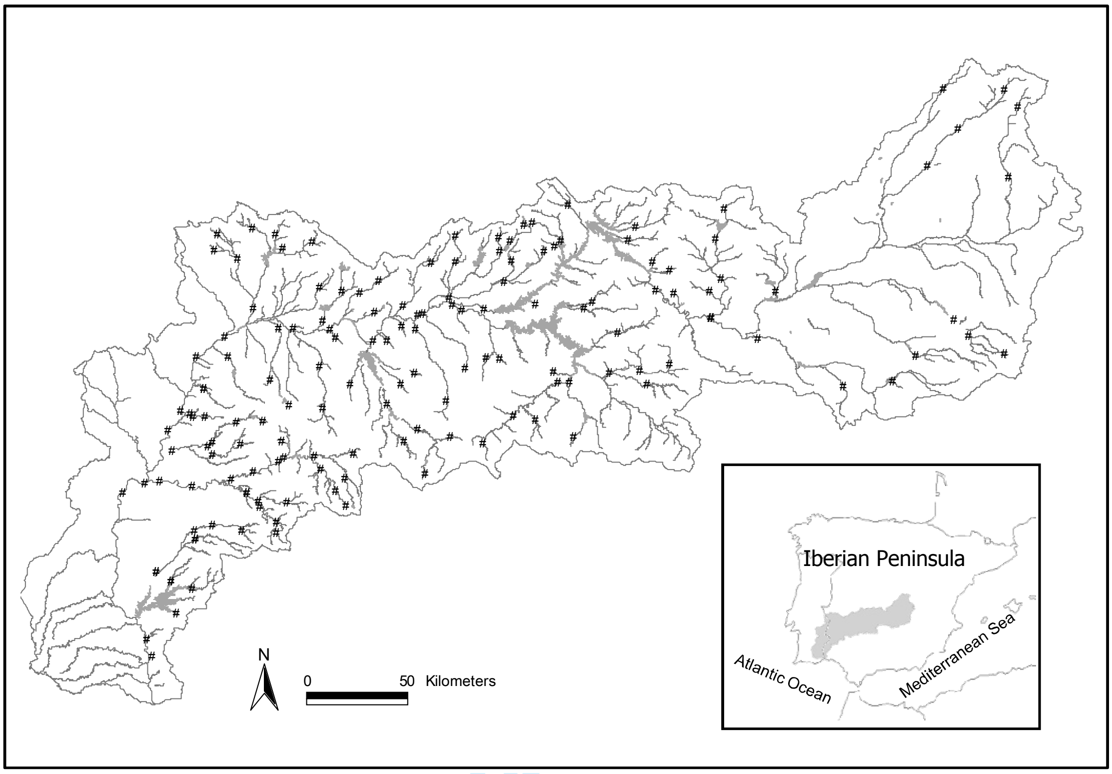
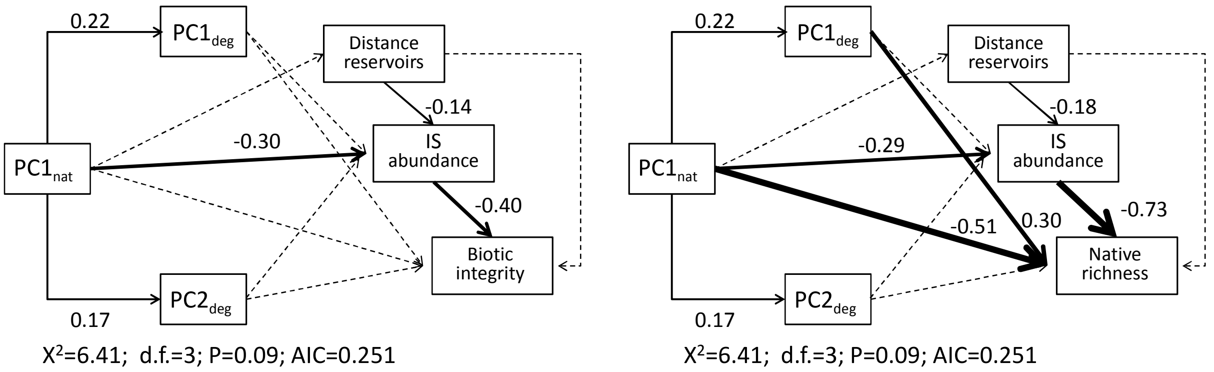
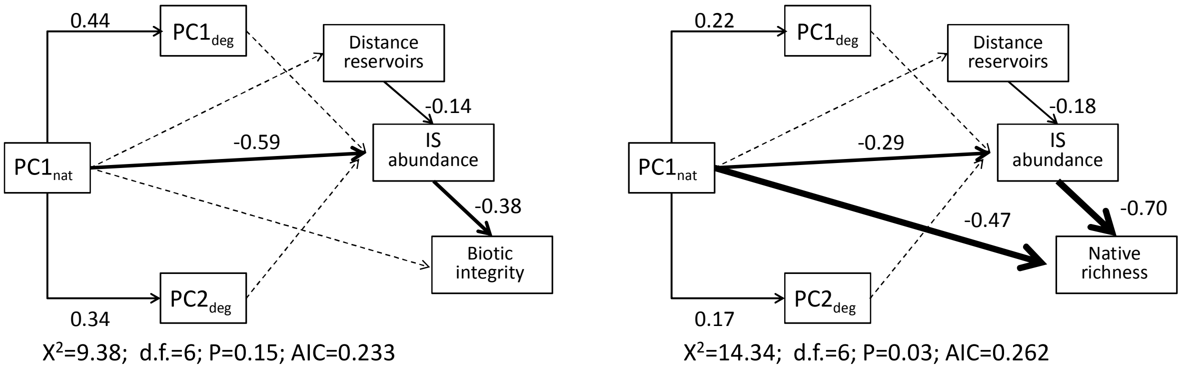


Figure 3. Scheme of different Structural equation Modeling (SEM) testing alternative pathways of invasive species and habitat degradation on native decline. The biotic integrity and the number of native freshwater fishes were used as a surrogate for measuring the relative biodiversity loss or community health. In the driver model it was excluded all direct effects of any human perturbation and the abundance of invasive species would be leading the process of biodiversity loss. On the other hand, the passenger model considers habitat degradation as the main source of native disturbance, while exotics do not have any relevant effect. The full model includes all potential paths between the variables considered. Standardized coefficients based on the correlation matrix for each path are showed. Dotted lines represent non significant effects and line thickness is proportional to their relative weight. The Chi-squared statistic, degrees of freedom (d.f.), p value and Akaike's information criterion (AIC) are also shown.

A) Full model



B) Driver model



C) Passenger model

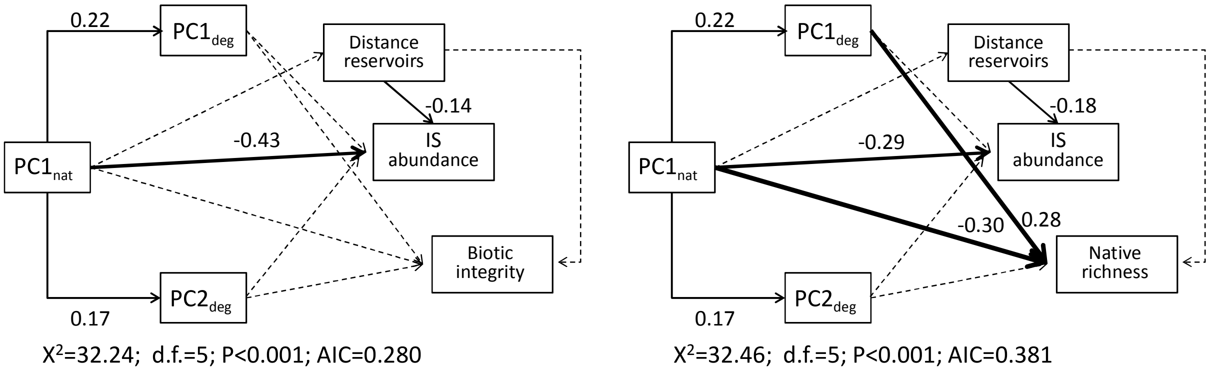


Figure 4. Relationship between the abundance of invasive species (covariable) and the biotic integrity (dependent) along different natural and perturbation gradients (factors). Each plot represents a portion of the gradient, corresponding to the four categories used in the ANCOVAS analyses.